

From **THE DEPARTMENT OF ONCOLOGY-PATHOLOGY**

Karolinska Institutet, Stockholm, Sweden

Studies of targeted therapy in breast cancer using
trastuzumab: HER2 testing and trastuzumab treatment –
clinical and economic evaluation.

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ABSTRACT

The incidence in breast cancer has more than tripled in Sweden since the 1960-ies. During the same time period the 5-year survival rate has increase from around 65% to almost 90%. The survival increase is mainly related to medical treatments (endocrine treatments, chemotherapy in different combinations), radiotherapy and breast cancer screening. Two thirds of costs for breast cancer are not related to these therapies, but to costs outside of the health care system (e.g. early retirement and premature death). Continued improvements in therapies would therefore be of great gain for the outcome and overall cost of breast cancer. One of these improvements has been the discovery of the Human Epidermal Growth Factor Receptor 2 (HER2) in the nineteen eighties and the development of the monoclonal antibody directed against HER2 (trastuzumab) in the nineteen nineties. Trastuzumab is registered for treatment of HER2 positive early and metastatic breast cancer. HER2 diagnosis and trastuzumab treatment are dependent on each other, as diagnosis is meaningless without the treatment and treatment is meaningless without diagnosis. This relationship is usually called co-dependent technologies.

The aim of this thesis was to determine how a targeted patient population is optimally managed in terms of co-dependent technologies.

The results demonstrate that HER2 testing is not optimally carried out in all situations. In 151 patients we found a 10% change in HER2 status between primary tumours and relapses (19% from HER2+ to HER2- and 6% from HER2- to HER2+). Patients with a change in HER2 status had a significant 5.47 (95% CI 2.01–14.91) increased risk of dying compared to patient with stable positive HER2 status (86% of these patients received trastuzumab treatment). In another study, there was a 32% change in 459 patients in Oestrogen Receptor (ER) status (ER+ to ER- 24.6% ER- to ER+ 7.8%). Also ER change is related to worse prognosis and patients with ER negative systemic relapses had a significant twofold (95% CI 1.39-2.87) increased risk of dying compared to patients with ER positive relapses. This shows that it is clinically relevant to re-test relapses for tumour marker status, and changes may offer additional treatment options. To be able to follow-up and monitor usage and outcome in clinical practice (clinical effectiveness as opposed to use in clinical trials: clinical efficacy) treatment with trastuzumab should be used according to guidelines, although we found large differences in usage between Health Care Regions (HCRs) in Sweden (years 2000-2004 300% difference between North HCR and South HCR, years 2006-2008 40% difference between Stockholm-Gotland and South-East and West HCRs). Another important aspect is that re-testing of HER2 status before trastuzumab treatment in the metastatic setting is cost-effective (USD 56,000 -USD 67,000 per Quality Adjusted Life Year and USD 39,000 – USD 46,000) and should therefore always be done.

Diagnosis of HER2 status and treatment with trastuzumab is a challenge and this thesis points to the complexity of co-dependent technologies. The knowledge created could be used for the introduction of future co-dependent technologies in order to gain the most benefit, both to patients and to society.

LIST OF PUBLICATIONS

- I** **HER2 status in a population derived breast cancer cohort – discordances during tumour progression.**
Wilking, U. Karlsson, E. Skoog, L. Hatschek, T. Lidbrink, E. Elmberger, G. Johansson, H. Lindstrom, L. Bergh, J.
Breast Cancer Res Treat 125(2): 553-561.
- II** **Clinically used breast cancer markers are instable throughout tumor progression**
Lindström LS, Karlsson E, **Wilking U**, Johansson U, Lidbrink E, Hatschek T, Skoog L, Bergh J.
Submitted, under revision for resubmission
- III** **Trastuzumab use in Breast Cancer (BC) patients in the six Health Care Regions in Sweden.**
Wilking, U. Jonsson, B. Wilking, N. Bergh, J.
Acta Oncol 49(6): 844-850.
- IV** **Cost-effectiveness of Re-Testing HER2 Status in Metastatic Breast Cancer patients before offering trastuzumab treatment**
Bernow M and **Wilking U**, Jönsson B, Wilking N, Bergh J
Manuscript

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ABBREVIATIONS

AI	Aromatase Inhibitors
ASR	Age-standardised rate is based on age structure for years 2000-2025 according to WHO's calculations.
BP	British Pounds
CEA	Cost Effectiveness Analysis
CER	Comparative Effectiveness Research
CHF	Congestive Heart Failure
CI	Confidence Intervals
CISH	Chromogenic In Situ Hybridisation
CUA	Cost Utility Analysis
CVM	Contingent Valuation Method
CYP	Cytochrome P450
DCE	Discrete Choice Experimentation
DFS	Disease Free Survival
EMA	European Medicines Agency
ER	Oestrogen Receptor
EU	European Union
FDA	Food and Drug Administration
FISH	Fluorescence In Situ Hybridization
FNA	Fine Needle Aspirate
GDP	Gross Domestic Product
HCR	Health Care Region. There are six HCRs in Sweden: North HCR, Stockholm-Gotland HCR, Uppsala-Örebro HCR, South HCR, South-East HCR, West HCR,
HER2	Human Epidermal Growth Factor Receptor 2
HTA	Health Technology Assessment
ICC	Immunocytochemistry
IHC	Immunohistochemistry
ISH	In Situ Hybridisation
LYG	Life Years Gained
NICE	National Institute for Health and Clinical Excellence
OS	Overall Survival

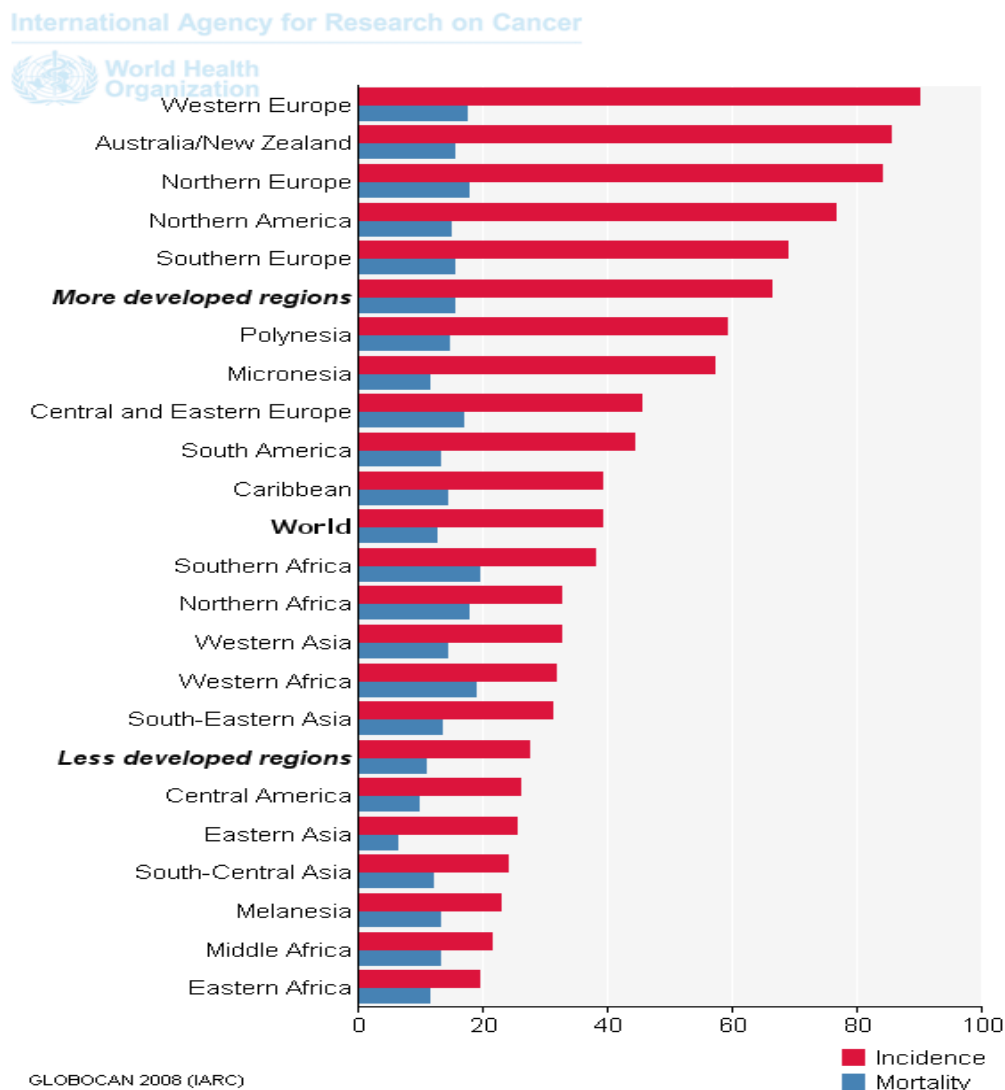
PFS	Progression Free Survival
pCR	pathologic Complete Response
PR	Progesterone Receptor
SEK	Swedish Crowns (svenska kronor)
SNP	Single-Nucleotide Polymorphism
QALY	Quality Adjusted Life Year
QoL	Quality of Life
USD	USA dollars – USD 1 = SEK 7.19 (7th January 2011)
VEGF	Vascular Endothelial Growth Factor
WTP	Willingness To Pay

1 INTRODUCTION

1.1 Breast cancer epidemiology

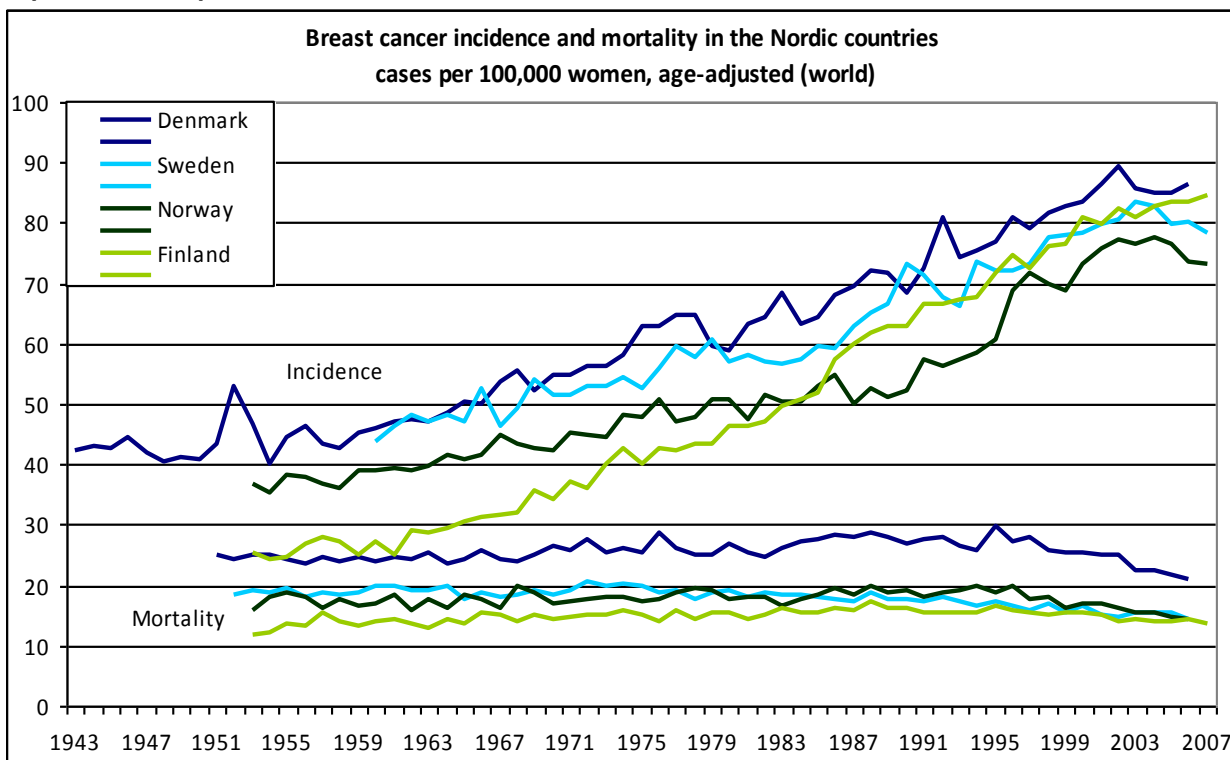
Breast cancer is the most common malignancy among women, with an estimated 1.4 million women diagnosed with the disease globally in the year 2008. The incidence of breast cancer has increased over the years; incidence in Europe was around 320,000 women in 1984 and around 424,000 in 2008. This increase is estimated to continue with a projected incidence in Europe of 465,000 women in 2020 (Ferlay et al., 2010). For Sweden the incidence numbers were approximately 2,500, 5,000 and 7,000 women in 1960, 1990 and 2008, respectively (Socialstyrelsen, 2010).

Figure 1 Global incidence and mortality in breast cancer (ASR)



In the USA the incidence in 2008 was 182,000 and the Age Standardise Rates (ASR) (WHO, 2001) was 76; Swedish ASR was 79 in 2008, see figure 1 (Ferlay et al., 2010). The ASR for 2002 and 2008 were 37.4 and 39.0 in the World and 82.5 and 84 in Northern Europe, respectively (Ferlay et al., 2010, Parkin et al., 2005).

Figure 2 Incidence and mortality in the Nordic countries 1950-2007 (world ASR)



From (Wilking N and Kasteng, 2009)

The increased incidence in Sweden (and most other “Western countries”) is mainly due to the combined effects of “Western life style factors” as increasing age, overweight, screening, hormone replacement therapy and changed reproductive patterns (Kumle, 2008, McPherson et al., 2000, Veronesi et al., 2005). Development of adjuvant treatment modalities of breast cancer, starting in the 1970-ties, has resulted in a marked decline in relative mortality (Berry et al., 2005, Koscielny et al., 2009, Nystrom et al., 2002, Clarke et al., 2005, EBCTCG, 2005a). Recent data shows in fact that multidisciplinary management teams and adjuvant treatments account for the major part of the reduced mortality, while screening only accounts

for 1/3 of the reduced mortality (Kalager et al., 2010). Recent studies also discuss the reduced mortality in lower age groups being mainly related to access to treatment (Autier et al., 2011, Foukakis et al., 2011, Coleman et al., 2011).

Breast cancer survival is high in Sweden and mortality numbers are stable at approximately 1,500 women per year since 1990 (Socialstyrelsen, 2009, Sant et al., 2009, Talback et al., 2003). The relative increase in survival results in an increased prevalence of survivors. In 2002 the estimate was that more than 80,000 women in Sweden had had a previous diagnosis of breast cancer (Lidgren et al., 2007b).

Although almost 90% of all women in Sweden and around 80% in Europe with a breast cancer diagnosis will survive 5 years or more (Ferlay et al., 2010, Coleman et al., 2011), some women will have recurrence of the disease (Mirza et al., 2002). At the time of relapse it is important to distinguish between an isolated loco-regional recurrence versus metastatic disease (Schmoor et al., 2000, Koscielny et al., 2009, Malmstrom et al., 2003). Very few patients with metastatic breast cancer will be cured and the median survival time is around two years, although patients <60 years had a statistically significant increased survival in a population based study in Sweden (Foukakis et al., 2011). Around 50% of patients with local relapse and around 20% of patients with regional relapse will obtain long-term survival/cure (Rausei et al., 2010, Wapnir IL et al., 2006). As in the adjuvant situation, improved treatment methods (new drugs, improvements in supportive care) have resulted in increased life expectancy for patients with metastatic breast cancer (Kochhar et al., 2005, Feyer et al., 2008). An increased knowledge of tumour biology has also increased the understanding of which patient to treat, at what dose and when to treat. This is particularly important for the use of drugs in targeted patient populations (Bergh, 2009).

1.2 Biology of breast cancer.

Breast cancer is not one disease, but many diseases based on different prognostic and predictive factors. Prognostic factors are used for outcome measures in untreated patients. Predictive factors are used to define the likeliness of responding to a certain treatment.

The TNM staging system for Tumour size, Lymph node involvement and Metastasis is the most important prognostic tool and tumour size and lymph node involvement have mostly been regarded as the most important prognostic factors (Singletary and Greene, 2003). Below follow brief descriptions of the standard prognostic and predictive factors.

Age: Younger women with a breast cancer diagnosis have a worse outcome, even if prognosis has improved over time due to adjuvant treatments (Chung et al., 1996, Foukakis et al., 2011, EBCTCG, 2005a, Kheirelseid et al., 2011).

Tumour size: A primary tumour over 2 cm indicates a worse prognosis, with 20 year disease free survival, DFS, of 64% compared to 79% with tumours with a size less than 2cm (Quiet et al., 1995).

Lymph node involvement: In untreated patients with lymph nodes without tumour involvement, the 5 year survival rate is 85% and if there are more or equal to 4 lymph nodes with tumour involvement the 5 year survival rate is only 26% (Fisher et al., 1983).

Histology: The most common histological grading system is a joint score of tubule formation, mitotic count and nuclear pleomorphism and these are brought together and defined as well differentiated (Elston-Ellis I), moderately differentiated (Elston-Ellis II), poorly differentiated (Elston-Ellis III) (Elston and Ellis, 2002).

Oestrogen receptor (ER) status and progesterone receptor (PR) status: ER positive breast cancer is the most common breast cancer sub-type, and around 50-80% (related to age) of all women belong to this sub-type (Clark et al., 1984). Oestrogen binding to ERs can promote cell growth in breast cancer (Osborne, 1998). A positive ER status is also a predictor of response to endocrine therapy (Jordan, 1988, Ryden et al., 2005, EBCTCG, 2011). A positive PR status is a prognostic factor, as it does not alter response to endocrine therapy (Dowsett et al., 2005, EBCTCG, 2011).

P53 is a tumour suppressor gene. Patients with breast cancer tumours with loss of p53 function (mostly by different types of mutations) have a worse prognosis (Hanahan and Weinberg, 2011, Petitjean et al., 2007).

Human Epidermal Growth Factor Receptor 2 (HER2): Details on HER2 are described under section 1.4, as this is one of the main focuses of this thesis. In

In addition to the molecular separation of breast cancers into several sub-groups, there are **signalling pathways** linked to gene expression, critical for cancer development and growth (Alvarez et al., 2010, Hanahan and Weinberg, 2011). These pathways affect the transcription of various genes via transmembrane proteins and intracellular enzymatic activities. One critical point in these pathways is the binding of a ligand (a substance with specific binding capacity), to a corresponding extracellular receptor (see figure 3). This ligand binding activates various enzyme systems, ultimately resulting in changes in cellular behaviour or growth. There are also feed-back mechanisms that have different effects on signalling (e.g. Ras, PI3K, Myc) and there are also factors that may circumvent cell growth regulation (e.g. P53, RB) (Hanahan and Weinberg, 2011) (see figure 3). The signalling pathways are presently being explored as therapeutic targets; targeted at the receptor level or further downstream. (Ross et al., 2003, Tsourdi et al., 2011, Arriola et al., 2011).

1.3 Pharmacogenomics

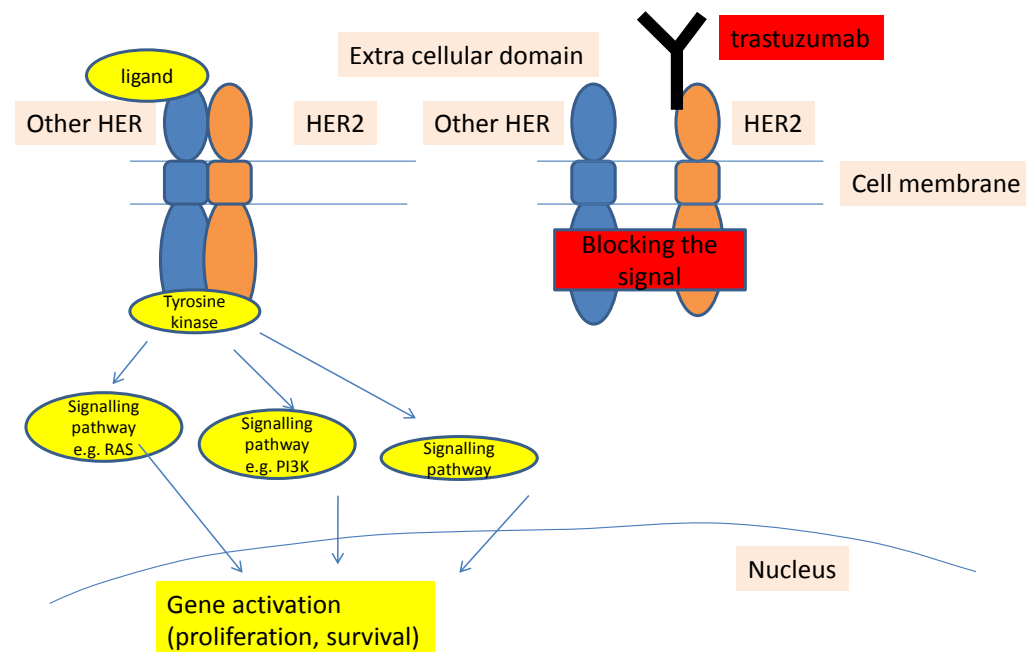
Pharmacogenomics is the influence of normal genetic variation or single nucleotide polymorphism (SNP) on drug response in patients by correlating gene expression with efficacy or toxicity of a drug (Sachidanandam et al., 2001, Diamandis et al., 2010).

