Diplomarbeit

Outcome of breast cancer patients with immunohistochemistry HER2 score 2 and 3 at the Division of Oncology, Graz
HER2 diagnostical and clinical refinement

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Graz, am 11.08.2020 *Daniel Steiner eh.*

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List of Abbreviations

%	Percent
AJCC	American Joint Committee on Cancer
ASCO	American Society of Clinical Oncology
BRCA1 and 2	Breast related cancer antigen 1 and 2
CAP	College of American Pathologists
CDK4/6	Cyclin-dependent kinase 4/6
CEP17	Chromosome 17 centromere
CI	Confidence interval
DCIS	Ductal carcinoma in situ
DDFS	Distant disease-free survival
Fig.	Figure
FISH	Fluorescence in situ hybridization
HER2	Human epidermal growth factor receptor 2
HR	Hormone receptor
IDC	Invasive ductal carcinoma
iDFS	Invasive disease-free survival
IHC	Immunohistochemistry
ILC	Invasive lobular carcinoma
ISH	In situ hybridization
LCIS	Lobular carcinoma in situ
mTOR	Mechanistic target of rapamycin
n	Number
OS	Overall survival
PARP	Polyadenosine diphosphate-ribose polymerase
pCR	Pathological complete response
PD-1	Programmed death 1
PD-L1	Programmed death ligand 1
PI3	Phosphoinositide 3-kinase
REDCap	Research Electronic Data Capture
RFS	Recurrence-free survival
STEEP	Standardized definition for efficacy end points
T-DM1	Trastuzumab-emtansine
TNM	Tumor, node, metastasis

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Abstract

Background: Breast cancer is the most common malignancy in women and one of the leading causes of cancer death worldwide. HER2 expressing breast cancer can be targeted with several different established therapeutic options, therefore definition and assessment of HER2 positivity is of clinical relevance.

Materials and Methods: We conducted a single-center, retrospective data analysis at the Division of Oncology in Graz. Patients were eligible having invasive breast cancer with a HER2 IHC score of 2+ or 3+ and being diagnosed or treated for breast cancer at our institution between 2006 and 2016. Our aim was to assess the patient composition regarding molecular subtypes and treatment setting. Co-primary end point was clinical outcome in different subgroups. Secondary end point was to assess the number of patients affected by the change in ASCO/CAP guidelines regarding HER2 positivity and whether this element influenced therapeutic decisions and outcomes.

Results: Of 611 patients included, 353 had a score of 2+ and 258 of 3+, resulting in 343 HER2 positive, 264 HER2 negative, i.e. 230 luminal-like and 34 triple-negative subtypes, and 4 unknown cases. Of HER2 positive patients, 168 were in adjuvant, 142 in neoadjuvant, and 33 in palliative setting, whereas 155, 77, and 32 HER2 negative patients were in these settings, respectively. OS at 10 years in (neo)adjuvant setting was more than 75%, OS at 5 years in palliative setting was less than 25%. OS at 10 years for HER2 positive patients in (neo)adjuvant setting was significantly better when patients received HER2 targeted therapy (81.38% and 63.48%; $p<0.005$). Of 353 patients with a score of 2+, 12 patients had a HER2/CEP17 ratio between 2.0 and 2.2 and were therefore affected by the guideline change. Due to the low case number, the influence on therapeutic decisions and outcomes could not be determined.

Conclusion: Breast cancer patients with a HER2 IHC score of 2+ and 3+ are a diverse population in different settings. In our study, HER2 targeted therapy was a crucial component in treating HER2 positive patients, leading to significant changes in outcomes. The clinical importance of the change of the ASCO/CAP guidelines could not be determined.

Zusammenfassung

Hintergrund: Brustkrebs ist die häufigste Krebsform bei Frauen und eine der führenden Krebstodesursachen weltweit. Da HER2 positiver Brustkrebs mit unterschiedlichen etablierten Therapieoptionen behandelt werden kann, ist die Definition und die Erfassung von HER2 Positivität klinisch relevant.