A SNP is a normal DNA sequence variation occurring when a single nucleotide in the genome (or other shared sequence) differs between individuals or between paired chromosomes in an individual (Sachidanandam et al., 2001). Variations in the DNA sequences can affect diseases response to pathogens, chemicals, drugs, vaccines, and other agents (Cooper DN, 2005, Farmer et al., 2009, Diamandis et al., 2010). Pharmacogenomics are also thought to be key enablers in the concept of personalized medicine (Wang et al., 2011, Diamandis et al., 2010)

Pharmacogenomics aims at developing rational means to “optimize” treatment, with respect to genotype of each individual. It explores biological markers that may define the right dose, treatment time and avoid adverse reactions in each patient (Wang et al., 2011, Albain et al., 2010, Farmer et al., 2009, Diamandis et al., 2010). The biological markers in responders in the early clinical trials could then be used in larger pivotal studies (Pusztai, 2007). The most commonly known genes that are

responsible for variances in drug metabolism and response, is the cytochrome P450 (CYP) genes (Gomes et al., 2009, Hart et al., 2008). Codeine, tamoxifen, and warfarin are examples that follow the CYP metabolic pathways. Cancer related genes targeted for treatment are for example EGFR (treatments: cetuximab, erlotinib, gefitinib, panitumumab), K-Ras (treatments: cetuximab, panitumumab) and HER2 (treatments: trastuzumab, lapatinib) (Wang et al., 2011).

Figure 4 HER2 signalling pathway and blocking of dimerization by trastuzumab



1.4 Human Epidermal Growth Factor Receptor 2 in Breast Cancer

The Human Epidermal Growth Factor Receptor 2 (HER2) is a cell membrane surface-bound receptor tyrosine kinase (Coussens et al., 1985, Hanahan and Weinberg, 2011, Slamon et al., 1987). HER2 is one of four members of the HER family. The HER2 gene is located on chromosome 17q21-q22 (Coussens et al., 1985). HER2 is normally involved in the signal transduction pathways controlling cell growth and differentiation (Stern et al., 1986). A normal epithelial cell contains two

copies of the HER2 gene and is defined positive when the gene is amplified. The normal number of HER2 on the cell surface is below 20,000 and is defined HER2 positive at levels over 2,300,000 (Ross et al., 2003).

HER2 has an independent prognostic value (Slamon et al., 1987, Winstanley et al., 1991, Seshadri et al., 1993, Al-azawi et al., 2011) and is associated with a general poor prognosis, with statistically significant shorter survival time (Ross et al., 2003, Sjogren et al., 1998, Revillion et al., 1998). HER2 positive breast cancer indicates higher sensitivity to chemotherapy and lower sensitivity to hormonal treatments (Dowsett, 2001, Nunes and Harris, 2002). The aspect of heterogeneity of HER2 in breast cancer tissue is not clear, but there seem to be different sub-groups of HER2, with different characteristics (Wu et al., 2008, Vance et al., 2009, Andersson et al., 2004, Staaf et al., 2010). HER2 positive early breast cancer has also been investigated using gene expression analysis. There are three sub-types of HER2 positive breast cancer and one of the sub-types has been described to have worse outcome (Staaf et al., 2010). There is data on extra cellular HER2 as an indicator of prognosis, but this technique is not yet recognised, possibly due to the small series in the reports (Meng et al., 2004, Fehm et al., 2004, Hayashi et al., 2011, Nunes and Harris, 2002).

HER2 positivity is also linked to response to HER2 targeted drugs; the monoclonal antibody trastuzumab, directed against the extracellular domain of HER2, (Baselga J, 1996), the HER1-HER2 tyrosine kinase inhibitors lapatinib and neratinib (Wood et al., 2004, Bose and Ozer, 2009), the HER dimerization inhibitor pertuzumab (Adams et al., 2006) and trasuzumab / cytotoxic drug conjugates, TDM1 (Lewis Phillips et al., 2008). This thesis is focused on trastuzumab.

1.4.1 Development of HER2 analysis methods in breast cancer

The HER2 status is determined by testing of tumour tissue or blood (extracellular HER2). In the earliest studies, the HER2 status was determined using Southern blot (sequencing method, to reveal the number of DNA copies) (Ross et al., 2003, Slamon et al., 1987). Testing strategies for HER2 were later developed in order to assess the amount of HER2 protein and number of gene copies (Pauletti et al., 2000, Pauletti et al., 1996, Liu et al., 1992, Muss et al., 1994, Tsuda et al., 2001, Larsimont et al., 2002, Bergqvist et al., 2007). The methods were standardized and analyses are nowadays done with Immunohistochemistry (IHC) assays (see table 1) at most cancer centres (protein screening method).

Table 1 IHC HER2 cut-off levels

Level 1)	Description of cell surface staining 1)	No of HER2 2)
0 HER2 negative	No staining, or less than 10% staining of tumour cells	<20,000
1 HER2 negative	Weak, or hardly visible membrane staining in >10% of tumour cells. Membrane staining not complete.	~100,000
2 HER2 equivocal	Weak to moderate complete membrane staining >10% of tumour cells	~500,000
3 HER2 positive	Strong complete membrane staining in >30% of tumour cells	≥2,300,000

1) (DAKO, 2011)

2) (Ross et al., 2003)

The results in the Swedish laboratory quality control program showed that IHC results have “good correlation” between laboratories, with kappa-values of 0.67 and 0.77 in year 2005 and year 2006, respectively (Ryden et al., 2009).

Confirmation of IHC results

Tumours with HER2 borderline result (IHC 2+) are confirmed with a test of gene amplification using an in-situ hybridization method (ISH); fluorescence in-situ hybridization FISH is the most recommended method (Wolff et al., 2007, Jacobs et al., 1999b, Jacobs et al., 1999a, Couturier et al., 2000, Birner et al., 2001). FISH has higher sensitivity (the proportion of correct HER2 positives), as well as higher specificity (the proportion of correct HER2 negatives). IHC has 92.6% sensitivity for IHC 2+ and 3+ compared to FISH, and 98.8% specificity for IHC 3+ compared to FISH and the overall concordance rate was 96.1% for IHC 3+ compared to FISH in the Yazij study (Yaziji et al., 2004). If the equivocal results with IHC (2+) are included, the specificity is reduced to almost 50% compared to FISH (Tsuda et al., 2001, Jacobs et al., 1999b, Yaziji et al., 2004). In an overview by Elkin shows that around 7% of IHC 0/1+ are FISH positive (Elkin et al., 2004).

There are some negative factors with the FISH method that in many centres has resulted in a decision to use FISH only for confirmation. The FISH method is more expensive and time consuming compared to IHC (Yaziji et al., 2004, Jacobs et al., 1999a) and requires a dedicated pathologist, and thus it is recommended that all confirmatory FISH testing is done by few laboratories and well trained staff (Perez et

al., 2002, Wolff et al., 2007, Mackay et al., 2011). The Swedish inter laboratory quality control results for FISH had a “very good correlation” with kappa-values of 0.92 and 0.96 in year 2005 and year 2006, respectively (Ryden et al., 2009).

Some centres are using another ISH method, Chromogenic In Situ Hybridisation (CISH) for screening instead of IHC (a separate confirmation FISH is not usually required), or as confirmation for equivocal IHC instead of FISH (Tanner et al., 2000). If CISH is used instead of IHC for screening, this will mean that the total time required for testing will be shorter and the result is more reliable compared to IHC (Gong et al., 2009b). The advantages with the CISH method are that the DNA probe is detected using a simple IHC-like peroxidase reaction and the CISH requires no specific fluorescence microscope. The results with CISH are also concordant with FISH (concordance 97-100%) (Gong et al., 2009a, Gong et al., 2009b, Arena et al., 2010). As with FISH, CISH detects gene amplified IHC equivocal, 2+ results (Meijer et al., 2011).

1.4.2 HER2 diagnostics in early breast cancer and metastatic breast cancer

The early studies assumed that HER2 status was stable during disease progression, and HER2 status was therefore only analysed in primary tumour tissue (Slamon et al., 1987, Cobleigh et al., 1999, Baselga J, 1996). The first data by Slamon in 1987 showed a 30% proportion of HER positive breast cancer patients. NB! In one cohort of patients 18% of tumours were HER2 positive and in another cohort 40% of tumours were HER2 positive (Slamon et al., 1987). Studies from Sweden show 14-19% HER2 positive early breast cancers in a screening environment (Ryden et al., 2009, Sjogren et al., 1998, Borg et al., 1989). The proportion of patients with HER2 positive metastatic breast cancer is around 30% (Wilking et al., 2011). The differences in the proportion of HER2 positive early breast cancers and metastatic breast cancers reflects the fact that HER2 positive breast cancers are more aggressive and these patients have an increased risk of developing distant metastases. However, studies indicate that tumour cells are not stable, and changes in tumour behaviour are seen during tumour development. Between 0-38% of patients will have altered HER2 status in recurrences (see table 2).

Table 2 HER2 in early breast cancer and recurrence in the same patient

Table 2: HER-2/neu in primary breast cancers with intraindividual comparisons with the corresponding recurrences								
Publication/ Abstract Author	Patients Number	Discordant Patients Number Percent		Site of relapse				Methods for receptor determination
				Local relapse		Systemic relapse		
				Number	Percent	Number	Percent	
Neihans 1993	30	1	3			30	100	Immunoperoxidase Technique
Masood 2000	56	1	2			56	100	IHC
Edgerton 2003	113	19	17	20		93		IHC/ FISH
Shimizu 2000	21	0	0			21	100	IHC/ Sandwich Enzyme Immunoassay
Tanner 2001	46	0	0	33		12		IHC/ CISH
Gancberg 2002	107	10	9			107	100	IHC/ FISH
Sekido 2003	44	2	5			*		IHC/ FISH
Luftner 2004	80	10	13			*		IHC
Meng 2004	24	9	38			*		Immunofluorescence Staining
Zidan 2005	58	8	14			*		IHC/ FISH
Lipton 2005	240	61	25			*		‡HER-2/neu Immunosorbent Assay
Gong 2005	60	2	3	43		17		FISH
Solomayer 2006	45	17	38			45	100	IHC/ FISH/ Double Immunofluorescence
Pectasides 2006	16	6	38			16	100	IHC/ CISH
Wilking 2008	155	18	12			*		IHC/ FISH
Tapia 2007	105	8	8			105	100	FISH
MacFarlane 2008	160	9	6			*		IHC
Lidtke 2009	528	72	14			*		IHC/ FISH
Leitzel 2009	27	5	19			27		Proximity-Based Quantifying Protein
Lower 2009	382	127	33			382		IHC
Chan 2009	116	18	16	45		71		IHC/ FISH
Broom 2009	100	0	0			100	100	§

* The number of samples within each category not clear from corresponding article
‡ HER-2/neu Oncoprotein Enzyme-Linked Immunosorbent Assay
§ Methods for receptor determination not clear from article

From (Lindström et al., 2010)

These results are being challenged and there are discussions whether the changes are artefacts or reflect biology and therefore are of real clinical importance (Khasraw et al., 2011, Pusztai et al., 2010).

1.5 Breast Cancer treatment

The breast cancer diseases are treated with different technologies for short or long periods of time. The most important treatments are surgery, radiotherapy (EBCTCG, 2005b), chemotherapies and endocrine therapies (Benson et al., 2009).

The types of treatments offered, are based on the prognostic and predictive factors described earlier. As also described earlier, medical treatments have contributed the

most to the increased survival of breast cancer. The two major medical treatment types, for patients with increased risk of recurrence, are chemotherapy combinations and endocrine therapies. Adjuvant treatments are also guided by a risk-benefit analysis, where risks of recurrence are weighted against the adverse effects of treatment (Goldhirsch et al., 2011, EBCTCG, 2005a, EBCTCG, 2011).

Chemotherapy inhibits cell growth by different mechanisms of action.

Chemotherapy in various combinations has been developed over more than 40 years and combinations containing an anthracycline or a taxane are regarded to be most effective. Adjuvant chemotherapy provides a long term survival increase of absolute 15% and 10% in patients with lymph node positive disease and lymph node negative disease, respectively (EBCTCG, 2005a). The later updates show that the survival advantage remained (EBCTCG, 2007).

Endocrine therapy blocks the effect of hormones on cell proliferation. In post menopausal women, with hormone dependent disease, tamoxifen is most widely used, partly replaced by the Aromatase Inhibitors (AI) (anastrozol, exemestane, letrozol) (Burstein et al., 2010). In pre menopausal women, with hormone dependent disease, increased survival can be achieved by tamoxifen alone, or combined with ovarian ablation (surgery, radiotherapy or a gonadotropin-releasing hormone, GNRH-agonist) (EBCTCG, 2005a). Tamoxifen provide over 30% reduced 15-year mortality (EBCTCG, 2011) and AIs reduces the risk of relapse with around 4% compared to tamoxifen, but no gain is seen in Overall Survival (OS) (Burstein et al., 2010, Dowsett et al., 2010).

1.5.1 Treatment for HER2 positive breast cancer

1.5.1.1 Trastuzumab

The monoclonal antibody, trastuzumab (Herceptin ®, F. Hoffman-La Roche Ltd Basel Switzerland), targets the extracellular domain of the HER2 protein and blocks the corresponding down-stream signalling pathway (see figure 4). Trastuzumab was registered in the EU in year 2000 for the treatment of HER2 positive metastatic breast cancer, having already been approved in USA in 1998. The adjuvant indication was approved in 2006 by EMA for EU (shortest process ever, around two months), followed by the FDA approval for USA.

Trastuzumab has relatively few side effects, with cardiac side effects as the major concern. The frequency of Congestive Heart Failure (CHF) is in the order of 2-4.3% of patients and LVEF drop (symptomatic and asymptomatic) is found in around 4-27% of patients (this varies largely between reports, related to the set levels of LVEF drop) (Dang et al., 2010, Perez et al., 2008, Suter et al., 2007, Slamon et al., 2001). The cardiac toxicity is often reversible and around 50% of patients can restart with trastuzumab treatment again after LVEF recovery (Dang et al., 2010, Perez et al., 2008). This is in contrast to the anthracycline related cardiac toxicity, which is not reversible and carries a long time risk of cardiac failure (Mulrooney et al., 2009).

1.5.1.2 Trastuzumab in Metastatic Breast Cancer

The Phase II data showed tumour responses with single agent trastuzumab in 11-15% of patients (Baselga J, 1996, Cobleigh et al., 1999, Vogel et al., 2001). The combination of trastuzumab and taxane based chemotherapies have been studied in metastatic breast cancer. In those studies the addition of trastuzumab provided statistically significant survival improvements in HER2 positive patients by 5-8.5 months. In this patient population the median survival time was 20-22.5 months without trastuzumab treatment (Marty et al., 2005, Slamon et al., 2001). In the study by Slamon the cardiac dysfunctions were seen in 27% of patients randomised to the combination of an anthracycline and trastuzumab (Slamon et al., 2001).

Trastuzumab may also be combined with vinorelbine for metastatic breast cancer with similar survival and fewer side effects compared with the standard docetaxel combination (Andersson et al., 2011).

NB! In an interesting comparison between targeting patients with HER2 positive disease versus treating an unselected patient population, Simon calculated that 23,586 patients would have been required to detect similar survival differences in the studies instead of the 469 patients included (Simon and Maitournam, 2004).

In a study in postmenopausal metastatic breast cancer patients, with HER2 and ER positive primary cancer (the TAnDEM studie), trastuzumab in combination with the AI anastrozole increased progression free survival (PFS) from 2.4 months (95% CI 2.0- 4.6 months) without trastuzumab to 4.8 months (95% CI 3.7-7.0 months) with trastuzumab (P= 0.0007), although the OS difference was not significant (70% of

patients in the anastrozole alone arm crossed over to treatment with trastuzumab) (Kaufman et al., 2009).

1.5.1.3 Trastuzumab in Early Breast Cancer

Adjuvant trastuzumab treatment for 12 months in combination/sequence with different types of chemotherapy results in an absolute disease free survival improvement of 6-12%, and a relative recurrence risk reduction of around 50% in women with HER2 positive early breast cancer (Gianni et al., 2011, Romond et al., 2005, Slamon, 2009, Smith et al., 2007, Piccart-Gebhart et al., 2005, Perez et al., 2011). In the latest update at 4 years, the OS benefit of trastuzumab remained (HR 0.61 95% CI 0.50-0.75) (Perez et al., 2011), although this benefit was not seen in another adjuvant study, with a 4-year HR 0.85 (95% CI 0.70-1.04; p=0.11) (Gianni et al., 2011). There is also data on a shorter treatment time of 9 weeks, but the initial effect on survival (3 year PFS 89% vs. 78% without trastuzumab) was reduced with time (5 year HR for recurrence 0.65, 95% CI 0.38-1.12 p=0.12). Thus, the recommended adjuvant treatment time remains at 12 months.

1.5.1.4 Trastuzumab in comparison to lapatinib in Breast Cancer

The HER1 and HER2 tyrosine kinase inhibitor lapatinib has obtained conditional approval in the EU for treatment of metastatic breast cancer patients, failing first line treatment with chemotherapy and trastuzumab. However, an update of the feasibility study revealed a reduced PFS time compared with the initial report (Cameron et al., 2008, Geyer et al., 2006). In addition, the German randomized GBG 26 study showed that maintaining trastuzumab, but shifting chemotherapy to capecitabine, after progression could result in similar effects (median PFS 5.6 months in the capecitabine group and 8.2 months in the capecitabine+trastuzumab group), although with no statistical significant OS survival gain (capecitabine: 20.4 months, 95% CI 17.8-24.7, capecitabine+trastuzumab: 25.5 months, 95% CI 19.0-30.7, P = 0.257) in this study including only 156 patients (von Minckwitz et al., 2009). Blackwell and colleagues demonstrated that the combination of trastuzumab and lapatinib provided better PFS (HR 0.73, 95% CI 0.57-0.93) compared to lapatinib alone, in patients progressing on trastuzumab, although there was no statistically significant difference in OS (HR 0.75, 95% CI 0.53-1.07) (Blackwell et al., 2010). The neoadjuvant GBG 44 study also demonstrated that trastuzumab provides higher

response rates (31.3% pathologic Complete Response, pCR) compared to lapatinib (21.7% pCR, P=0.05) (Untch M et al., 2010). In The **Adjuvant Lapatinib And/Or Trastuzumab Treatment Optimisation** study (ALTTO), it was just decided to offer trastuzumab to all patients who initially were randomised to lapatinib, indicating increased recurrence rates or toxicity to lapatinib (personal communication September 2011).

1.5.1.5 Novel treatment in HER2 positive Breast Cancer

Pertuzumab is a monoclonal antibody, the first of its class in a line of agents called "HER dimerization inhibitors". By binding to HER2, it inhibits the dimerization of HER-mediated signaling pathways, which is hypothesized to result in slowed tumour growth (Adams et al., 2006). The response rate in trastuzumab failing metastatic breast cancer patients is around 24% (Complete Responses, CR, 7.6%, 80% CI 3.7-13.6%, Partial Response, PR, 16.7%, 80% CI 10.9-24.1) (Baselga et al., 2010). Neo-adjuvant results show that the combination of pertuzumab, trastuzumab and docetaxel obtained higher proportion of pCR (88% p= 0.014) compared to the combination without pertuzumab (80% pCR) (Gianni L et al., 2010).

1.6 Health Economic aspects

In Sweden (population 9.4 million) direct costs (health care related) of breast cancer were in 2002 estimated to be SEK 900 million (USD 125 million) and indirect costs (all other costs) to be SEK 2.1 billion (USD 292 million) (Lidgren et al., 2007a). As a reference, the estimated cost of breast cancer in USA was USD 13.9 billion in 2006 (NCI., 2010). This cost translates to a total cost (direct + indirect costs) per incident case in Sweden to USD 57,000 and in USA to USD 76,000 (NB! These figures should be read with caution and reflect differences in the health care systems' expenditure).

We have seen increasing costs for cancer drugs over the last decade, as there have been introductions of several new innovative drugs. Data shows that this has been a correct approach, as usage of new cancer drugs result in a statically significant reduced mortality and can be of good value for society (Luce et al., 2006, Lichtenberg, 2011, Lichtenberg et al., 2009). In general, investments in health care are of good value for money, especially investments in breast cancer (Luce et al., 2006).

Health economic studies are important as a basis for developing guidelines for resource allocation in health care systems (Jonsson, 1997, Karlsson and Johannesson, 1996), reflecting the limited resources in health care systems (Eckerlund et al., 1995). However, assessments and appraisals of new cancer drugs have surfaced a new policy issue of clinical consequences, since reimbursement decisions may delay access to new therapies. An example is the appraisal of trastuzumab in the UK (two years after approval) (NICE, 2002). In general, the use of Health Technology Assessments (HTA) is important for an overall perspective of the benefit of an intervention. HTA has developed over many decades and should include all aspects of the intervention (e.g. demographics, disease panoramas, treatment efficacy, effectiveness variation in health practices, cost-effectiveness, social and ethical aspects) (Jonsson, 2009). It is also important that health economic studies are made relevant for the country or the situation, as health care systems differ (Drummond et al., 2005). One important aspect in assessing value for money is the perspective on cost. The guiding principle should be to include all relevant

costs in the estimates, also costs outside of the health care system (Johannesson M et al., 2009, Lidgren et al., 2007a).

Decisions about clinical strategies must take all factors into account and develop knowledge on the effectiveness of an intervention (use in clinical practice, as opposed to the use in clinical trials) (Frueh, 2010, Nilsen, 2011). Comparative Effectiveness Research (CER) is the direct comparison of existing health care interventions to determine which work best for which patients and which pose the greatest benefits and harm (Berry, 2011, Steinfort et al., 2011). At the American Society for Clinical Oncology this year the plans for a large study on CER in adjuvant breast cancer patients was presented (Ramsey, 2011). This is an important step in the direction towards true effectiveness evaluations.

1.6.1 The co-dependent technologies HER2 testing and trastuzumab treatment

The testing for HER2 and treatment with trastuzumab has important health economic implications. The first is about optimal testing strategies. Which test should be used, and what cut-off points should be set for confirmatory testing? The answer to this question depends on the characteristics of the available tests (sensitivity, specificity) (Wolff et al., 2007, Yaziji et al., 2004), as well as to costs. The second aspect relates to the optimal treatment strategy, taking into account outcome in terms of survival and quality of life, side effects, as well as to costs in relation to the defined targeted patient population. Trastuzumab treatment costs and outcome are also related to the length of treatment (Joensuu et al., 2009, Joensuu et al., 2006, Slamon, 2009). These two aspects are not independent and the optimal testing and treatment strategies have to be analysed together. HER2 testing and trastuzumab treatment is an example of co-dependent technologies. Testing without treatment is meaningless unless a special value is assigned to the information about prognosis. Treatment can be undertaken without testing, but cost-effectiveness will be lower; perhaps even so low that it is not worthwhile. Price can of course be adjusted to reflect cost-effectiveness, but the benefit will always be less favourable when patients with no or minor benefit are exposed to treatment.

1.6.2 Cost of HER2 testing and treatment with trastuzumab

In Sweden the HER2 testing cost is around SEK 1,800 (USD 250) and SEK 5,300 (USD 750) for IHC and FISH, respectively (Sjukvårdsregionen, 2011). According to the Swedish guidelines, every patient with early breast cancer should be HER2 tested with IHC and confirmed with FISH for IHC borderline and positive results. The guideline also state that recurrent sites should be HER2 tested (SweBCG, 2011). HER2 testing of recurring breast cancer is uncommon in other countries, even if data shows that tumours change in HER2 status (as well as in other tumour markers). (Lindström et al., 2010). If testing is carried out on all 7,300 women diagnosed per year in Sweden, the cost of testing early breast cancers would be over SEK 40 million (USD 5.5 million) and for metastatic breast cancers (FISH for all) around SEK 8 million (USD 1 million). Testing cost is also a one off cost and therefore far out priced by treatment cost (see below), as this is an ongoing cost.

Breast cancer is a very drug intense disease (around 10% of direct costs, increasing due to new innovative drugs). Even so, in Sweden only about 1/3 of the total costs of the disease relate to direct costs (e.g. hospitalisation and drugs) and 2/3 relate to indirect costs, such as loss of work capacity, sick leave and mortality (Lidgren et al., 2007a). Total spending on cancer drugs in Sweden is at a similar level (12%), but the indirect costs are lower and constitute around 50% of cost (Cancerfonden, 2006). The cost of the trastuzumab drug is around SEK 25,000 (USD 3,850) per month in Sweden and the total sale for trastuzumab for 2010 was around SEK 323 million (USD 45 million). The cost of trastuzumab treatment is an additional cost to the previous standard of care, as the drug was first in class for the treatment of HER2 positive breast cancer. There are health economic studies showing that HER2 testing and treatment with trastuzumab in breast cancer patients in Sweden is cost-effective with an ICER of SEK 485,000 to SEK 560,000 in the metastatic breast cancer setting and SEK 333,000 to SEK 385,000 in the adjuvant setting (Lidgren et al., 2008a, Lidgren et al., 2008b). Without the HER2 testing trastuzumab treatment would probably never reach a cost-effectiveness level, as the numbers of patients required for statistically significant survival benefit would never have been reached in the clinical trials (Simon and Maitournam, 2004).

1.6.3 Quality Adjusted Life Years

Quality Adjusted Life Year (QALY) is a measure of disease burden, including both the quality and the quantity of life lived (Doctor et al., 2004). In health economics it is used in assessing the value for money of a medical intervention (Jonsson, 1990).

The QALY is based on the number of years of life that would be added by the intervention, Life Years Gained (LYG). Each year in perfect health is assigned the value of 1.0 down to a value of 0.0 for death. If the extra years would not be lived in full health, then the extra life-years are given a value between 0 and 1 to account for this.

The advantages of using QALY as the outcome measure in economic evaluations, is that it can combine different consequences into one measure, and that makes comparisons possible between interventions affecting Quality of Life (QoL) with interventions affecting life expectancy. The acceptance of QALY differs and is not always useful, for instance in relation to survival in cancer (Tengs, 2004).

1.6.4 Cost effectiveness Analysis

Cost Effectiveness Analysis (CEA) is used to make informed decisions about allocation of resources to achieve the greatest improvement in health in a budget constrained environment (Jonsson, 1993). CEA compares the costs and outcomes of two or more interventions. Normally CE is expressed as a ratio, where the denominator is the change in effect, e.g. LYGs or QALYs gained (also called Cost Utility Analysis, CUA), and the numerator is the change in cost. The ratio is Incremental Cost Effectiveness Ratio (ICER). To make a correct calculation of ICER it is important that each intervention is compared to the next most effective, non-dominated intervention. The concept of dominance refers to the fact that some alternatives are inferior to others. There are two types of dominance. Simple dominance occurs when an intervention, in absolute terms, is more effective and less costly than another. Extended dominance occurs when an intervention with higher effect has a lower ICER, i.e. the more effective intervention costs more in absolute terms but is less costly per gained effect unit.

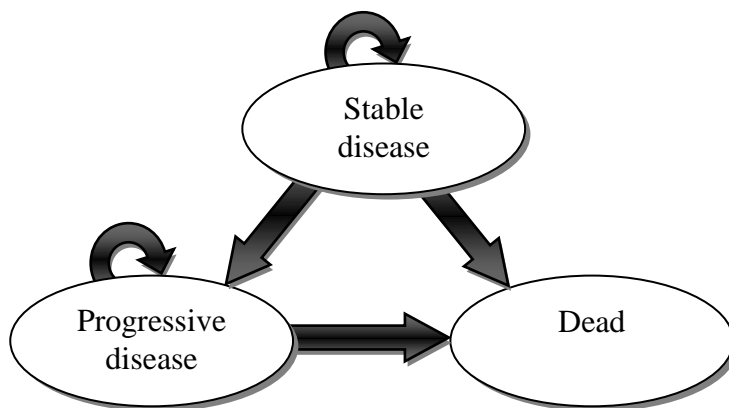
One important methodological consideration when calculating cost-effectiveness ratios is what costs to include. While the guiding principle should be to include all costs (but only once), practices vary. Health care payers in many countries will not include all the societal costs in their studies, although the total value of an

intervention should include the societal perspective (Johannesson M et al., 2009). In Sweden on the other hand, it is recommended to use the societal perspective for reimbursement evaluations (Läkemedelsförmånsverket, 2003).

1.6.5 Modelling technique / Markov model

A model is a simplified description of a complex process and the consequences of different actions. The Markov model is a transition model, used to study health states that recur and change over time. These health state changes are called transitions and are described by probabilities. The decision process is not linear, so the evolving disease pattern must be accounted for in the transitions. For example, a Markov model could be used for analyzing the progression and relapse of a chronic disease and treatment outcomes over a specific period of time (Sonnenberg and Beck, 1993).

Figure 6 The Markov model



1.6.6 Willingness to pay

In economics, the willingness to pay (WTP) is the maximum amount a person/society would be willing to pay, sacrifice or exchange in order to receive a benefit or to avoid something undesired.

It is difficult to set a benchmark level of WTP, even if attempts have been made to make estimates (Culyer et al., 2007, Eichler et al., 2004). For example, the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom set a level in 2008 for health care of BP 20,000- 30,000 per QALY. A study that translated data from road accidents to health care, estimated the Swedish WTP for a QALY at SEK

655,000 (USD 91,100) (Persson and Hjelmgren, 2003). In USA the levels of USD 100,000 to USD 300,000 in health care for a QALY saved are discussed (Braithwaite et al., 2008).

The more society is willing to pay for improvements in health, the more society is willing to pay for interventions that will improve health. An intervention will not be adopted if the WTP per QALY gained is not at an acceptable level. If the price per QALY gained is set at the optimal level, CEA will maximise the use of social welfare (Johannesson M et al., 2009).

2 RATIONALS FOR THIS THESIS

In the future, many cancers will be (some already are) defined by different genetic factors besides the standard diagnostic criteria (so far mostly based on different morphology criteria). Diagnoses and treatments of breast cancer are becoming more complex, due to the different sub-groups (Goldhirsch et al., 2011). There are already some important breakthroughs (e.g. trastuzumab for the HER2 positive sub-group), based on this new knowledge, established from different gene expression defined sub-groups (Sorlie et al., 2001).

The introduction of a drug like trastuzumab, based on a predictive marker is nothing new. Tamoxifen and ER status is the first example in breast cancer (EBCTCG, 2005a, EBCTCG, 2011, Rutqvist, 1990). The new aspect is that co-dependent technologies are developed based on knowledge from disease biology and that they are introduced jointly to the health care system.

A diagnostic test combined with a targeted treatment raises questions on the test procedures and the related treatment options. The test is important, as it may offer a prognostic value. It may also offer a predictive value, as it may reduce the number of patients required in the pivotal clinical studies and provides information on which patients could benefit from treatment (Simon and Maitournam, 2004). The treatment is important as it may affect outcome with increased response rates and prolonged survival (Marty et al., 2005, Romond et al., 2005, Slamon, 2009, Slamon et al.,

2001). Thus, it is important to make best use of the knowledge accrued of how these patient populations should be managed in terms of co-dependent technologies.

In Sweden we have a personal identification system for each of the nation's inhabitants. It is a unique ten digit identification number, making it possible to track individuals through their whole life, in any logistic system. This makes Sweden very well suited for follow-up and observational studies.

3 AIMS OF THIS THESIS

The overall aim of this thesis was to determine how a targeted patient population is optimally managed.

The aims of the papers included were:

Paper 1 (HER2 status in primary tumours and recurrences).

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

Paper 2 (Clinically used breast cancer markers are instable throughout tumour progression).

Assessing intra-individual ER status, PR status and HER2 status in primary tumour and first relapse and later relapses, and relate altered and unchanged oestrogen receptor status to survival, in a large homogenous cohort of breast cancer patients.

Paper 3 (Uptake and use of trastuzumab in Sweden during the years 2000-2008).

Study the introduction and usage of trastuzumab in breast cancer patients in the six Health Care Regions (HCRs) in Sweden.

Paper 4 (Cost-effectiveness of HER2 re-testing and trastuzumab treatment in patients with metastatic breast cancer).

Study cost-effectiveness of re-testing HER2 status in metastatic breast cancer tumour samples from patients before offering trastuzumab treatment in a Swedish setting.

4 MATERIALS AND METHODS

This thesis is based on data from registries, databases and retrospective studies of available data in databases and patient records. The projects required were approved by the Ethics Committee at the Karolinska Institutet.

The following data sources were used for the entire thesis:

- Data on breast cancer patients retrieved from the Breast Cancer Registry in Stockholm, with recurrent diagnosis years 1997-2007.
- Data from the Swedish Cancer Registry and Statistics Sweden.
- Sales of trastuzumab for years 2000-2008 from Apoteksbolaget (Pharmacy wholesaler)
- Price list for the Stockholm County from 1 April 2010.
- Encoded observational patient database with 225 metastatic breast cancer patients, diagnosed with metastatic disease years 1997-2007 in Stockholm.
- HER2 status results, diagnosis and treatment details on patients with relapse diagnosis at the Karolinska University Hospital in Stockholm.
- HER2 status results from the pathology departments in Linköping (South East HCR) and Lund (South HCR). (no individual data)
- Database on HER2 status and other data retrieved from patient records in three out of the six health care regions (HCR) in Sweden: Umeå (North HCR), Uppsala (Uppsala-Örebro HCR) and Stockholm (Stockholm-Gotland HCR), in total data from ca. 600 patients (supportive data).

For the individual projects the following materials and methods were used:

For paper 1 (HER2 status in primary tumours and recurrences), data on the Stockholm metastatic breast cancer patient cohort years 1997-2007 were obtained from the Regional Breast Cancer Registry in Stockholm. HER2 status results for these patients were retrieved from the Departments of Pathology at the Karolinska University Hospital in Stockholm and St Görans hospital. Data on trastuzumab treatment for these patients was retrieved from individual patient files.

IHC/ICC analyses were carried out on primary tumours and recurrence (internal control consisted of four breast cancer cell lines: BT474 3+, MDA453 2+, RT4 1+,

and 5637 0). FISH was carried out if the IHC/ICC protein level was 2+ or 3+, or direct FISH.

Paper 2 (Clinically used breast cancer markers are instable throughout tumour progression): Data on the Stockholm metastatic breast cancer patient cohort years 1997-2007 was obtained from the Regional Breast Cancer Registry in Stockholm (bilateral and primary metastatic disease excluded). ER and PR status were assessed either by monoclonal antibody-based biochemical methods ($\geq 0,05$ fmol/ μ g DNA positive), by immunohistochemistry (IHC) or by immunocytochemistry (ICC) ($\geq 10\%$ positive) (Lofgren et al., 2003). IHC/ICC methods were used for determination of HER2, confirmed by FISH for HER2 IHC/ICC equivocal 2+ and positive 3+.

Paper 3 (Uptake and usage of trastuzumab in Sweden): Data from the Swedish Cancer Registry, the Cause of Death registry by the National Board of Health and Welfare (Socialstyrelsen) were used. For general statistics, Statistics Sweden was used. Sales data was received from Apoteksbolaget (Pharmacy wholesaler). Data from the pathology departments in Umeå, Uppsala, Stockholm Linköping and Lund were retrieved from the local databases.

Paper 4 (Cost-effectiveness of HER2 re-testing and trastuzumab treatment in patients with metastatic breast cancer): The price list for Stockholm County was used for cost estimates. Effects were taken from the Marty study (Marty et al., 2005). Quality of Life (QoL) data was taken from the Lidgren study in 2007 on QoL in different states of breast cancer (Lidgren et al., 2007b). HER2 testing results and test specifics were taken from the estimates by Elkin in 2004 (Elkin et al., 2004). Six different testing/treatment strategies were used (see table 3), with the reference base case of no re-testing and no trastuzumab treatment.

Table 3 Re-testing and treatment strategies for HER-2 positive Metastatic Breast Cancer

Strategy	Testing of metastasis		Treatment	
	IHC	FISH	Trastuzumab + Chemo	Chemo
0	-	-	-	All
1	-	-	If primary tumour was HER2+	Others
2	Yes	-	If IHC 3+	Others
3	Yes	-	If IHC 2+ or 3+	Others
4	Yes	If IHC 2+ or 3+	If FISH+	Others
5	-	Yes	If FISH+	Others

4.1 Statistical methods

Paper 1 (HER2 status in primary tumours and recurrences): The Agreement between assessments was calculated by kappa statistics, according to Landis and Koch (Landis and Koch, 1977). For the survival analyses, follow-up started at the date of breast cancer diagnosis and continued either until date of death or until censoring (end of follow-up of December 31, 2007). A univariate analysis (Kaplan-Meier method) was used for overall survival. The risk of dying was modelled by use of a multivariable proportional hazard (Cox) model, adjusted for age, year of diagnosis, oestrogen and progesterone receptor status (Schoenfeld, 1982). The proportional hazard assumption was assessed using Schoenfeld's test statistics (Schoenfeld, 1982). An arbitrary level of 5% statistical significance was used and 95% confidence intervals (CI) were calculated.

Paper 2 (Clinically used breast cancer markers are instable throughout tumour progression): Univariate analyses of overall survival from either primary tumour diagnosis or relapse were performed in patients according to intra-individual ER status in primary tumour and relapse, using the Kaplan-Meier method. The risk of dying in relation to ER status in primary tumour and relapse was modelled using a multivariable proportional hazard (Cox) model, adjusting for potential confounding factors (age and calendar year of primary breast cancer diagnosis, PR status, tumour stage, adjuvant hormonal therapy and adjuvant chemotherapy). The proportional hazard assumption for the main exposure variable

was assessed using Schoenfeld's test statistics. An arbitrary level of 5% statistical significance (two-tailed) was used.

Paper 3 (Uptake and usage of trastuzumab in Sweden): The analyses were based on the following assumptions: 25% HER2 positive metastatic breast cancer patients (Slamon et al., 1987, Wilking et al., 2011). and 38 weeks treatment time (Slamon et al., 2001) (and our database described earlier), 14% HER2 positive early breast cancer patients (Ryden et al., 2009) and 52 weeks treatment time (based on drug label).

Sales data on trastuzumab was used to calculate the actual use. The "optimal" use was based on the total number of patients in need of treatment, based on incidence- and mortality data for each HCR.

Paper 4 (Cost-effectiveness of HER2 re-testing and trastuzumab treatment in patients with metastatic breast cancer): We used a Markov state transition model to simulate six different strategies for re-testing and treatment (see table 1 in Materials and Methods). The analyses were based on treatment guidelines according to the label and published data (Elkin et al., 2004, Marty et al., 2005). QALY weights and risks of progression and breast cancer death were taken from a previous study (Lidgren et al., 2008b). Outcomes are measured as LYG and QALYs gained and costs, including both direct and indirect costs. For the modeling we used the program TreeAge Pro 2009 (TreeAge Software, Inc., Williamstown, MA). Analyses were performed with life time perspective and both costs and outcomes were discounted at 3%.

5 RESULTS

Paper 1 (HER2 status in primary tumours and recurrence): The total sample cohort consisted of 1181 breast cancer patients with recurrent disease. Baseline patient and tumour characteristics are described in table 4.

Table 4 Patient and tumour characteristics

Patient group (total in category)	Median age (range)	Median time to recurrence in months (range)	Clinical stage % 1)				Histop. grade (Elston) 2)		ER/PR positive % 3)	Adj. Chemo. %	Adj. Hormone treatment %	Adj. trast . %
			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	55	0
HER2 Positive stable (35)	51 (28-83)	22 (0-65)	17	60	17	0	3	8	14	80	34	11
HER2 change (15)	56 (30-78)	22.3 (7.9-97.9)	25	50	25	0	1	4	25	63	56	1
Total sample (excluding study sample) (1021)	56 (23-97)	64.3	44	37	14	1	-	-	34	37	55	NA

- 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 an Elston grade 1 cancer.
- 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.

In 368 patients HER2 status was analyzed in a recurrence (mostly from Fine Needle Aspirates (FNA) samples). In 151 of these patients, HER2 status was also available from primary tumour samples. The sites of recurrence are listed in table 5.