Material und Methoden: Wir führten an der Klinischen Abteilung für Onkologie in Graz eine retrospektive Datenanalyse durch. Eingeschlossen wurden PatientInnen, welche an invasivem Brustkrebs mit einem HER2 IHC-Score von 2+ oder 3+ erkrankt waren und zwischen 2006 und 2016 an unserer Institution mit Brustkrebs diagnostiziert oder deshalb behandelt wurden. Unser Ziel war es, die Zusammensetzung dieser PatientInnen hinsichtlich der molekularen Subtypen und des Behandlungssettings zu beurteilen. Co-primärer Endpunkt waren klinische Outcome-Parameter in verschiedenen Untergruppen. Der sekundäre Endpunkt war die Erhebung der PatientInnenzahl, die von der Änderung der ASCO/CAP-Leitlinien bezüglich der HER2-Positivität betroffen war, und die Beurteilung, ob diese Änderung therapeutische Entscheidungen und Ergebnisse beeinflusste.

Ergebnisse: Von 611 PatientInnen, die in die Analyse einbezogen wurden, hatten 353 einen HER2 IHC-Score von 2+ und 258 von 3+, woraus sich 343 HER2-positive, 264 HER2-negative, d.h. 230 luminal-like und 34 triple-negative Subtypen, und 4 unbekannte Fälle ergaben. Von den HER2-positiven PatientInnen befanden sich 168 in adjuvantem, 142 in neoadjuvantem und 33 in palliativem Setting, während sich 155, 77 und 32 HER2-negative PatientInnen in ebendiesen Settings befanden. Die Gesamtüberlebensrate nach 10 Jahren in (neo)adjuvantem Setting betrug mehr als 75%, die Gesamtüberlebensrate nach 5 Jahren in palliativem Setting weniger als 25%. Die Gesamtüberlebensrate nach 10 Jahren für HER2-positive PatientInnen in (neo-)adjuvantem Setting war signifikant besser, wenn die PatientInnen eine gezielte HER2-Therapie erhielten (81,38% und 63,48%; $p<0,005$). Von 353 PatientInnen mit einem HER2 IHC-Score von 2+ wiesen 12 PatientInnen ein HER2/CEP17-Verhältnis zwischen 2,0 und 2,2 auf und waren daher von der Änderung der Leitlinien betroffen. Aufgrund der geringen Fallzahl konnte der Einfluss auf therapeutische Entscheidungen und Ergebnisse nicht ermittelt werden.

Fazit: BrustkrebspatientInnen mit einem HER2 IHC-Score von 2+ und 3+ stellen eine vielfältige Population in unterschiedlichen Settings dar. In unserer Studie war die zielgerichtete HER2-Therapie eine entscheidende Komponente bei der Behandlung HER2-positiver PatientInnen und führte zu einem signifikanten Überlebensvorteil. Die klinische Bedeutung der Änderung der ASCO/CAP-Leitlinien konnte nicht bestimmt werden.

1 Introduction

Breast carcinoma is a clonal disease which can exist for years as non-invasive or invasive but non-metastatic before being detected.[1] It emerges from epithelial cells of the human mammae, in most cases from the cells of the terminal duct lobular unit.[2]

1.1 Epidemiology

Breast cancer is the most common malignancy in women and one of the leading causes of cancer death worldwide.[3,4] In 2019, there will be 271,270 estimated new cases and 42,260 estimated deaths (numbers for both sexes) due to breast cancer in the US.[3] While the incidence remains relatively stable[3,5], mortality rates have declined considerably.[1]

1.2 Etiology

One main risk factor for breast cancer is age, as shown by a rising incidence with age but with a decreasing slope at the beginning of menopause. Hormonal factors, e.g. early menarche, late age at menopause, nulliparity or late age at first pregnancy, play a substantial role. Furthermore, hormone replacement therapy with combined estrogen and progesterone increases breast cancer risk.[1]