Table 5 Sites of recurrence

Site of recurrence	Numbers	Proportion %
Bone/bone marrow	45	30
Liver	24	16
Local	27	18
Lung/pleura	15	10
Axillary lymph nodes	13	9
Skin (not local)	10	7
Other sites	9	7
Supra clavicular lymph nodes	8	5
Total	151	100

IHC analyses were carried out on 144 primary tumours and 86 samples were analysed with FISH. In the recurrence samples the corresponding numbers were 84 for IHC/ICC and 102 for FISH.

In 10% (15 patients) of all patients HER2 status changed from primary tumour to recurrence. In 19% (8 patients) of the patients with HER2 positive early breast cancer the relapse was HER2 negative and 6% (7 patients) of the patients with HER2 negative early breast cancer had HER2 positive relapse. Intra-patient agreement in HER2 status was 76% (95% CI 64-87%), and the disagreement was 10% (95% CI 5-15%).

In the group of patients with unchanged HER2 negative status 24% and of the patients with a change in HER2 status 6% had triple negative (ER negative, PR negative, HER2 negative) primary breast cancer.

The multivariable Cox analysis showed a significant increased risk of dying in the patient group with changed HER2 status compared with patients with concordant positive HER2 status (see table 6).

Table 6 Risk of death in breast cancer patients related to intra-individual HER2 status in primary tumour and relapse.

Intra-individual HER2 status in primary tumour and relapse	Patients		Deaths	Overall survival from breast cancer diagnosis to death or censoring	Overall survival from relapse to death or censoring
	No	%	Overall	Adjusted* HR (95% CI)	Adjusted* HR (95% CI)
Her-2 status					
Positive	34	23.1	18	1.0 ref.	1.0 ref.
Discordant	14	9.5	10	5.47 (2.01-14.91)	3.22 (1.18-8.77)
Negative	99	67.3	71	1.85 (0.99-3.45)	2.38 (1.27-4.43)

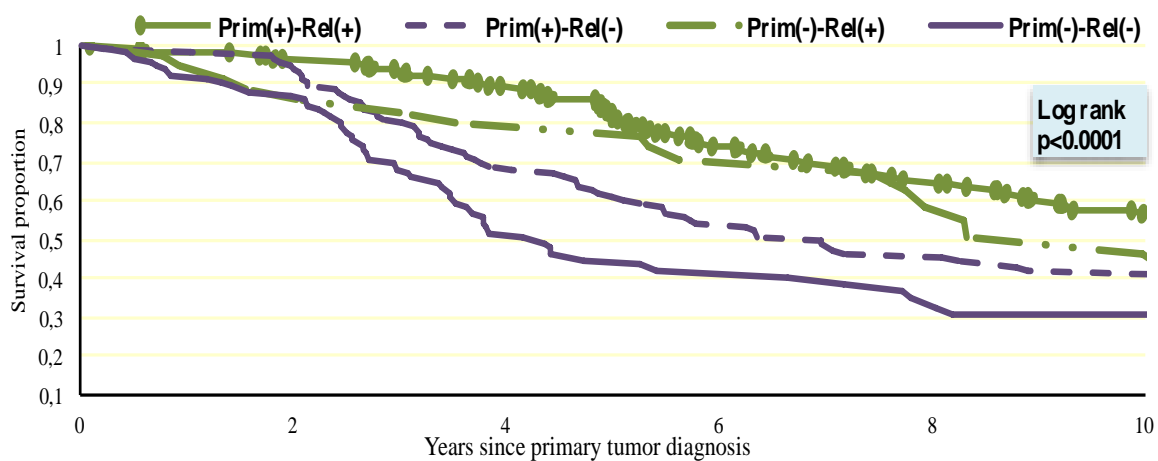
*Adjusted for age and year of diagnosis, oestrogen receptor status, progesterone receptor status and stage

Trastuzumab was administered to 77% of the patients with HER positive primary tumours (12% adjuvant and 82% for recurrence disease, 6% unknown date). For patients with HER2 positive metastatic disease, 76% received trastuzumab treatment (94% recurrent disease, 6% unknown date). For patients with stable HER2 positive status, 86% received trastuzumab (13% adjuvant 77% recurrence, 10% unknown date) and only 33% of patients with changed HER2 status received trastuzumab.

Paper 2 (Clinically used breast cancer markers are instable throughout tumour progression) ER, PR and HER2 status was found in a total of 845, 836 and 356 patients, respectively, of the 1010 breast cancer patients diagnosed with relapse from 1997 through 2007 (bilateral and primary MBC excluded). Of these patients, 71.9% were ER positive, 58.8% PR positive and 27.9% HER2 positive. In 459, 430, and 104 patients, respectively, ER, PR and HER2 status was assessed in both primary tumour and first relapse. A change in 32.4% (ER), 40.7% (PR) and 14.5% (HER2) of patients was seen. In 119 (ER), 116 (PR) and 32 (HER2) patients, respectively information from multiple relapses was available. When comparing several relapses, 33.6% (ER), 30.1% (PR) and 15.7% (HER2) of patients showed altered marker status. Furthermore, 16.0% of patients had changed ER status from

positive to negative and 12.6% had changed from negative to positive. Actually, 5% of patients had altered ER status back and forth between relapses.

Figure 6 Overall breast cancer survival (primary tumour diagnosis to death or censoring). Intra-individual ER status in primary tumour and relapse (both local and systemic relapses included)



In the Cox proportional hazard survival model from early breast cancer diagnosis to death or censoring at end of follow-up, patients with change in ER status from positive to negative, had a statistically significant increased hazard ratio (HR) for dying, including both local and systemic relapses (HR 1.61, 95% CI 1.17-2.21) and including systemic relapses only (HR 2.00, 95% CI 1.39-2.87), compared to patients with stable positive ER status.

Paper 3 (Uptake and usage of trastuzumab in Sweden) shows that the use of trastuzumab differs greatly between HCRs in Sweden. During the years 2000 to 2004, when the drug was approved only for treatment of metastatic breast cancer, there were very large differences in usage between HCRs. For example the South HCR had 30% of the usage of the North HCR (see figure 7). The usage during years 2006-2008, with both metastatic and adjuvant use, is shown in figure 8.

Figure 7 Proportion usage years 200-2004 in patients (MBC only)

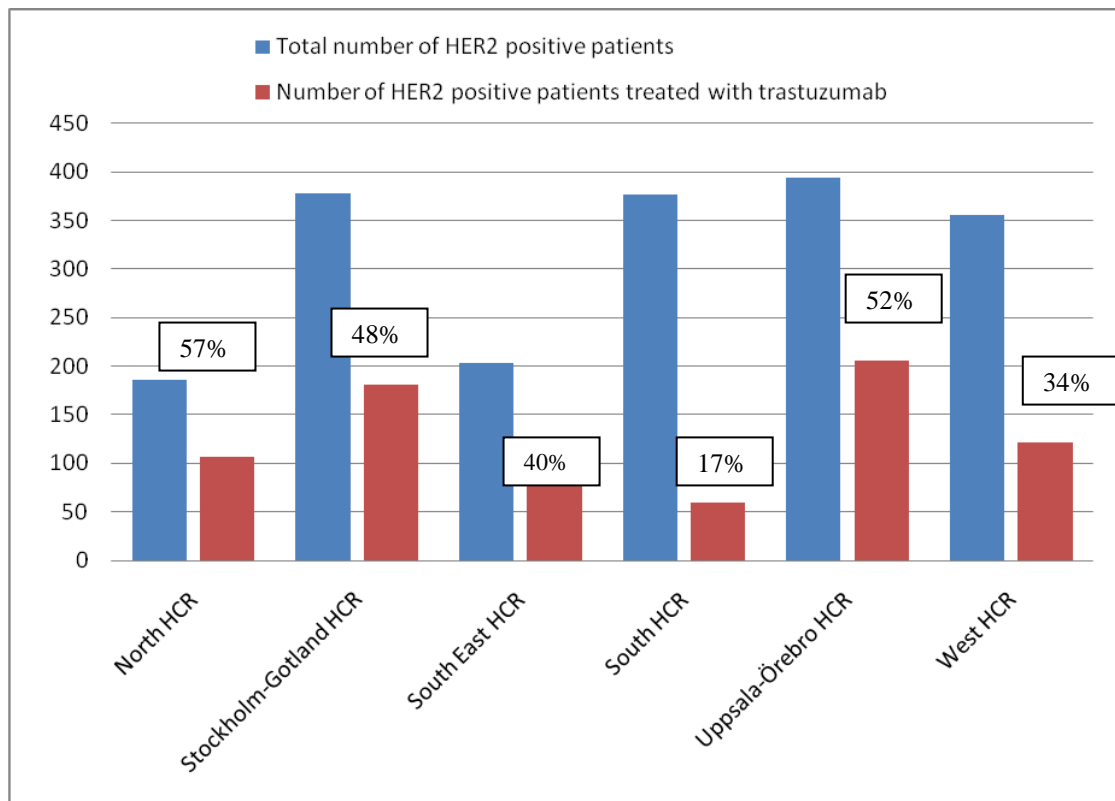
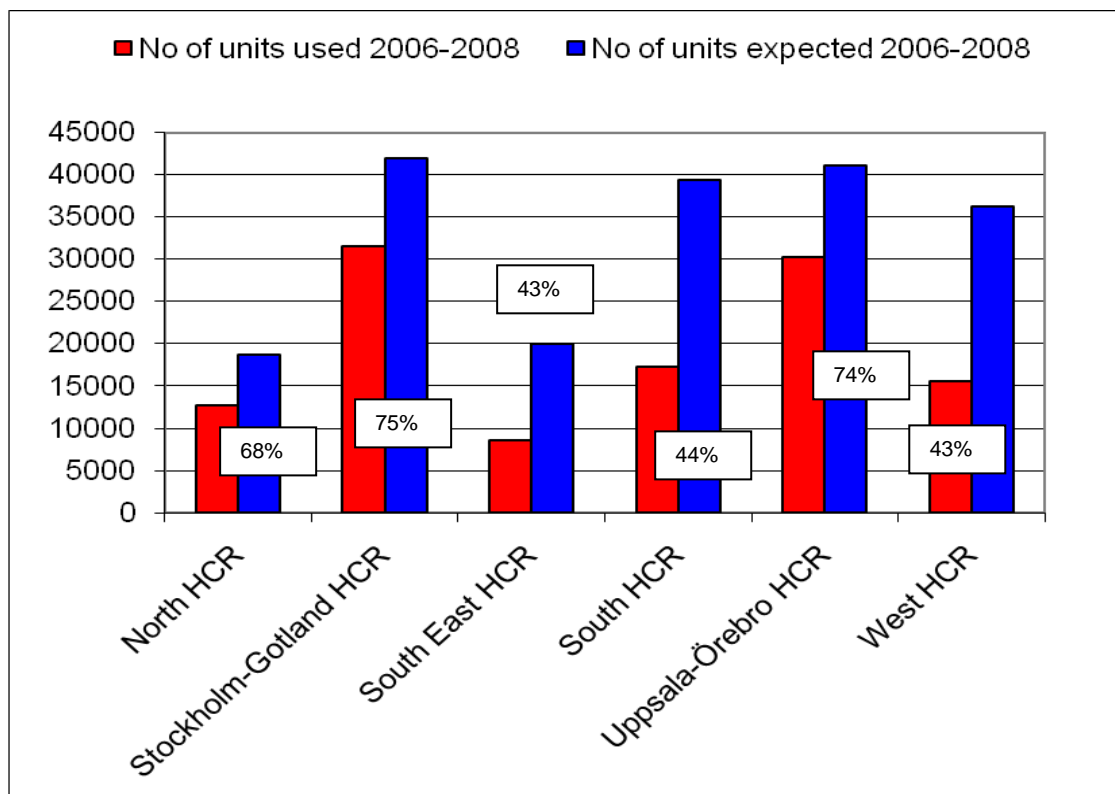


Figure 8 Proportion usage years 2006-2008, in units (MBC and EBC)



Paper 4 (Cost-effectiveness of HER2 re-testing and trastuzumab treatment in patients with metastatic breast cancer): Please see table 3 in materials and methods for definition of strategies. The baseline strategy, not to re-test HER2 status, and treat all patients with chemotherapy only (Strategy 0), was least effective and least costly. To re-test all relapses with IHC and to FISH test IHC 2+ and 3+ (Strategy 4) had the lowest ICER compared to Strategy 0. The detailed results are presented in table 7.

Table 7 ICER of re-testing HER2 status in metastatic breast cancer patients

Strategy	QALYs gained	LYG	Cost USD SEK	ICER per QALY USD SEK	ICER per LYG USD SEK
0 No re-testing, chemotherapy alone	1.309	1.911	41,380 297,500		
1 No re-testing. Trastuzumab to previously known HER2 positive patients	1.501	2.192	53,030 381,300	Dominated ²⁾	Dominated ²⁾
2 IHC re-testing. Trastuzumab to IHC 3+ patients	1.466	2.140	50,400 362,400	Dominated ²⁾	Dominated ²⁾
3 IHC re-testing. Trastuzumab to IHC 2+ and 3+ patients	1.525	2.226	56,200 404,100	Dominated ¹⁾	Dominated ¹⁾
4 IHC and FISH re-testing. FISH confirmation of IHC 2+ and 3+. Trastuzumab to FISH positive patients.	1.525	2.226	53,490 384,600	56,230 404,300	38,510 276,900
5 FISH re-testing of all patients. Trastuzumab to FISH positive patients.	1.543	2.253	54,730 393,500	66,620 479,000	45,630 328,100

1) Simple Dominance 2) Extended Dominance

6 DISCUSSION

The papers will be discussed one by one, except for the two first papers. They will be discussed together, as these papers contain related results.

Paper 1 (HER2 status in primary tumours and recurrence) and Paper 2 (Clinically used breast cancer markers are instable throughout tumour progression)

The main finding in Paper 1 is that there are discordances in HER2 status between primary tumour and recurrent sites in 6% of primary HER2 negative tumours and 19% in primary HER2 positive tumours, which reflects a discordance rate of 10% for the entire study population. Paper 2 demonstrates a 32.4% change in ER status, 41% change in PR status and 15% change in HER2 status. The small differences in HER2 status between the two papers reflect differences in study design (Lindström et al., 2010).

In the multivariable analysis in paper 1 we noted (although a small sample size) a statistically significant increased risk of dying for patients with changed HER2 status, compared to patients with unchanged positive HER2 status (adjusted for age and year of diagnosis, ER status, PR status and stage). In paper 2 patients with stable ER positive tumours have similar survival as patients with a change from primary ER negative tumours to ER positive relapses, whereas patients with ER negative relapses (no matter of primary ER status) have a statistically significant worse overall survival (adjusted for age, year of diagnosis, PR, tumour stage, adjuvant endocrine therapy and adjuvant chemotherapy).

Findings in relation to other data

As demonstrated in other studies, adjuvant therapies could enhance clonal selection and patients with loss of ER and HER2 most likely show resistance to endocrine therapy and trastuzumab. (Lipton et al., 2005, Pectasides et al., 2006, Munzone et al., 2006, Mittendorf et al., 2009). Results also show that there seems to be an inborn clonal selection, as patients without prior treatment have alterations in tumours (Lindström et al., 2010, Chambers et al., 2002). Other research groups have also shown that it is important to re-test HER2 and ER status in recurrences

and metastases, as re-testing may avoid the risk of incorrect diagnosis of metastatic breast cancers (e.g. benign condition or other malignancy) (Amir and Clemons, 2009, Cottu et al., 2008, Kaufmann and Pusztai, 2010, Shah et al., 2009, Simmons et al., 2009).

Of the patients in paper 1 with stable HER2 positive status, 86% received trastuzumab treatment (11% as adjuvant treatment) which may have had a positive effect on outcome in this patient group, as shown in previous studies (Marty et al., 2005, Slamon et al., 2001). Furthermore, the majority of relapse samples were analysed with FISH and Dowsett and colleagues showed that FISH HER2 positive tumours respond to trastuzumab treatment irrespective of the IHC result (Dowsett et al., 2009). The better outcome for patients with stable positive HER2 status in paper 1 is in line with a publication on neoadjuvant treatment with trastuzumab, in which patients retaining a positive HER2 status had better prognosis compared with patients with changed HER2 status (Mittendorf et al., 2009).

Strengths and weaknesses

Data sources

The overall strength with the patient samples in the two papers is that they are derived from a population based cohort from the Stockholm catchment area. The obvious drawback of the two papers is that they present retrospective data. This is also illustrated by the fact that there are differences in the base line characteristics between the study sample and the total sample (excluding the study sample) in paper 1, see table 4. The study sample also received adjuvant treatment at a higher degree, indicating more aggressive disease, as shown in the much shorter time to recurrence. The sample size in paper 1 may seem small, although the very few larger studies demonstrate similar results, which also strengthen our study (see also table 2) (Liedtke et al., 2009, Lower et al., 2009). There are a few studies including patients at diagnosis of relapse (Pectasides et al., 2006, Thomson et al., 2010).

Technical issues

There are also issues with both sampling techniques and analysis methods. The choice of sampling technique may be of importance, and there are advantages and disadvantages with both Fine Needle Aspirate (FNA) and Core Needle Biopsy (CNB) (Barra Ade et al., 2008), but from our experience the FNA technique is well suited for tumour sampling, although CNB is better than FNA at preoperative diagnosis of screen-detected breast cancer as it misses fewer cancers (Lieske et al., 2006). However, combining FNAC resulted in a better preoperative diagnosis rate. It is also very important to correctly target the areas for sampling of recurrences and therefore collaboration between the pathologist, radiologist and oncologist is essential (Lindström et al., 2010).

There is an issue with false results with IHC/ICC (sensitivity 92.6%, specificity 98.8% IHC 3+ and 50% IHC 2+ and 3+, 7% of IHC 0/1+ FISH positive) (Elkin et al., 2004, Yaziji et al., 2004). The majority of FNAs were analysed with FISH for HER2. The testing was done at one laboratory and by very few and well trained personnel (different staff for the ER/PR and HER2 analyses), which would support the reliability of the results (Ryden et al., 2009, Wilking et al., 2011, Perez et al., 2002, Roche et al., 2002). The HER2 IHC/ICC analyses were carried out with 2-3 antibodies from 2000 to March 2005. There was also an internal control which consisted of four breast cancer cell lines. This will most probably enhance the reliability of the IHC/ICC results.

Clinical relevance

The observations in papers 1 and 2 have been challenged and questions are raised whether the change in tumour markers are artefacts or of real clinical importance. Some claim that it will only be feasible to take a tumour sample when there are clinical indications to do so, i.e. when a previous analysis was expected to be false or if there is suspicion for a changed response to treatment (Khasraw et al., 2011, Pusztai et al., 2010). On the other hand, we found that there are clinical implications for the change, as survival is affected, both in relation to ER change and to HER2 change. Conflicting results also show that tumours and recurrences are either heterogeneous or homogenous. Foci with variable morphology and marker expressions show that cancers could either be heterogeneous for HER2 status, or

different clone expansions may explain the altered findings in recurrent lesions (Andersson et al., 2004, Wu et al., 2008, Staaf et al., 2010).

Table 8 Methods for assessing ER and PR status in paper 2

Table 4S: Measurement method to assess ER and PR status in primary tumor and relapse

Measurement method	ER				
	IHC or ICC		Immunoassay		Total
	Number	Percent	Number	Percent	
Primary tumor					
IHC or ICC	153	33.3	5	1.1	158
Immunoassay	258	56.2	43	9.4	301
Total	411	89.5	48	10.5	459

Measurement method	PR				
	IHC or ICC		Immunoassay		Total
	Number	Percent	Number	Percent	
Primary tumor					
IHC or ICC	134	31.2	2	0.5	136
Immunoassay	249	57.9	45	10.5	294
Total	383	89.1	47	10.9	430

The results in paper 2 are based on both biochemical receptor determinations and IHC/ICC (see table 8). Even if different methods were used, there were similar ER concordant and discordant percentages. Furthermore, data from the Karolinska Hospital (based on 683 patients with diagnosis during the same time period as the present study) revealed a concordance value of 88% between the IHC and the cytosol based assays (Lofgren et al., 2003). The findings in paper 2 therefore strongly indicate that the observed ER changes are reflections of true biological alterations and not related to methodological factors.

Conclusions

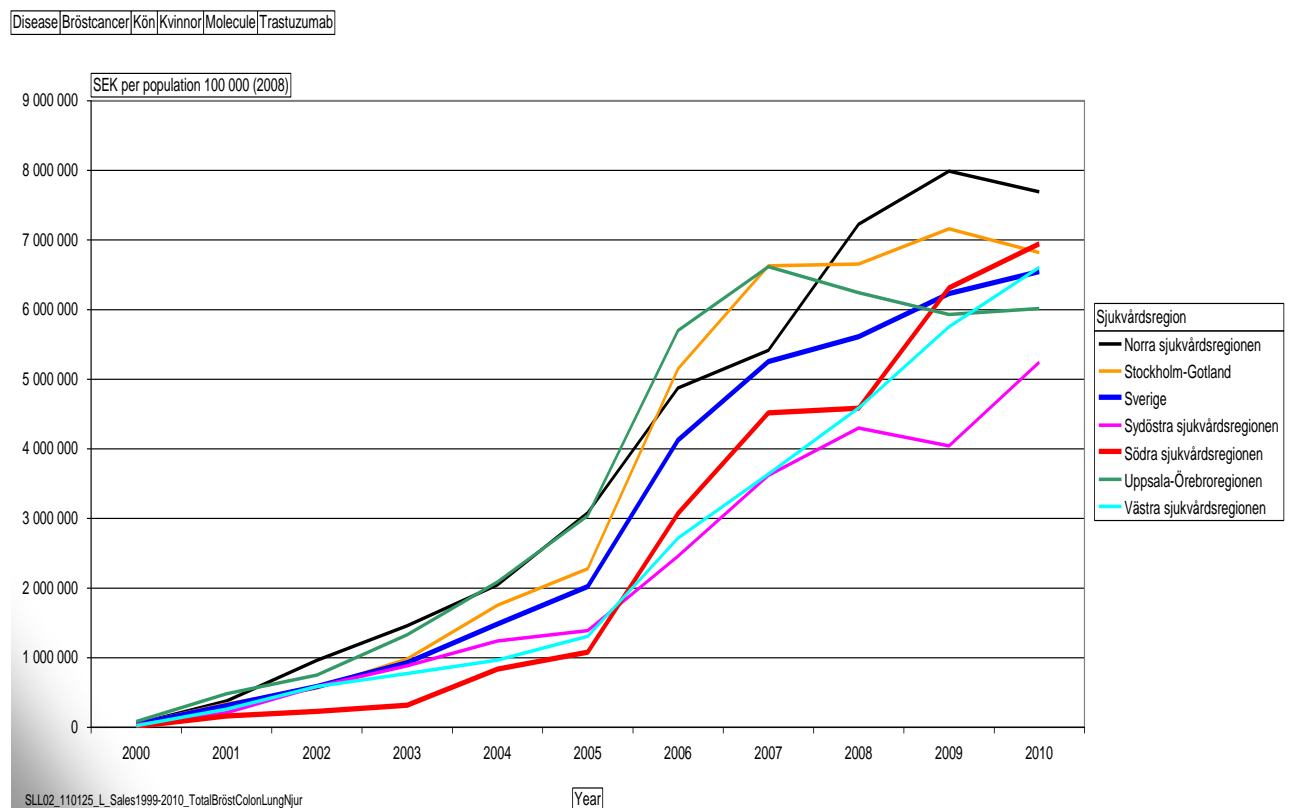
The findings in papers 1 and 2 broaden the understanding of tumour biology and demonstrate that some tumour markers seem to lack stability throughout tumour progression. Changes in tumour marker status have prognostic and predictive implications. In other words, our results show that it is essential to retest any recurrence for tumour marker status in order to be able to offer correct treatment, as over treatment will increase the risk of side effects and under-treatment may result in a missed treatment effect. Further (prospective) studies are required.

Paper 3 (Uptake and usage of trastuzumab in Sweden)

Paper 3 shows that the use of trastuzumab was low during the first years after approval; 11% received treatment in year 2000 and the usage in the HCRs was 17-57% for the period 2000-2004. After early breast cancer introduction of trastuzumab in 2005 (curative intent) there were still large differences between HCRs (43-75% of all HER2 positive breast cancer patients treated). These differences remained, even eight years after introduction.

Figure 9 Population based sales in the six HCRs in Sweden years 2000-2010.

Black – North HCR, orange – Stockholm-Gotland HCR, blue- Sweden average, pink, South East HCR, Red-South HCR, green Uppsala-Örebro HCR, Turquoise- West HCR



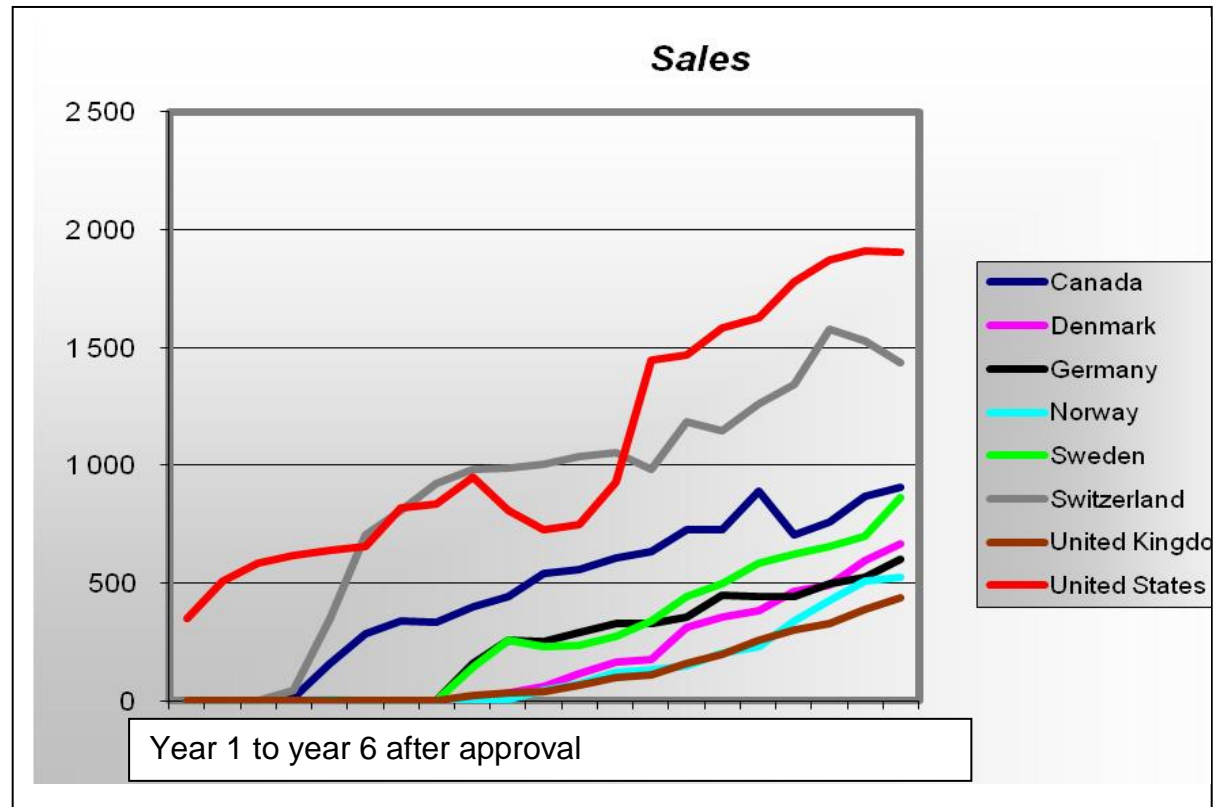
As seen from figure 9, there are large differences in sales over time. The adjuvant introduction in 2005 is illustrated with a “leap up” in usage in all HCRs. In the latter years the level of difference was reduced to 40% from 300% during the first four years. One can also observe some changes after year 2008. For instance, the North HCR, Stockholm-Gotland HCR and Uppsala-Örebro HCR have reached a plateau, even if we do not know if this is the maximum expected usage. The three other HCRs are still increasing their usage and only the future will tell when these HCRs have reached their maximum level of usage.

It is worth mentioning that early introduction of adjuvant treatment may have resulted in fewer relapses during the time period. Early introduction will reduce the number of patients in need of treatment for metastatic disease, as we know from the adjuvant data that trastuzumab treatment will reduce the risk of recurrence with around 40% after 5 years (Slamon, 2009).

Findings in relation to other data

These differences are also seen in the introduction and uptake of trastuzumab between countries, with an almost fourfold difference between the countries in Europe with the lowest use (UK approx. EUR 6,000) and the highest use (Switzerland approx. EUR 22,000) related to mortality, as seen in figure 10 (Wilking, 2008). The difference in usage is seen also for other cancer drugs, with large differences between countries and drugs. In general, countries with a lower income per capita have a slower uptake, despite the fact that drugs account for a large part of total health care spending in these countries. (Wilking N et al., 2009).

Figure 10 Trastuzumab sales per death in selected countries the first six years after approval in each country.



From (Wilking, 2008)

Strengths and weaknesses

Used databases

All trastuzumab sales are handled through the wholesaler system, and thus, all sales in the HCRs in Sweden are captured. The validation study of the breast cancer registration in Sweden shows that the registration is of good quality (Holmberg et al., 2009).

Some of the factors which could have contributed to the differences in usage between HCRs are listed below.

Proportion of patients with HER2 positive tumours

The proportion of HER2 early positive breast cancer patients in Sweden vary in the reports between 14 and 19% (Ryden et al., 2005, Slamon et al., 1987, Sjogren et al., 1998) and many studies in MBC refer to a level of 30%, or higher (Slamon et al.,

1987, Wilking et al., 2011). The proportion of HER2 positive early breast cancer patients differed between HCRs (North HCR 11.4% South HCR 14.6% HER2 positive). This is most probably not a real difference, but is most likely related to the difference in proportion tested (North HCR 100% and South HCR 84%). The levels of 14% and 25% HER2 positive early breast cancers and metastatic breast cancers were used in the estimates not to overestimate the number of breast cancer patients in need of treatment. Taken together this indicates that differences in HER2 positivity between HCRs does not explain the different levels of usage.

Treatment length

Treatment lengths vary largely for metastatic breast cancer. The pivotal clinical study had a median treatment time of 36-40 weeks, with a range of 1-171 weeks (Slamon et al., 2001) and, in the encoded database, based on observational data of metastatic breast cancer patients, the average treatment time was 52 weeks and the median 36 weeks (range 1-324 weeks). Some HCRs may have continued to treat with trastuzumab beyond progression, substituting the cytotoxic agent, while others may have stopped trastuzumab treatment at progression or earlier. There is recent data from a randomised study showing that trastuzumab given beyond progression will significantly prolong TTP and PFS (von Minckwitz et al., 2009, Blackwell et al., 2010) and this is now a recommended approach by many, although it is not based on the highest level of evidence (SweBCG, 2011, Cardoso et al., 2010).

Patients with HER2 negative tumours

There is some data on minor effects of trastuzumab in patients with HER2 negative metastatic breast cancers (Ardavanis et al., 2008). If this is hidden positive HER2 (extracellular HER2) (Meng et al., 2004, Pusztai et al., 2010), or if there are other mechanisms behind is not known (Ardavanis et al., 2008, Fornier et al., 2005).

There is also an ongoing prospective study evaluating trastuzumab in HER2 IHC 1+ and 2+ HER2 high risk early breast cancer patients (node negative or node positive) (NSABP(B-47), 2011). Some HCRs may therefore have treated single patients with HER2 negative or IHC 1+ or 2+ tumours, either intentionally, or unintentionally due to methodological deficiencies.

Small tumours

Data shows that patients with small, node-negative HER2 positive early breast cancer may benefit from adjuvant trastuzumab treatment (Curigliano et al., 2009, Gonzalez-Angulo et al., 2009, Untch et al., 2008) and HCRs may offer treatment to some of these patients with higher risk of recurrence.

Cardiac risk and age

The perception of the cardiac risk (CHF occur in 0.38-4.3% of patients) with trastuzumab treatment may differ between HCRs and some HCRs may not treat patient above a certain age or with pre existing cardiac conditions (Dang et al., 2010, Perez et al., 2008, Suter et al., 2007, Ewer et al., 2005). We see that older people have poorer survival rates and, in general, this is probably based on lower proportion of treated patients in older age groups (Foukakis et al., 2011).

Budget factors

Trastuzumab treatment comes at a relatively high price, compared to the previous standard of care (SEK 25,000 per month) and this cost is added to other treatment costs. The trastuzumab price likely results in differences in priorities, based on different budget constrain in the HCRs. The cardiac monitoring during trastuzumab treatment may also be considered to be a budget burden, although our estimate in paper 4 of the monthly cost for cardiac monitoring was only USD 120 (SEK 864). Furthermore, one of the smallest HCRs (low taxation base), the North HCR has the highest usage. This indicates that there are other factors involved.

Conclusions

There are differences between HCRs in budget priorities, interpretations of published data and national and international guidelines, resulting in differences in the size of the aimed target population, resulting in different treatment patterns.

Paper 4 (Cost-effectiveness of HER2 re-testing and trastuzumab treatment in patients with metastatic breast cancer)

Paper 4 shows that re-testing of HER2 status in metastatic breast cancer patients before initiating trastuzumab is cost-effective, with a cost per QALY gained between USD 56,000 and USD 67,000 and cost per LYG between USD 39,000 and USD 46,000, based on a switch of 10% in HER2 status in either direction (from HER2 + to HER2 – and from HER2 – to HER2 +).

The consequences of the two switches in HER2 status are different. If patients switching to negative are treated there will be no benefits, only costs for trastuzumab and side effects (testing has a cost-saving effect) (Simon and Maitournam, 2004). A switch from HER2 negative to HER2 positive will add costs and side effects of treatment, but will also improve outcome. Even if the HER2 test and trastuzumab were costless, re-testing would be a dominant strategy and should always be done.

Findings in relation to other data

A previous Swedish cost-effectiveness study on trastuzumab treatment in metastatic breast cancer patients showed higher ICER compared to our results, although this study did not include re-testing of relapses in the analyses. The results in both that study and our study were sensitive to change in IHC sensitivity and specificity and improving the IHC method would affect the results in our study (Lidgren et al., 2008b). The early studies concluded that trastuzumab usage in metastatic breast cancer is not cost-effective, but the improved survival may most likely alter the conclusion (Norum et al., 2005), as is seen in our study, based on the Marty study (Marty et al., 2005).

There is no international consensus on HER2 testing of metastatic breast cancer, although the Swedish guidelines for breast cancer treatment recommend re-testing of HER2 status in metastatic breast cancer patients and trastuzumab treatment on the basis of HER status of re-testing (SweBCG, 2011).

Strengths and weaknesses

Change in HER status

The used proportion of HER2 change between primary tumour and relapse of 10% is a conservative level. One could argue that this low level of change in HER2 would not make any difference, but our sensitivity analysis showed that the change had to drop below 5% before re-testing could be questioned. Actually it seems that the level of change is higher as showed at ASCO 2010 Lindstrom presented a summary on available studies, and switch proportion in HER2 status ranged from 0 to 38% and the total reported numbers of patients with change in HER2 status were 403 of the 2,512 included in the studies (Lindström et al., 2010).

Data sources

One could argue that local data sources should be chosen to be able to achieve the most relevant results (Ferrusi et al., 2011). We used QoL data from a Swedish study (Lidgren et al., 2007b), although the survival data was taken from the study by Marty (Marty et al., 2005), as there are no later studies including an untreated metastatic patient population. We also used resource data and costs from the Stockholm County. One could also argue that data from Swedish clinical practice should be used. As a consequence of this, we made a sensitivity analysis on data from the encoded database, based on observational data of metastatic breast cancer patients from Stockholm. In the analyses treatment costs were higher, as treatments were given longer (both trastuzumab/chemotherapy and progression drugs). Results were otherwise similar, if the assumption holds that no survival gain is achieved from the longer treatment durations (see table 9).

Table 9 Sensitivity analyses based on data from encoded metastatic breast cancer database.

Strategy	QALYs gained	Cost (USD)	ICER (USD/QALY)
0	1.309	39,000	
2	1.466	49,040	Dominated**
1	1.501	52,090	Dominated**
4	1.525	52,420	62,350
3	1.525	55,770	Dominated*
5	1.543	53,770	72,750

*Simple dominance. **Extended dominance.

Patients with HER2 negative breast cancer

Some may suggest that only patients with HER2 negative early breast cancer should undergo HER2 testing of tumour samples in the metastatic setting (Pusztai et al., 2010). The results showed that re-testing of only metastatic breast cancer patients, who had HER2 negative early breast cancer, was dominated by re-testing tumour samples of all metastatic breast cancer patients.

There is data showing that trastuzumab may have an effect in HER2 negative patients, but most probably these patients have either false negative HER2 results or hidden HER2 positive disease (i.e. extra cellular HER2) (Ardavanis et al., 2008, Fournier et al., 2005). If later research would show a substantial benefit of trastuzumab in HER2 negative metastatic breast cancer patients, similar to the effect on HER2 positive metastatic breast cancer patients, we would have to revise our results. As mentioned before, there is actually an ongoing study in early breast cancer patients with HER2 IHC 1+ and 2+ disease (NSABP(B-47), 2011).

Conclusions

Paper 4 shows that re-testing of HER2 status in metastatic breast cancers is cost-effective. It is also important to carry out testing from a clinical perspective, as both under- and over-treatment is avoided.

7 CONCLUDING REMARKS

Optimal management of small targeted patient populations is a challenge, as with the HER2 positive breast cancer patient population, and there are many aspects to consider.

Our studies revealed that it is important to define the patient population correctly, in primary and metastatic settings. There is a lack of stability of both HER2 and hormone receptors, being specific targets for anti HER2 and endocrine agents. Our studies also revealed that there are clinical and economic rationales for re-biopsying breast cancer patients with clinical and/or radiological diagnosed recurrences.

The knowledge created in this thesis could be used for the introduction of future co-dependent technologies, in order to gain the most health benefits, both to patients and to society, which should be applicable to upcoming new targeted agents and related diagnostic procedures.

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10 REFERENCES

- ADAMS, C. W., ALLISON, D. E., FLAGELLA, K., PRESTA, L., CLARKE, J., DYBDAL, N., MCKEEVER, K. & SLIWKOWSKI, M. X. 2006. Humanization of a recombinant monoclonal antibody to produce a therapeutic HER dimerization inhibitor, pertuzumab. *Cancer Immunol Immunother*, 55, 717-27.
- AL-AZAWI, D., LEONG, S., WONG, L., KAY, E., HILL, A. D. & YOUNG, L. 2011. HER-2 positive and p53 negative breast cancers are associated with poor prognosis. *Cancer Invest*, 29, 365-9.
- ALBAIN, K. S., BARLOW, W. E., SHAK, S., HORTOBAGYI, G. N. & LIVINGSTON, R. B. Y., I. T. RAVDIN, P. BUGARINI, R. BAEHNER, F. L. DAVIDSON, N. E. SLEDGE, G. W. WINER, E. P. HUDIS, C. INGLE, J. N. PEREZ, E. A. PRITCHARD, K. I. SHEPHERD, L. GRALOW, J. R. YOSHIZAWA, C. ALLRED, D. C. OSBORNE, C. K. HAYES, D. F. 2010. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*, 11, 55-65.
- ALVAREZ, R. H., VALERO, V. & HORTOBAGYI, G. N. 2010. Emerging targeted therapies for breast cancer. *J Clin Oncol*, 28, 3366-79.
- AMIR, E. & CLEMONS, M. 2009. Should a biopsy be recommended to confirm metastatic disease in women with breast cancer? *Lancet Oncol*, 10, 933-5.
- ANDERSSON, J., LINDERHOLM, B., BERGH, J. & ELMBERGER, G. 2004. 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*, 12, 14-20.
- ANDERSSON, M., LIDBRINK, E., BJERRE, K., WIST, E., ENEVOLDSEN, K., JENSEN, A. B., KARLSSON, P., TANGE, U. B., SORENSEN, P. G., MOLLER, S., BERGH, J. & LANGKJER, S. T. 2011. Phase III randomized study comparing docetaxel plus trastuzumab with vinorelbine plus trastuzumab as first-line therapy of metastatic or locally advanced human epidermal growth factor receptor 2-positive breast cancer: the HERNATA study. *J Clin Oncol*, 29, 264-71.
- ARDAVANIS, A., KOUNTOURAKIS, P., KYRIAKOU, F., MALLIOU, S., MANTZARIS, I., GAROUFALI, A., YIOTIS, I., SCORILAS, A., BAZIOTIS, N. & RIGATOS, G. 2008. Trastuzumab plus paclitaxel or docetaxel in HER-2-negative/HER-2 ECD-positive anthracycline- and taxane-refractory advanced breast cancer. *Oncologist*, 13, 361-9.
- ARENA, V., PENNACCHIA, I., VECCHIO, F. M. & CARBONE, A. 2010. "CISH the FISH" for HER2: our laboratory experience. *Am J Clin Pathol*, 134, 347-8.
- ARRIOLA, E., CANADAS, I., ARUMI-URIA, M., DOMINE, M., LOPEZ-VILARINO, J. A., ARPI, O., SALIDO, M., MENENDEZ, S., GRANDE, E., HIRSCH, F. R., SERRANO, S., BELLOSILLO, B., ROJO, F., ROVIRA, A. & ALBANELL, J. 2011. MET phosphorylation predicts poor outcome in small cell lung carcinoma and its inhibition blocks HGF-induced effects in MET mutant cell lines. *Br J Cancer*.
- AUTIER, P., BONIOL, M., GAVIN, A. & VATTEN, L. J. 2011. Breast cancer mortality in neighbouring European countries with different levels of screening but similar access to treatment: trend analysis of WHO mortality database. *BMJ*, 343, d4411.
- BARRA ADE, A., GOBBI, H., DE, L. R. C. A., GOUVEA, A. P., DE LUCENA, C. E., REIS, J. H. & COSTA E SILVA, S. Z. 2008. A comparison of aspiration cytology and core needle biopsy according to tumor size of suspicious breast lesions. *Diagn Cytopathol*, 36, 26-31.
- BASELGA, J., GELMON, K. A., VERMA, S., WARDLEY, A., CONTE, P., MILES, D., BIANCHI, G., CORTES, J., MCNALLY, V. A., ROSS, G. A., FUMOLEAU, P. & GIANNI, L. 2010. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *J Clin Oncol*, 28, 1138-44.