In postmenopausal women, increased body weight leads to a rise in incidence of breast cancer, whereas physical activity lowers the risk in this subgroup as well as in premenopausal women.[2] Another risk factor is alcohol, which shows a dose-response relationship, but increases breast cancer risk even at low levels of consumption.[6] Both active and passive smoking lead to a modest increase in risk.[7]

Apart from a seemingly multi-gene germline susceptibility which can explain family history as a moderate risk factor, properly inherited single nucleotide polymorphisms lead to a remarkably high lifetime risk of breast cancer. Breast related cancer antigen 1 and 2 (BRCA1 and 2) are the most relevant of these, leading to a substantially higher rate of breast cancer and ovarian cancer in affected patients.[1]

1.3 Localization

About half of all cases of breast cancer occur in the upper outer quadrant of the breast, while the lower inner quadrant is least frequently affected.[2]

1.4 Clinical Presentation

Breast cancer is difficult to distinguish from various types of benign breast disease by signs and symptoms alone. Common presentations include breast deformities, lumps which may be associated with pain, and nipple abnormalities, i.e. inversion, discharge, retraction, or eczema.[2,4,5] The introduction of mammography as a screening method has led to an increase in patients presenting asymptomatically.[2]

1.5 Histological Subtypes

Based on histology, there are several different subtypes of breast cancer: invasive ductal carcinoma (IDC) not otherwise specified and invasive lobular carcinoma (ILC) are the most common, while numerous other special types of breast cancer include: tubular carcinoma, mucinous carcinoma, signet-ring cell carcinoma, mucinous cystadenocarcinoma, carcinomas with neuroendocrine differentiation, invasive papillary carcinoma, invasive micropapillary carcinoma, apocrine carcinoma, secretory carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, sebaceous carcinoma, invasive cribriform carcinoma, medullary carcinoma, metaplastic carcinomas, clear cell carcinoma, lipid-rich carcinoma, metastatic carcinoma, and inflammatory carcinoma.[8] IDC, the largest subset, is a heterogenous group of tumors without the features of a specific subgroup.[2] One characteristic of ILC, which accounts for five to fifteen percent of all invasive breast cancers,[2] is the absence of cohesion within the cells, and about 50% of all cases show mutations in E-cadherin gene.[9] A diagnosis of inflammatory carcinoma is based on lymphangiosis carcinomatosa cutis,[8] but more commonly it is a clinical decision which describes the inflamed appearance of the skin.[9] Metastatic carcinoma does not involve a primary carcinoma of breast tissue, but metastases from a different primary tumor site to the breast.

1.6 Breast Biomarkers

1.6.1 Hormone Receptors

Estrogen is a key player in the regulation of growth and differentiation of breast tissue. Values of estrogen receptor together with progesterone receptor work as markers for the response to endocrine therapies.[2] These therapies can modulate the growth of breast cancer, slowing or even stopping the progression of hormone receptor positive tumors.[10] The American Society of Clinical Oncology (ASCO) and the College of American Pathologists

(CAP) have issued guidelines on hormone receptor testing in 2010, recommending assessments of estrogen and progesterone receptor values in every invasive breast cancer as well as in recurring carcinomas.[11]

1.6.2 Grade

As with other tumors, the grading of breast cancer builds on an assessment of formation of the tubules/glands, numbers of mitoses, and nuclear pleomorphism. A higher grade indicates poorer differentiation, with grade 1 representing good differentiation and grade 3 representing poor differentiation.[2]

1.6.3 Ki67

The expression of Ki67, a cell cycle regulated protein, forms the basis of proliferation scoring in pathology breast cancer assessments.[12] However, there is no standardized procedure for assessing and interpreting this marker, therefore its usefulness in clinical cancer care is strongly disputed.[12,13]