- BASELGA J, T. D., MENDELSON J, ET AL. 1996. Phase II study of weekly intravenous recombinant humanized anti- p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. . *J Clin Oncol*, , 737-744.
- BENSON, J. R., JATOI, I., KEISCH, M., ESTEVA, F. J., MAKRIS, A. & JORDAN, V. C. 2009. Early breast cancer. *Lancet*, 373, 1463-79.
- BERGH, J. 2009. Quo vadis with targeted drugs in the 21st century? *J Clin Oncol*, 27, 2-5.
- BERGQVIST, J., OHD, J. F., SMEDS, J., KLAAR, S., ISOLA, J., NORDGREN, H., ELMBERGER, G. P., HELLBORG, H., BJOHLE, J., BORG, A. L., SKOOG, L. & BERGH, J. 2007. Quantitative real-time PCR analysis and microarray-based RNA expression of HER2 in relation to outcome. *Ann Oncol*, 18, 845-50.
- BERRY, D. A. 2011. Bayesian approaches for comparative effectiveness research. *Clin Trials*.
- BERRY, D. A., CRONIN, K. A., PLEVITIS, S. K., FRYBACK, D. G., CLARKE, L., ZELEN, M., MANDELBLATT, J. S., YAKOVLEV, A. Y., HABBEMA, J. D. & FEUER, E. J. 2005. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med*, 353, 1784-92.
- BIRNER, P., OBERHUBER, G., STANI, J., REITHOFER, C., SAMONIGG, H., HAUSMANINGER, H., KUBISTA, E., KWASNY, W., KANDIOLER-ECKERSBERGER, D., GNANT, M. & JAKESZ, R. 2001. Evaluation of the United States Food and Drug Administration-approved scoring and test system of HER-2 protein expression in breast cancer. *Clin Cancer Res*, 7, 1669-75.
- BLACKWELL, K. L., BURSTEIN, H. J., STORNILOLO, A. M., RUGO, H., SLEDGE, G., KOEHLER, M., ELLIS, C., CASEY, M., VUKELJA, S., BISCHOFF, J., BASELGA, J. & O'SHAUGHNESSY, J. 2010. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol*, 28, 1124-30.
- BORG, A., LINELL, F., IDVALL, I., JOHANSSON, S., SIGURDSSON, H., FERNO, M. & KILLANDER, D. 1989. HER2/neu amplification and comedo type breast carcinoma. *Lancet*, 1, 1268-9.
- BOSE, P. & OZER, H. 2009. Neratinib: an oral, irreversible dual EGFR/HER2 inhibitor for breast and non-small cell lung cancer. *Expert Opin Investig Drugs*, 18, 1735-51.
- BRAITHWAITE, R. S., MELTZER, D. O., KING, J. T., JR., LESLIE, D. & ROBERTS, M. S. 2008. What does the value of modern medicine say about the \$50,000 per quality-adjusted life-year decision rule? *Med Care*, 46, 349-56.
- BURSTEIN, H. J., PRESTRUD, A. A., SEIDENFELD, J., ANDERSON, H., BUCHHOLZ, T. A., DAVIDSON, N. E., GELMON, K. E., GIORDANO, S. H., HUDIS, C. A., MALIN, J., MAMOUNAS, E. P., ROWDEN, D., SOLKY, A. J., SOWERS, M. R., STEARNS, V., WINER, E. P., SOMERFIELD, M. R. & GRIGGS, J. J. 2010. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*, 28, 3784-96.
- CAMERON, D., CASEY, M., PRESS, M., LINDQUIST, D., PIENKOWSKI, T., ROMIEU, C. G., CHAN, S., JAGIELLO-GRUSZFELD, A., KAUFMAN, B., CROWN, J., CHAN, A., CAMPONE, M., VIENS, P., DAVIDSON, N., GORBOUNOVA, V., RAATS, J. I., SKARLOS, D., NEWSTAT, B., ROYCHOWDHURY, D., PAOLETTI, P., OLIVA, C., RUBIN, S., STEIN, S. & GEYER, C. E. 2008. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. *Breast Cancer Res Treat*, 112, 533-43.
- CANCERFONDEN. 2006. *Cancerfondsrapporten* [Online]. [Accessed].
- CARDOSO, F., SENKUS-KONEFKA, E., FALLOWFIELD, L., COSTA, A. & CASTIGLIONE, M. 2010. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, 21 Suppl 5, v15-9.
- CHAMBERS, A. F., GROOM, A. C. & MACDONALD, I. C. 2002. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer*, 2, 563-72.
- CHUNG, M., CHANG, H. R., BLAND, K. I. & WANEBO, H. J. 1996. Younger women with breast carcinoma have a poorer prognosis than older women. *Cancer*, 77, 97-103.

- CLARK, G. M., OSBORNE, C. K. & MCGUIRE, W. L. 1984. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. *J Clin Oncol*, 2, 1102-9.
- CLARKE, M., COLLINS, R., DARBY, S., DAVIES, C., ELPHINSTONE, P., EVANS, E., GODWIN, J., GRAY, R., HICKS, C., JAMES, S., MACKINNON, E., MCGALE, P., MCHUGH, T., PETO, R., TAYLOR, C. & WANG, Y. 2005. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet*, 366, 2087-106.
- COBLEIGH, M. A., VOGEL, C. L., TRIPATHY, D., ROBERT, N. J., SCHOLL, S., FEHRENBACHER, L., WOLTER, J. M., PATON, V., SHAK, S., LIEBERMAN, G. & SLAMON, D. J. 1999. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol*, 17, 2639-48.
- COLEMAN, M. P., FORMAN, D., BRYANT, H., BUTLER, J., RACHET, B., MARINGE, C., NUR, U., TRACEY, E., COORY, M., HATCHER, J., MCGAHAN, C. E., TURNER, D., MARRETT, L., GJERSTORFF, M. L., JOHANNESSEN, T. B., ADOLFSSON, J., LAMBE, M., LAWRENCE, G., MEECHAN, D., MORRIS, E. J., MIDDLETON, R., STEWARD, J. & RICHARDS, M. A. 2011. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. *Lancet*, 377, 127-38.
- COOPER DN, P. D. S., AND NADIA A. CHUZHANOVA 2005. The Human Gene Mutation Database UNIT 1.13 (HGMD) and Its Exploitation in the Study of Mutational Mechanisms. *Current Protocols in Bioinformatics (2005)* 1.13.1-1.13.20.
- COTTU, P. H., ASSELAH, J., LAE, M., PIERGA, J. Y., DIERAS, V., MIGNOT, L., SIGAL-ZAFRANI, B. & VINCENT-SALOMON, A. 2008. Intratumoral heterogeneity of HER2/neu expression and its consequences for the management of advanced breast cancer. *Ann Oncol*, 19, 595-7.
- COUSSENS, L., YANG-FENG, T. L., LIAO, Y. C., CHEN, E., GRAY, A., MCGRATH, J., SEEBURG, P. H., LIBERMANN, T. A., SCHLESSINGER, J., FRANCKE, U. & ET AL. 1985. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*, 230, 1132-9.
- COUTURIER, J., VINCENT-SALOMON, A., NICOLAS, A., BEUZEBOC, P., MOURET, E., ZAFRANI, B. & SASTRE-GARAU, X. 2000. Strong correlation between results of fluorescent in situ hybridization and immunohistochemistry for the assessment of the ERBB2 (HER-2/neu) gene status in breast carcinoma. *Mod Pathol*, 13, 1238-43.
- CULYER, A., MCCABE, C., BRIGGS, A., CLAXTON, K., BUXTON, M., AKEHURST, R., SCULPHER, M. & BRAZIER, J. 2007. Searching for a threshold, not setting one: the role of the National Institute for Health and Clinical Excellence. *J Health Serv Res Policy*, 12, 56-8.
- CURIGLIANO, G., VIALE, G., BAGNARDI, V., FUMAGALLI, L., LOCATELLI, M., ROTMENSZ, N., GHISINI, R., COLLEONI, M., MUNZONE, E., VERONESI, P., ZURRIDA, S., NOLE, F. & GOLDFIRSCH, A. 2009. Clinical relevance of HER2 overexpression/amplification in patients with small tumor size and node-negative breast cancer. *J Clin Oncol*, 27, 5693-9.
- DAKO. 2011. *HercepTest* <http://www.dako.com/se/download.pdf?objectid=120980003> [Online]. [Accessed].
- DANG, C., LIN, N., MOY, B., COME, S., SUGARMAN, S., MORRIS, P., ABBRUZZI, A., CHEN, C., STEINGART, R., PATIL, S., NORTON, L., WINER, E. & HUDIS, C. 2010. Dose-dense doxorubicin and cyclophosphamide followed by weekly paclitaxel with trastuzumab and lapatinib in HER2/neu-overexpressed/amplified breast cancer is not feasible because of excessive diarrhea. *J Clin Oncol*, 28, 2982-8.
- DIAMANDIS, M., WHITE, N. M. & YOUSEF, G. M. 2010. Personalized medicine: marking a new epoch in cancer patient management. *Mol Cancer Res*, 8, 1175-87.
- DOCTOR, J. N., BLEICHRODT, H., MIYAMOTO, J., TEMKIN, N. R. & DIKMEN, S. 2004. A new and more robust test of QALYs. *J Health Econ*, 23, 353-67.

- DOWSETT, M. 2001. Overexpression of HER-2 as a resistance mechanism to hormonal therapy for breast cancer. *Endocr Relat Cancer*, 8, 191-5.
- DOWSETT, M., CUZICK, J., WALE, C., FORBES, J., MALLON, E. A., SALTER, J., QUINN, E., DUNBIER, A., BAUM, M., BUZDAR, A., HOWELL, A., BUGARINI, R., BAEHNER, F. L. & SHAK, S. 2010. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol*, 28, 1829-34.
- DOWSETT, M., CUZICK, J., WALE, C., HOWELL, T., HOUGHTON, J. & BAUM, M. 2005. Retrospective analysis of time to recurrence in the ATAC trial according to hormone receptor status: an hypothesis-generating study. *J Clin Oncol*, 23, 7512-7.
- DOWSETT, M., PROCTER, M., MCCASKILL-STEVENS, W., DE AZAMBUJA, E., DAFNI, U., RUESCHOFF, J., JORDAN, B., DOLCI, S., ABRAMOVITZ, M., STOSS, O., VIALE, G., GELBER, R. D., PICCART-GEBHART, M. & LEYLAND-JONES, B. 2009. Disease-free survival according to degree of HER2 amplification for patients treated with adjuvant chemotherapy with or without 1 year of trastuzumab: the HERA Trial. *J Clin Oncol*, 27, 2962-9.
- DRUMMOND, M., MANCA, A. & SCULPHER, M. 2005. Increasing the generalizability of economic evaluations: recommendations for the design, analysis, and reporting of studies. *Int J Technol Assess Health Care*, 21, 165-71.
- EBCTCG 2005a. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*, 365, 1687-717.
- EBCTCG 2005b. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet*, 366, 2087-106.
- EBCTCG. Year. The worldwide overview: new results for systemic adjuvant therapies. *In: San Antonio Breast Cancer Symposium, 2007.*
- EBCTCG 2011. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*, 378, 771-84.
- ECKERLUND, I., JOHANNESSEN, M., JOHANSSON, P. O., TAMBOUR, M. & ZETHRAEUS, N. 1995. Value for money? A contingent valuation study of the optimal size of the Swedish health care budget. *Health Policy*, 34, 135-43.
- EICHLER, H. G., KONG, S. X., GERTH, W. C., MAVROS, P. & JONSSON, B. 2004. Use of cost-effectiveness analysis in health-care resource allocation decision-making: how are cost-effectiveness thresholds expected to emerge? *Value Health*, 7, 518-28.
- ELKIN, E. B., WEINSTEIN, M. C., WINER, E. P., KUNTZ, K. M., SCHNITT, S. J. & WEEKS, J. C. 2004. HER-2 testing and trastuzumab therapy for metastatic breast cancer: a cost-effectiveness analysis. *J Clin Oncol*, 22, 854-63.
- ELSTON, C. W. & ELLIS, I. O. 2002. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. C. W. Elston & I. O. Ellis. *Histopathology* 1991; 19; 403-410. *Histopathology*, 41, 151-2, discussion 152-3.
- EWER, M. S., VOOLETICH, M. T., DURAND, J. B., WOODS, M. L., DAVIS, J. R., VALERO, V. & LENIHAN, D. J. 2005. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. *J Clin Oncol*, 23, 7820-6.
- FARMER, P., BONNEFOI, H., ANDERLE, P., CAMERON, D., WIRAPATI, P., BECETTE, V., ANDRE, S., PICCART, M., CAMPONE, M., BRAIN, E., MACGROGAN, G., PETIT, T., JASSEM, J., BIBEAU, F., BLOT, E., BOGAERTS, J., AGUET, M., BERGH, J., IGGO, R. & DELORENZI, M. 2009. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. *Nat Med*, 15, 68-74.

- FEHM, T., JAGER, W., KRAEMER, S., SOHN, C., SOLOMAYER-MEYBERG, G., SOLOMAYER, E. F., KUREK, R., WALLWIENER, D. & GEBAUER, G. 2004. Changes of serum HER2 status during clinical course of metastatic breast cancer patients. *Anticancer Res*, 24, 4205-10.
- FERLAY, J., SHIN, H. R., BRAY, F., FORMAN, D., MATHERS, C. & PARKIN, D. M. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, 127, 2893-917.
- FERRUSI, I. L., LEIGHL, N. B., KULIN, N. A. & MARSHALL, D. A. 2011. Do economic evaluations of targeted therapy provide support for decision makers? *J Oncol Pract*, 7, 36s-45s.
- FEYER, P., KLEEBERG, U. R., STEINGRABER, M., GUNTHER, W. & BEHRENS, M. 2008. Frequency of side effects in outpatient cancer care and their influence on patient satisfaction--a prospective survey using the PASQOC questionnaire. *Support Care Cancer*, 16, 567-75.
- FISHER, B., BAUER, M., WICKERHAM, D. L., REDMOND, C. K., FISHER, E. R., CRUZ, A. B., FOSTER, R., GARDNER, B., LERNER, H., MARGOLESE, R. & ET AL. 1983. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. *Cancer*, 52, 1551-7.
- FORNIER, M. N., SEIDMAN, A. D., SCHWARTZ, M. K., GHANI, F., THIEL, R., NORTON, L. & HUDIS, C. 2005. Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate. *Ann Oncol*, 16, 234-9.
- FOUKAKIS, T., FORNANDER, T., LEKBERG, T., HELLBORG, H., ADOLFSSON, J. & BERGH, J. 2011. Age-specific trends of survival in metastatic breast cancer: 26 years longitudinal data from a population-based cancer registry in Stockholm, Sweden. *Breast Cancer Res Treat*.
- FRUEH, F. W. 2010. Real-world clinical effectiveness, regulatory transparency and payer coverage: three ingredients for translating pharmacogenomics into clinical practice. *Pharmacogenomics*, 11, 657-60.
- GEYER, C. E., FORSTER, J., LINDQUIST, D., CHAN, S., ROMIEU, C. G., PIENKOWSKI, T., JAGIELLO-GRUSZFELD, A., CROWN, J., CHAN, A., KAUFMAN, B., SKARLOS, D., CAMPONE, M., DAVIDSON, N., BERGER, M., OLIVA, C., RUBIN, S. D., STEIN, S. & CAMERON, D. 2006. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*, 355, 2733-43.
- GIANNI L, PIENKOWSKI T, IM Y-H, ROMAN L, TSENG L-M, L. M.-C., LLUCH-HERNANDEZ A & SEMIGLAZOV V, S. T., ROSS G. . Year. Neoadjuvant Pertuzumab (P) and Trastuzumab (H): Antitumor and Safety Analysis of a Randomized Phase II Study ('NeoSphere'). *In*: [HTTP://WWW.ABSTRACTS2VIEW.COM/SABCS10/VIEW.PHP?NU=SABCS10L_1000](http://www.abstracts2view.com/sabcs10/view.php?nu=sabcs10l_1000), ed. San Antonio Breast Cancer Symposium, 2010.
- GIANNI, L., DAFNI, U., GELBER, R. D., AZAMBUJA, E., MUEHLBAUER, S., GOLDBIRSCH, A., UNTCH, M., SMITH, I., BASELGA, J., JACKISCH, C., CAMERON, D., MANO, M., PEDRINI, J. L., VERONESI, A., MENDIOLA, C., PLUZANSKA, A., SEMIGLAZOV, V., VRDOLJAK, E., ECKART, M. J., SHEN, Z., SKIADOPOULOS, G., PROCTER, M., PRITCHARD, K. I., PICCART-GEBHART, M. J. & BELL, R. 2011. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol*, 12, 236-44.
- GOLDBIRSCH, A., WOOD, W. C., COATES, A. S., GELBER, R. D., THURLIMANN, B. & SENN, H. J. 2011. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol*, 22, 1736-47.

- GOMES, A. M., WINTER, S., KLEIN, K., TURPEINEN, M., SCHAEFFELER, E., SCHWAB, M. & ZANGER, U. M. 2009. Pharmacogenomics of human liver cytochrome P450 oxidoreductase: multifactorial analysis and impact on microsomal drug oxidation. *Pharmacogenomics*, 10, 579-99.
- GONG, Y., SWEET, W., DUH, Y. J., GREENFIELD, L., FANG, Y., ZHAO, J., TARCO, E., SYMMANS, W. F., ISOLA, J. & SNEIGE, N. 2009a. Chromogenic in situ hybridization is a reliable method for detecting HER2 gene status in breast cancer: a multicenter study using conventional scoring criteria and the new ASCO/CAP recommendations. *Am J Clin Pathol*, 131, 490-7.
- GONG, Y., SWEET, W., DUH, Y. J., GREENFIELD, L., TARCO, E., TRIVEDI, S., SYMMANS, W. F., ISOLA, J. & SNEIGE, N. 2009b. Performance of chromogenic in situ hybridization on testing HER2 Status in breast carcinomas with chromosome 17 polysomy and equivocal (2+) herceptest results: a study of two institutions using the conventional and new ASCO/CAP scoring criteria. *Am J Clin Pathol*, 132, 228-36.
- GONZALEZ-ANGULO, A. M., LITTON, J. K., BROGLIO, K. R., MERIC-BERNSTAM, F., RAKKHIT, R., CARDOSO, F., PEINTINGER, F., HANRAHAN, E. O., SAHIN, A., GURAY, M., LARSIMONT, D., FEOLI, F., STRANZL, H., BUCHHOLZ, T. A., VALERO, V., THERIAULT, R., PICCART-GEHART, M., RAVDIN, P. M., BERRY, D. A. & HORTOBAGYI, G. N. 2009. High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. *J Clin Oncol*, 27, 5700-6.
- HANAHAN, D. & WEINBERG, R. A. 2011. Hallmarks of cancer: the next generation. *Cell*, 144, 646-74.
- HART, S. N., WANG, S., NAKAMOTO, K., WESSELMAN, C., LI, Y. & ZHONG, X. B. 2008. Genetic polymorphisms in cytochrome P450 oxidoreductase influence microsomal P450-catalyzed drug metabolism. *Pharmacogenet Genomics*, 18, 11-24.
- HAYASHI, N., NAKAMURA, S., TOKUDA, Y., SHIMODA, Y., YAGATA, H., YOSHIDA, A., OTA, H., HORTOBAGYI, G. N., CRISTOFANILLI, M. & UENO, N. T. 2011. Prognostic value of HER2-positive circulating tumor cells in patients with metastatic breast cancer. *Int J Clin Oncol*.
- HOLMBERG, L., DUFFY, S. W., YEN, A. M., TABAR, L., VITAK, B., NYSTROM, L. & FRISELL, J. 2009. Differences in endpoints between the Swedish W-E (two county) trial of mammographic screening and the Swedish overview: methodological consequences. *J Med Screen*, 16, 73-80.
- JACOBS, T. W., GOWN, A. M., YAZIJI, H., BARNES, M. J. & SCHNITT, S. J. 1999a. Comparison of fluorescence in situ hybridization and immunohistochemistry for the evaluation of HER-2/neu in breast cancer. *J Clin Oncol*, 17, 1974-82.
- JACOBS, T. W., GOWN, A. M., YAZIJI, H., BARNES, M. J. & SCHNITT, S. J. 1999b. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol*, 17, 1983-7.
- JOENSUU, H., BONO, P., KATAJA, V., ALANKO, T., KOKKO, R., ASOLA, R., UTRIAINEN, T., TURPEENIEMI-HUJANEN, T., JYRKKIO, S., MOYKKYNNEN, K., HELLE, L., INGALSUO, S., PAJUNEN, M., HUUSKO, M., SALMINEN, T., AUVINEN, P., LEINONEN, H., LEINONEN, M., ISOLA, J. & KELLOKUMPU-LEHTINEN, P. L. 2009. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer Trial. *J Clin Oncol*, 27, 5685-92.
- JOENSUU, H., KELLOKUMPU-LEHTINEN, P. L., BONO, P., ALANKO, T., KATAJA, V., ASOLA, R., UTRIAINEN, T., KOKKO, R., HEMMINKI, A., TARKKANEN, M., TURPEENIEMI-HUJANEN, T., JYRKKIO, S., FLANDER, M., HELLE, L., INGALSUO, S., JOHANSSON, K., JAASKELAINEN, A. S., PAJUNEN, M., RAUHALA, M., KALEVA-KEROLA, J., SALMINEN, T., LEINONEN, M., ELOMAA, I. & ISOLA, J. 2006. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med*, 354, 809-20.

- JOHANNESSON M, JONSSON B, JONSSON L, KOBELT G & N, Z. 2009. *Why should Economic Evaluation of Medical Innovations Have a Societal Perspective?* [Online]. [Accessed].
- JONSSON, B. 1990. Quality of life--economic aspects. *Scand J Prim Health Care Suppl*, 1, 93-6.
- JONSSON, B. 1993. Economic evaluation of health care technologies. *Acta Endocrinol (Copenh)*, 128 Suppl 2, 50-4.
- JONSSON, B. 1997. Economic evaluation of medical technologies in Sweden. *Soc Sci Med*, 45, 597-604.
- JONSSON, E. 2009. History of Health Technology assessment i Sweden. 25.
- JORDAN, V. C. 1988. The development of tamoxifen for breast cancer therapy: a tribute to the late Arthur L. Walpole. *Breast Cancer Res Treat*, 11, 197-209.
- KALAGER, M., ZELEN, M., LANGMARK, F. & ADAMI, H. O. 2010. Effect of screening mammography on breast-cancer mortality in Norway. *N Engl J Med*, 363, 1203-10.
- KARLSSON, G. & JOHANNESSON, M. 1996. The decision rules of cost-effectiveness analysis. *Pharmacoeconomics*, 9, 113-20.
- KAUFMAN, B., MACKEY, J. R., CLEMENS, M. R., BAPSY, P. P., VAID, A., WARDLEY, A., TJULANDIN, S., JAHN, M., LEHLE, M., FEYEREISLOVA, A., REVIL, C. & JONES, A. 2009. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol*, 27, 5529-37.
- KAUFMANN, M. & PUSZTAI, L. 2010. Use of standard markers and incorporation of molecular markers into breast cancer therapy: consensus recommendations from an International Expert Panel. *Cancer*.
- KHASRAW, M., BROGI, E. & SEIDMAN, A. D. 2011. The need to examine metastatic tissue at the time of progression of breast cancer: is re-biopsy a necessity or a luxury? *Curr Oncol Rep*, 13, 17-25.
- KHEIRELSEID, E. A., BOGGS, J. M., CURRAN, C., GLYNN, R. W., DOOLEY, C., SWEENEY, K. J. & KERIN, M. J. 2011. Younger age as a prognostic indicator in breast cancer: A cohort study. *BMC Cancer*, 11, 383.
- KOCHHAR, R., HOWARD, E. M., UMBREIT, J. N. & LAU, S. K. 2005. Metaplastic breast carcinoma with squamous differentiation: molecular and clinical analysis of six cases. *Breast J*, 11, 367-9.
- KOSCIELNY, S., ARRIAGADA, R., ADOLFSSON, J., FORNANDER, T. & BERGH, J. 2009. Impact of tumour size on axillary involvement and distant dissemination in breast cancer. *Br J Cancer*, 101, 902-7.
- KUMLE, M. 2008. Declining breast cancer incidence and decreased HRT use. *Lancet*, 372, 608-10.
- LANDIS, J. R. & KOCH, G. G. 1977. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics*, 33, 363-74.
- LARSIMONT, D., DI LEO, A., ROUAS, G., PAESMANS, M., FERREIRA-FILHO, F., BERNARD, C., CARDOSO, F., VERHEST, A., PICCART, M. J. & GANCBERG, D. 2002. HER-2/neu evaluation by immunohistochemistry and fluorescence in situ hybridization in breast cancer: implications for daily laboratory practice. *Anticancer Res*, 22, 2485-90.
- LEWIS PHILLIPS, G. D., LI, G., DUGGER, D. L., CROCKER, L. M., PARSONS, K. L., MAI, E., BLATTLER, W. A., LAMBERT, J. M., CHARI, R. V., LUTZ, R. J., WONG, W. L., JACOBSON, F. S., KOEPPEN, H., SCHWALL, R. H., KENKARE-MITRA, S. R., SPENCER, S. D. & SLIWKOWSKI, M. X. 2008. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res*, 68, 9280-90.
- LICHTENBERG, F. R. 2011. Despite steep costs, payments for new cancer drugs make economic sense. *Nat Med*, 17, 244.

- LICHTENBERG, F. R., GROOTENDORST, P., VAN AUDENRODE, M., LATREMOUILLE-VIAU, D. & LEFEBVRE, P. 2009. The impact of drug vintage on patient survival: a patient-level analysis using Quebec's provincial health plan data. *Value Health*, 12, 847-56.
- LIDGREN, M., JONSSON, B., REHNBERG, C., WILLKING, N. & BERGH, J. 2008a. Cost-effectiveness of HER2 testing and 1-year adjuvant trastuzumab therapy for early breast cancer. *Ann Oncol*, 19, 487-95.
- LIDGREN, M., WILKING, N. & JONSSON, B. 2007a. Cost of breast cancer in Sweden in 2002. *Eur J Health Econ*, 8, 5-15.
- LIDGREN, M., WILKING, N., JONSSON, B. & REHNBERG, C. 2007b. Health related quality of life in different states of breast cancer. *Qual Life Res*, 16, 1073-81.
- LIDGREN, M., WILKING, N., JONSSON, B. & REHNBERG, C. 2008b. Cost-effectiveness of HER2 testing and trastuzumab therapy for metastatic breast cancer. *Acta Oncol*, 47, 1018-28.
- LIEDTKE, C., BROGLIO, K., MOULDER, S., HSU, L., KAU, S. W., SYMMANS, W. F., ALBARRACIN, C., MERIC-BERNSTAM, F., WOODWARD, W., THERIAULT, R. L., KIESEL, L., HORTOBAGYI, G. N., PUSZTAI, L. & GONZALEZ-ANGULO, A. M. 2009. Prognostic impact of discordance between triple-receptor measurements in primary and recurrent breast cancer. *Ann Oncol*, 20, 1953-8.
- LIESKE, B., RAVICHANDRAN, D. & WRIGHT, D. 2006. Role of fine-needle aspiration cytology and core biopsy in the preoperative diagnosis of screen-detected breast carcinoma. *Br J Cancer*, 95, 62-6.
- LINDERHOLM, B., LINDH, B., TAVELIN, B., GRANKVIST, K. & HENRIKSSON, R. 2000. p53 and vascular-endothelial-growth-factor (VEGF) expression predicts outcome in 833 patients with primary breast carcinoma. *Int J Cancer*, 89, 51-62.
- LINDSTRÖM, L., HOWELL S, ÅSTRÖM G, WILKING U, LIDBRINK E, ARMSTRONG A, KARLSSON E, HATSCHEK T & J, B. 2010. Controversies in the Management of metastatic breast cancer: Biologic evaluation of Breast Cancer - Should metastases Be Biosied? *ASCO annual meeting Educational Book*, 2010.
- LIPTON, A., LEITZEL, K., ALI, S. M., DEMERS, L., HARVEY, H. A., CHAUDRI-ROSS, H. A., EVANS, D., LANG, R., HACKL, W., HAMER, P. & CARNEY, W. 2005. Serum HER-2/neu conversion to positive at the time of disease progression in patients with breast carcinoma on hormone therapy. *Cancer*, 104, 257-63.
- LIU, E., THOR, A., HE, M., BARCOS, M., LJUNG, B. M. & BENZ, C. 1992. The HER2 (c-erbB-2) oncogene is frequently amplified in in situ carcinomas of the breast. *Oncogene*, 7, 1027-32.
- LOFGREN, L., SKOOG, L., VON SCHOULTZ, E., TANI, E., ISAKSSON, E., FERNSTAD, R., CARLSTROM, K. & VON SCHOULTZ, B. 2003. Hormone receptor status in breast cancer--a comparison between surgical specimens and fine needle aspiration biopsies. *Cytopathology*, 14, 136-42.
- LOWER, E. E., GLASS, E., BLAU, R. & HARMAN, S. 2009. HER-2/neu expression in primary and metastatic breast cancer. *Breast Cancer Res Treat*, 113, 301-6.
- LUCE, B. R., MAUSKOPF, J., SLOAN, F. A., OSTERMANN, J. & PARAMORE, L. C. 2006. The return on investment in health care: from 1980 to 2000. *Value Health*, 9, 146-56.
- LÄKEMEDELSFÖRMÅNSVERKET, T. O. 2003. *Läkemedelsförmånsnämndens allmänna råd om ekonomiska utvärderingar* [Online]. [Accessed].
- MACKAY, A., WEIGELT, B., GRIGORIADIS, A., KREIKE, B., NATRAJAN, R., A'HERN, R., TAN, D. S., DOWSETT, M., ASHWORTH, A. & REIS-FILHO, J. S. 2011. Microarray-based class discovery for molecular classification of breast cancer: analysis of interobserver agreement. *J Natl Cancer Inst*, 103, 662-73.
- MALMSTROM, P., HOLMBERG, L., ANDERSON, H., MATTSSON, J., JONSSON, P. E., TENNVALL-NITTBY, L., BALLDIN, G., LOVEN, L., SVENSSON, J. H., INGVAR, C., MOLLER, T., HOLMBERG, E. & WALLGREN, A. 2003. Breast conservation surgery, with and without radiotherapy, in women with lymph node-negative breast cancer: a randomised clinical trial in a population with access to public mammography screening. *Eur J Cancer*, 39, 1690-7.

- MARTY, M., COGNETTI, F., MARANINCHI, D., SNYDER, R., MAURIAC, L., TUBIANA-HULIN, M., CHAN, S., GRIMES, D., ANTON, A., LLUCH, A., KENNEDY, J., O'BYRNE, K., CONTE, P., GREEN, M., WARD, C., MAYNE, K. & EXTRA, J. M. 2005. Randomized 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. *J Clin Oncol*, 23, 4265-74.
- MCPHERSON, K., STEEL, C. M. & DIXON, J. M. 2000. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *BMJ*, 321, 624-8.
- MEIJER, S. L., WESSELING, J., SMIT, V. T., NEDERLOF, P. M., HOOIJER, G. K., RUIJTER, H., ARENDS, J. W., KLIFFEN, M., VAN GORP, J. M., STERK, L. & VAN DE VIJVER, M. J. 2011. HER2 gene amplification in patients with breast cancer with equivocal IHC results. *J Clin Pathol*.
- MENG, S., TRIPATHY, D., SHETE, S., ASHFAQ, R., HALEY, B., PERKINS, S., BEITSCH, P., KHAN, A., EUHUS, D., OSBORNE, C., FRENKEL, E., HOOVER, S., LEITCH, M., CLIFFORD, E., VITETTA, E., MORRISON, L., HERLYN, D., TERSTAPPEN, L. W., FLEMING, T., FEHM, T., TUCKER, T., LANE, N., WANG, J. & UHR, J. 2004. HER-2 gene amplification can be acquired as breast cancer progresses. *Proc Natl Acad Sci U S A*, 101, 9393-8.
- MILLER, L. D., SMEDS, J., GEORGE, J., VEGA, V. B., VERGARA, L., PLONER, A., PAWITAN, Y., HALL, P., KLAAR, S., LIU, E. T. & BERGH, J. 2005. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A*, 102, 13550-5.
- MIRZA, N. Q., VLASTOS, G., MERIC, F., BUCHHOLZ, T. A., ESNAOLA, N., SINGLETARY, S. E., KUERER, H. M., NEWMAN, L. A., AMES, F. C., ROSS, M. I., FEIG, B. W., POLLOCK, R. E., MCNEESE, M., STROM, E. & HUNT, K. K. 2002. Predictors of locoregional recurrence among patients with early-stage breast cancer treated with breast-conserving therapy. *Ann Surg Oncol*, 9, 256-65.
- MITTENDORF, E. A., WU, Y., SCALTRITI, M., MERIC-BERNSTAM, F., HUNT, K. K., DAWOOD, S., ESTEVA, F. J., BUZDAR, A. U., CHEN, H., EKSAMBI, S., HORTOBAGYI, G. N., BASELGA, J. & GONZALEZ-ANGULO, A. M. 2009. Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. *Clin Cancer Res*, 15, 7381-8.
- MULROONEY, D. A., YEAZEL, M. W., KAWASHIMA, T., MERTENS, A. C., MITBY, P., STOVALL, M., DONALDSON, S. S., GREEN, D. M., SKLAR, C. A., ROBISON, L. L. & LEISENRING, W. M. 2009. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. *BMJ*, 339, b4606.
- MUNZONE, E., CURIGLIANO, G., ROCCA, A., BONIZZI, G., RENNE, G., GOLDBIRSCHE, A. & NOLE, F. 2006. Reverting estrogen-receptor-negative phenotype in HER-2-overexpressing advanced breast cancer patients exposed to trastuzumab plus chemotherapy. *Breast Cancer Res*, 8, R4.
- MUSS, H. B., THOR, A. D., BERRY, D. A., KUTE, T., LIU, E. T., KOERNER, F., CIRRINCIONE, C. T., BUDMAN, D. R., WOOD, W. C., BARCOS, M. & ET AL. 1994. c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med*, 330, 1260-6.
- NCI: N. C. I. 2010. Cancer Trends Progress Reports 2009/2010 Update. http://progressreport.cancer.gov/doc_detail.asp?pid=1&did=2009&chid=95&coid=926&mid=
- NICE. 2002. *The clinical effectiveness and cost effectiveness of trastuzumab for breast cancer* [Online]. [Accessed].
- NILSEN, P. 2011. Praktikbaserad forskning kan ge forskningsbaserad praktik. *Läkartidningen*, 108, 1672-1673.
- NORUM, J., RISBERG, T. & OLSEN, J. A. 2005. A monoclonal antibody against HER-2 (trastuzumab) for metastatic breast cancer: a model-based cost-effectiveness analysis. *Ann Oncol*, 16, 909-14.

- NSABP(B-47). 2011. *Protocol B-47 A Randomized Phase III Trial of Adjuvant Therapy Comparing Chemotherapy Alone (Six Cycles of Docetaxel Plus Cyclophosphamide or Four Cycles of Doxorubicin Plus Cyclophosphamide Followed by Weekly Paclitaxel) to Chemotherapy Plus Trastuzumab in Women with Node-Positive or High-Risk Node-Negative HER2-Low Invasive Breast Cancer* [Online]. [Accessed].
- NUNES, R. A. & HARRIS, L. N. 2002. The HER2 extracellular domain as a prognostic and predictive factor in breast cancer. *Clin Breast Cancer*, 3, 125-35; discussion 136-7.
- NYSTROM, L., ANDERSSON, I., BJURSTAM, N., FRISELL, J., NORDENSKJOLD, B. & RUTQVIST, L. E. 2002. Long-term effects of mammography screening: updated overview of the Swedish randomised trials. *Lancet*, 359, 909-19.
- OSBORNE, C. K. 1998. Steroid hormone receptors in breast cancer management. *Breast Cancer Res Treat*, 51, 227-38.
- OTTESEN, G. L., GRAVERSEN, H. P., BLICHERT-TOFT, M., CHRISTENSEN, I. J. & ANDERSEN, J. A. 2000. Carcinoma in situ of the female breast. 10 year follow-up results of a prospective nationwide study. *Breast Cancer Res Treat*, 62, 197-210.
- PARKIN, D. M., BRAY, F., FERLAY, J. & PISANI, P. 2005. Global cancer statistics, 2002. *CA Cancer J Clin*, 55, 74-108.
- PAULETTI, G., DANDEKAR, S., RONG, H., RAMOS, L., PENG, H., SESHADRI, R. & SLAMON, D. J. 2000. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol*, 18, 3651-64.
- PAULETTI, G., GODOLPHIN, W., PRESS, M. F. & SLAMON, D. J. 1996. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene*, 13, 63-72.
- PECTASIDES, D., GAGLIA, A., ARAPANTONI-DADIOTI, P., BOBOTA, A., VALAVANIS, C., KOSTOPOULOU, V., MYLONAKIS, N., KARABELIS, A., PECTASIDES, M. & ECONOMOPOULOS, T. 2006. HER-2/neu status of primary breast cancer and corresponding metastatic sites in patients with advanced breast cancer treated with trastuzumab-based therapy. *Anticancer Res*, 26, 647-53.
- PEREZ, E. A., ROCHE, P. C., JENKINS, R. B., REYNOLDS, C. A., HALLING, K. C., INGLE, J. N. & WOLD, L. E. 2002. HER2 testing in patients with breast cancer: poor correlation between weak positivity by immunohistochemistry and gene amplification by fluorescence in situ hybridization. *Mayo Clin Proc*, 77, 148-54.
- PEREZ, E. A., ROMOND, E. H., SUMAN, V. J., JEONG, J. H., DAVIDSON, N. E., GEYER, C. E., JR., MARTINO, S., MAMOUNAS, E. P., KAUFMAN, P. A. & WOLMARK, N. 2011. Four-Year Follow-Up of Trastuzumab Plus Adjuvant Chemotherapy for Operable Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Joint Analysis of Data From NCCTG N9831 and NSABP B-31. *J Clin Oncol*.
- PEREZ, E. A., SUMAN, V. J., DAVIDSON, N. E., SLEDGE, G. W., KAUFMAN, P. A., HUDIS, C. A., MARTINO, S., GRALOW, J. R., DAKHIL, S. R., INGLE, J. N., WINER, E. P., GELMON, K. A., GERSH, B. J., JAFFE, A. S. & RODEHEFFER, R. J. 2008. Cardiac safety analysis of doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab in the North Central Cancer Treatment Group N9831 adjuvant breast cancer trial. *J Clin Oncol*, 26, 1231-8.
- PERSSON, U. & HJELMGREN, J. 2003. [Health services need knowledge of how the public values health]. *Lakartidningen*, 100, 3436-7.
- PETITJEAN, A., ACHATZ, M. I., BORRESEN-DALE, A. L., HAINAUT, P. & OLIVIER, M. 2007. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene*, 26, 2157-65.

- PICCART-GEBHART, M. J., PROCTER, M., LEYLAND-JONES, B., GOLDBIRSCH, A., UNTCH, M., SMITH, I., GIANNI, L., BASELGA, J., BELL, R., JACKISCH, C., CAMERON, D., DOWSETT, M., BARRIOS, C. H., STEGER, G., HUANG, C. S., ANDERSSON, M., INBAR, M., LICHINITSER, M., LANG, I., NITZ, U., IWATA, H., THOMSEN, C., LOHRISCH, C., SUTER, T. M., RUSCHOFF, J., SUTO, T., GREATOREX, V., WARD, C., STRAEHLE, C., MCFADDEN, E., DOLCI, M. S. & GELBER, R. D. 2005. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*, 353, 1659-72.
- PUSZTAI, L. 2007. Limitations of pharmacogenomic predictor discovery in Phase II clinical trials. *Pharmacogenomics*, 8, 1443-8.
- PUSZTAI, L. 2008. Current status of prognostic profiling in breast cancer. *Oncologist*, 13, 350-60.
- PUSZTAI, L., VIALE, G., KELLY, C. M. & HUDIS, C. A. 2010. Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist*, 15, 1164-8.
- QUIET, C. A., FERGUSON, D. J., WEICHSELBAUM, R. R. & HELLMAN, S. 1995. Natural history of node-negative breast cancer: a study of 826 patients with long-term follow-up. *J Clin Oncol*, 13, 1144-51.
- RAMSEY, S. Year. Incorporating comparative effectiveness research study endpoints into the treatment for positive-node, endocrine-responsive breast cancer (RxPONDER) study. *In: ASCO, 2011 Chicago.*
- RAUSEI, S., ROVERA, F., DIONIGI, G., TORNESE, D., FACHINETTI, A., BONI, L. & DIONIGI, R. 2010. Predictors of loco-regional recurrence and cancer-related death after breast cancer surgery. *Breast J*, 16 Suppl 1, S29-33.
- REVILLION, F., BONNETERRE, J. & PEYRAT, J. P. 1998. ERBB2 oncogene in human breast cancer and its clinical significance. *Eur J Cancer*, 34, 791-808.
- ROCHE, P. C., SUMAN, V. J., JENKINS, R. B., DAVIDSON, N. E., MARTINO, S., KAUFMAN, P. A., ADDO, F. K., MURPHY, B., INGLE, J. N. & PEREZ, E. A. 2002. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst*, 94, 855-7.
- ROMOND, E. H., PEREZ, E. A., BRYANT, J., SUMAN, V. J., GEYER, C. E., JR., DAVIDSON, N. E., TAN-CHIU, E., MARTINO, S., PAIK, S., KAUFMAN, P. A., SWAIN, S. M., PISANSKY, T. M., FEHRENBACHER, L., KUTTEH, L. A., VOGEL, V. G., VISSCHER, D. W., YOTHERS, G., JENKINS, R. B., BROWN, A. M., DAKHIL, S. R., MAMOUNAS, E. P., LINGLE, W. L., KLEIN, P. M., INGLE, J. N. & WOLMARK, N. 2005. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*, 353, 1673-84.
- ROSS, J. S., FLETCHER, J. A., LINETTE, G. P., STEC, J., CLARK, E., AYERS, M., SYMMANS, W. F., PUSZTAI, L. & BLOOM, K. J. 2003. The HER-2/neu Gene and Protein in Breast Cancer 2003: Biomarker and Target of Therapy. *Oncologist*, 8, 307-325.
- RUTQVIST, L. E. 1990. The significance of hormone receptors to predict the endocrine responsiveness of human breast cancer. *Acta Oncol*, 29, 371-7.
- RYDEN, L., HAGLUND, M., BENDAHL, P. O., HATSCHEK, T., KOLARIC, A., KOVACS, A., OLSSON, A., OLSSON, H., STRAND, C. & FERNO, M. 2009. Reproducibility of human epidermal growth factor receptor 2 analysis in primary breast cancer: a national survey performed at pathology departments in Sweden. *Acta Oncol*, 48, 860-6.
- RYDEN, L., JONSSON, P. E., CHEBIL, G., DUFMATS, M., FERNO, M., JIRSTROM, K., KALLSTROM, A. C., LANDBERG, G., STAL, O., THORSTENSON, S. & NORDENSKJOLD, B. 2005. Two years of adjuvant tamoxifen in premenopausal patients with breast cancer: a randomised, controlled trial with long-term follow-up. *Eur J Cancer*, 41, 256-64.

- SACHIDANANDAM, R., WEISSMAN, D., SCHMIDT, S. C., KAKOL, J. M., STEIN, L. D., MARTH, G., SHERRY, S., MULLIKIN, J. C., MORTIMORE, B. J., WILLEY, D. L., HUNT, S. E., COLE, C. G., COGGILL, P. C., RICE, C. M., NING, Z., ROGERS, J., BENTLEY, D. R., KWOK, P. Y., MARDIS, E. R., YEH, R. T., SCHULTZ, B., COOK, L., DAVENPORT, R., DANTE, M., FULTON, L., HILLIER, L., WATERSTON, R. H., MCPHERSON, J. D., GILMAN, B., SCHAFFNER, S., VAN ETTEN, W. J., REICH, D., HIGGINS, J., DALY, M. J., BLUMENSTIEL, B., BALDWIN, J., STANGE-THOMANN, N., ZODY, M. C., LINTON, L., LANDER, E. S. & ALTSHULER, D. 2001. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*, 409, 928-33.
- SANT, M., ALLEMANI, C., SANTAQUILANI, M., KNIJN, A., MARCHESI, F. & CAPOCACCIA, R. 2009. EUROCARE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary. *Eur J Cancer*, 45, 931-91.
- SCHMOOR, C., SAUERBREI, W., BASTERT, G. & SCHUMACHER, M. 2000. Role of isolated locoregional recurrence of breast cancer: results of four prospective studies. *J Clin Oncol*, 18, 1696-708.
- SCHOENFELD, D. 1982. Partial residuals for the proportional hazards regression model. *Biometrika*, 69, 239-241.
- SESHADRI, R., FIRGAIRA, F. A., HORSFALL, D. J., MCCAUL, K., SETLUR, V. & KITCHEN, P. 1993. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. *J Clin Oncol*, 11, 1936-42.
- SHAH, S. P., MORIN, R. D., KHATTRA, J., PRENTICE, L., PUGH, T., BURLEIGH, A., DELANEY, A., GELMON, K., GULIANY, R., SENZ, J., STEIDL, C., HOLT, R. A., JONES, S., SUN, M., LEUNG, G., MOORE, R., SEVERSON, T., TAYLOR, G. A., TESCHENDORFF, A. E., TSE, K., TURASHVILI, G., VARHOL, R., WARREN, R. L., WATSON, P., ZHAO, Y., CALDAS, C., HUNTSMAN, D., HIRST, M., MARRA, M. A. & APARICIO, S. 2009. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. *Nature*, 461, 809-13.
- SIMMONS, C., MILLER, N., GEDDIE, W., GIANFELICE, D., OLDFIELD, M., DRANITSARIS, G. & CLEMONS, M. J. 2009. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases? *Ann Oncol*, 20, 1499-504.
- SIMON, R. & MAITOURNAM, A. 2004. Evaluating the efficiency of targeted designs for randomized clinical trials. *Clin Cancer Res*, 10, 6759-63.
- SINGLETARY, S. E. & GREENE, F. L. 2003. Revision of breast cancer staging: the 6th edition of the TNM Classification. *Semin Surg Oncol*, 21, 53-9.
- SJOGREN, S., INGANAS, M., LINDGREN, A., HOLMBERG, L. & BERGH, J. 1998. Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. *J Clin Oncol*, 16, 462-9.
- SJUKVÅRDSREGIONEN, S. 2011. *Regionala priser och ersättningar för Södra sjukvårdsregionen 2010*. [Online]. [Accessed].
- SLAMON, D. J. Year. 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. In: [HTTP://WWW.ABSTRACTS2VIEW.COM/SABCS09/VIEW.PHP?NU=SABCS09L_1752](http://www.abstracts2view.com/sabcs09/view.php?nu=sabcs09l_1752), ed. San Antonio Breast Cancer Symposium, 2009.
- SLAMON, D. J., CLARK, G. M., WONG, S. G., LEVIN, W. J., ULLRICH, A. & MCGUIRE, W. L. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, 235, 177-82.
- SLAMON, D. J., LEYLAND-JONES, B., SHAK, S., FUCHS, H., PATON, V., BAJAMONDE, A., FLEMING, T., EIERMANN, W., WOLTER, J., PEGRAM, M., BASELGA, J. & NORTON, L. 2001. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*, 344, 783-92.

- SMITH, I., PROCTER, M., GELBER, R. D., GUILLAUME, S., FEYEREISLOVA, A., DOWSETT, M., GOLDHIRSCH, A., UNTCH, M., MARIANI, G., BASELGA, J., KAUFMANN, M., CAMERON, D., BELL, R., BERGH, J., COLEMAN, R., WARDLEY, A., HARBECK, N., LOPEZ, R. I., MALLMANN, P., GELMON, K., WILCKEN, N., WIST, E., SANCHEZ ROVIRA, P. & PICCART-GEBHART, M. J. 2007. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet*, 369, 29-36.
- SOCIALSTYRELSEN 2009. Cancer i Siffror 2009. Socialstyrelsen.
- SOCIALSTYRELSEN 2010. Cancer incidence in Sweden 2009. *Official Statistics of Sweden*. Cancer Registry.
- SONNENBERG, F. A. & BECK, J. R. 1993. Markov models in medical decision making: a practical guide. *Med Decis Making*, 13, 322-38.
- SORLIE, T., PEROU, C. M., TIBSHIRANI, R., AAS, T., GEISLER, S., JOHNSEN, H., HASTIE, T., EISEN, M. B., VAN DE RIJN, M., JEFFREY, S. S., THORSEN, T., QUIST, H., MATESE, J. C., BROWN, P. O., BOTSTEIN, D., EYSTEIN LONNING, P. & BORRESEN-DALE, A. L. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*, 98, 10869-74.
- SOTIRIOU, C. & PUSZTAI, L. 2009. Gene-expression signatures in breast cancer. *N Engl J Med*, 360, 790-800.
- STAAF, J., RINGNER, M., VALLON-CHRISTERSSON, J., JONSSON, G., BENDAHL, P. O., HOLM, K., ARASON, A., GUNNARSSON, H., HEGARDT, C., AGNARSSON, B. A., LUTS, L., GRABAU, D., FERNO, M., MALMSTROM, P. O., JOHANNSSON, O. T., LOMAN, N., BARKARDOTTIR, R. B. & BORG, A. 2010. Identification of subtypes in human epidermal growth factor receptor 2--positive breast cancer reveals a gene signature prognostic of outcome. *J Clin Oncol*, 28, 1813-20.
- STEINFORT, D. P., VINCENT, J., HEINZE, S., ANTIPPA, P. & IRVING, L. B. 2011. Comparative effectiveness of radial probe endobronchial ultrasound versus CT-guided needle biopsy for evaluation of peripheral pulmonary lesions: A randomized pragmatic trial. *Respir Med*.
- STERN, D. F., HEFFERNAN, P. A. & WEINBERG, R. A. 1986. p185, a product of the neu proto-oncogene, is a receptorlike protein associated with tyrosine kinase activity. *Mol Cell Biol*, 6, 1729-40.
- SUTER, T. M., PROCTER, M., VAN VELDHUISEN, D. J., MUSCHOLL, M., BERGH, J., CARLOMAGNO, C., PERREN, T., PASSALACQUA, R., BIGHIN, C., KLIJN, J. G., AGEEV, F. T., HITRE, E., GROETZ, J., IWATA, H., KNAP, M., GNANT, M., MUEHLBAUER, S., SPENCE, A., GELBER, R. D. & PICCART-GEBHART, M. J. 2007. Trastuzumab-associated cardiac adverse effects in the herceptin adjuvant trial. *J Clin Oncol*, 25, 3859-65.
- SWEBCG, G. 2011. *National Guidelines on Breast Cancer treatment* [Online]. [Accessed].
- TALBACK, M., STENBECK, M., ROSEN, M., BARLOW, L. & GLIMELIUS, B. 2003. Cancer survival in Sweden 1960-1998--developments across four decades. *Acta Oncol*, 42, 637-59.
- TANNER, M., GANCBERG, D., DI LEO, A., LARSIMONT, D., ROUAS, G., PICCART, M. J. & ISOLA, J. 2000. Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples. *Am J Pathol*, 157, 1467-72.
- TENG, T. O. 2004. Cost-effectiveness versus cost-utility analysis of interventions for cancer: does adjusting for health-related quality of life really matter? *Value Health*, 7, 70-8.
- THOMSON, T. A., ZHOU, C., CEBALLOS, K. & KNIGHT, B. 2010. Tissue microarray for routine clinical breast biomarker analysis. The British Columbia Cancer Agency 2008 experience. *Am J Clin Pathol*, 133, 909-14.
- TSOURDI, E., RACHNER, T. D., RAUNER, M., HAMANN, C. & HOFBAUER, L. 2011. Denosumab for bone diseases - Translating bone biology into targeted therapy. *Eur J Endocrinol*.

- TSUDA, H., TANI, Y., HASEGAWA, T. & FUKUTOMI, T. 2001. Concordance in judgments among c-erbB-2 (HER2/neu) overexpression detected by two immunohistochemical tests and gene amplification detected by Southern blot hybridization in breast carcinoma. *Pathol Int*, 51, 26-32.
- UNTCH M, LOIBL S, BISCHOFF J, EIDTMANN H, KAUFMANN M & BLOHMER J-U. 2010. *Lapatinib vs Trastuzumab in Combination with Neoadjuvant Anthracycline-Taxane-Based Chemotherapy: Primary Efficacy Endpoint Analysis of the GEPARQUINTO STUDY (GBG 44)*. [Online]. [Accessed].
- UNTCH, M., GELBER, R. D., JACKISCH, C., PROCTER, M., BASELGA, J., BELL, R., CAMERON, D., BARI, M., SMITH, I., LEYLAND-JONES, B., DE AZAMBUJA, E., WERMUTH, P., KHASANOV, R., FENG-YI, F., CONSTANTIN, C., MAYORDOMO, J. I., SU, C. H., YU, S. Y., LLUCH, A., SENKUS-KONEFKA, E., PRICE, C., HASLBAUER, F., SUAREZ SAHUI, T., SRIMUNINIMIT, V., COLLEONI, M., COATES, A. S., PICCART-GEBHART, M. J. & GOLDHIRSCH, A. 2008. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. *Ann Oncol*, 19, 1090-6.
- URRUTICOECHEA, A., SMITH, I. E. & DOWSETT, M. 2005. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol*, 23, 7212-20.
- VANCE, G. H., BARRY, T. S., BLOOM, K. J., FITZGIBBONS, P. L., HICKS, D. G., JENKINS, R. B., PERSONS, D. L., TUBBS, R. R. & HAMMOND, M. E. 2009. Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. *Arch Pathol Lab Med*, 133, 611-2.
- WANG, L., MCLEOD, H. L. & WEINSHILBOUM, R. M. 2011. Genomics and drug response. *N Engl J Med*, 364, 1144-53.
- WAPNIR IL, ANDERSON SJ, MAMOUNAS EP, GEYER CE JR, JEONG JH, TAN-CHIU E, FISHER B & N., W. 2006. Prognosis after ipsilateral breast tumor recurrence and locoregional recurrences in five National Surgical Adjuvant Breast and Bowel Project node-positive adjuvant breast cancer trials. *J Clin Oncol*. 2006 May 1, 24, 2028-37.
- WENNMALM, K., OSTMAN, A. & BERGH, J. 2009. Stromal signature identifies basal breast cancers. *Nat Med*, 15, 237-8; author reply 238.
- VERONESI, U., BOYLE, P., GOLDHIRSCH, A., ORECCHIA, R. & VIALE, G. 2005. Breast cancer. *Lancet*, 365, 1727-41.
- WHO. 2001. *Age Standardization of Rates: A new WHO standard* [Online]. WHO. [Accessed 2001].
- WILKING N, B JÖNSSON, D HÖGBERG & JUSTO, N. 2009. *Comparator Report on Patient Access to Cancer Drugs in Europe* [Online]. [Accessed].
- WILKING N & KASTENG, F. 2009. *A review of breast cancer care and outcomes in 18 countries in Europe, Asia, and Latin America* [Online]. [Accessed].
- WILKING, U., KARLSSON, E., SKOOG, L., HATSCHEK, T., LIDBRINK, E., ELMBERGER, G., JOHANSSON, H., LINDSTROM, L. & BERGH, J. 2011. HER2 status in a population-derived breast cancer cohort: discordances during tumor progression. *Breast Cancer Res Treat*, 125, 553-61.
- WILKING, U. J., B WILKING, N BERGH, J. Year. Uptake of aromatase inhibitors (AI) and trastuzumab (T) during the first 5 years after market introduction. A North American and European comparison. In: [HTTP://WWW.ASCO.ORG/PORTAL/SITE/ASCOV2/GSASEARCH](http://www.asco.org/portal/site/asco/2/gsearch), ed. ASCO, 2008.
- WINSTANLEY, J., COOKE, T., MURRAY, G. D., PLATT-HIGGINS, A., GEORGE, W. D., HOLT, S., MYSKOV, M., SPEDDING, A., BARRACLOUGH, B. R. & RUDLAND, P. S. 1991. The long term prognostic significance of c-erbB-2 in primary breast cancer. *Br J Cancer*, 63, 447-50.
- WIRAPATI, P., SOTIRIOU, C., KUNKEL, S., FARMER, P., PRADERVAND, S., HAIBEKAINS, B., DESMEDT, C., IGNATIADIS, M., SENGSTAG, T., SCHUTZ, F., GOLDSTEIN, D. R., PICCART, M. & DELORENZI, M. 2008. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res*, 10, R65.

- VOGEL, C. L., COBLEIGH, M. A., TRIPATHY, D., GUTHEIL, J. C., HARRIS, L. N., FEHRENBACHER, L., SLAMON, D. J., MURPHY, M., NOVOTNY, W. F., BURCHMORE, M., SHAK, S. & STEWART, S. J. 2001. First-line Herceptin monotherapy in metastatic breast cancer. *Oncology*, 61 Suppl 2, 37-42.
- WOLFF, A. C., HAMMOND, M. E., SCHWARTZ, J. N., HAGERTY, K. L., ALLRED, D. C., COTE, R. J., DOWSETT, M., FITZGIBBONS, P. L., HANNA, W. M., LANGER, A., MCSHANE, L. M., PAIK, S., PEGRAM, M. D., PEREZ, E. A., PRESS, M. F., RHODES, A., STURGEON, C., TAUBE, S. E., TUBBS, R., VANCE, G. H., VAN DE VIJVER, M., WHEELER, T. M. & HAYES, D. F. 2007. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*, 131, 18-43.
- VON MINCKWITZ, G., DU BOIS, A., SCHMIDT, M., MAASS, N., CUFER, T., DE JONGH, F. E., MAARTENSE, E., ZIELINSKI, C., KAUFMANN, M., BAUER, W., BAUMANN, K. H., CLEMENS, M. R., DUERR, R., ULEER, C., ANDERSSON, M., STEIN, R. C., NEKLJUDOVA, V. & LOIBL, S. 2009. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a german breast group 26/breast international group 03-05 study. *J Clin Oncol*, 27, 1999-2006.
- WOOD, E. R., TRUESDALE, A. T., MCDONALD, O. B., YUAN, D., HASSELL, A., DICKERSON, S. H., ELLIS, B., PENNISI, C., HORNE, E., LACKEY, K., ALLIGOOD, K. J., RUSNAK, D. W., GILMER, T. M. & SHEWCHUK, L. 2004. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res*, 64, 6652-9.
- WU, J. M., FACKLER, M. J., HALUSHKA, M. K., MOLAVI, D. W., TAYLOR, M. E., TEO, W. W., GRIFFIN, C., FETTING, J., DAVIDSON, N. E., DE MARZO, A. M., HICKS, J. L., CHITALE, D., LADANYI, M., SUKUMAR, S. & ARGANI, P. 2008. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. *Clin Cancer Res*, 14, 1938-46.
- YAZIJI, H., GOLDSTEIN, L. C., BARRY, T. S., WERLING, R., HWANG, H., ELLIS, G. K., GRALOW, J. R., LIVINGSTON, R. B. & GOWN, A. M. 2004. HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA*, 291, 1972-7.