1.6.4 HER2

Human epidermal growth factor receptor 2 (HER2) is a well-studied oncogene in breast cancer which is associated with poor differentiation as well as a poor prognosis.[10] Furthermore, past reports have shown an association between HER2 positivity and metastases in lymph nodes, high rates of recurrence, and decreased responses to several systemic endocrine therapies and chemotherapies.[2,14] Multiple different pathways could lead to the involvement of this oncogene in the control of cell growth, differentiation, and survival.[15] Overexpression of HER2 can be assessed with immunohistochemistry[2,4] (IHC) and amplification can be assessed with fluorescence in situ hybridization (FISH), both of which show a correlation.[2] ASCO and CAP first issued guidelines on HER2 testing in 2006[14,16], with updates in 2013[17,18] and 2018[19,20]. These recommendations define an IHC score of 3+ as positive, indicating that more than 30% of invasive tumor cells show intense membrane staining; an IHC score of 2+ as equivocal, entailing a recommendation to perform a subsequent assay for HER2 gene amplification; and an IHC score of 1+ and 0 as negative.[14] Regarding HER2 gene amplification, there are differences between the first guideline and the updates.

In 2006, HER2 positivity tested via gene amplification was defined as a HER2/chromosome 17 centromere (CEP17) ratio of more than 2.2 or a HER2 gene copy number above 6.0, while a ratio of less than 1.8 or a gene copy number below 4.0 was defined as negative. The values in-between were characterized as equivocal, with the recommendation to recount, retest, or test with IHC.[14]

In 2013, the cut-off for positivity assessed via HER2/CEP17 ratio was changed from 2.2 to 2.0. Furthermore, specimens with a ratio of 2.0 or more and an average HER2 copy number of less than 4.0 signals per cell in a dual probe in situ hybridization (ISH) were defined as positive. In the case of equivocal results in a dual probe ISH, i.e. a ratio of less than 2.0 and a number between 4.0 and 6.0 signals per cell, recommendations included a reflex test with the same specimen using IHC, a test with an alternative ISH chromosome 17 probe, or a new test with new specimen.[17]

In the latest update published in 2018, the diagnostic procedure is as follows: IHC values of 0 and 1+ are considered negative, 3+ is considered positive, and 2+ is considered equivocal, requiring a reflex test with the same specimen using ISH or a test with new specimen using either IHC or ISH. A single probe ISH with an average HER2 copy number of 6.0 signals per cell or higher is positive; a number of less than 4.0 signals per cell is negative; and a number between 4.0 and 6.0 signals per cell is equivocal. Equivocal values with a concurrent IHC score of 3+ result in positivity, while scores of 1+ and 0 result in negativity, and a concurrent score of 2+ requires dual probe ISH. The latter splits specimens into five groups:[19]

- Group 1: a HER2/CEP 17 ratio of 2.0 or more and an average HER2 copy number of 4.0 or more per cell: HER2 positive
- Group 2: a ratio of 2.0 or more and a number of less than 4.0 signals per cell: requiring additional testing
- Group 3: a ratio of 2.0 or less and a number of 6.0 or more signals per cell: requiring additional testing
- Group 4: a ratio of 2.0 or less and a number between 4.0 and 6.0 signals per cell: requiring additional testing
- Group 5: a ratio of 2.0 or less and a number of less than 4.0 signals per cell: HER2 negative

Since several different established therapeutic options exist which target HER2,[21] the definition and assessment of HER2 positivity is of clinical relevance.

1.7 Classification

Breast carcinoma can be distinguished in non-invasive carcinoma in situ and invasive breast cancer.

1.7.1 Carcinoma in Situ

1.7.1.1 Ductal Carcinoma in Situ

Ductal carcinoma in situ (DCIS) is a term for breast epithelial cells which possess cytological characteristics of malignancy but proliferate in a non-invasive way, i.e. within the ducts without infiltrating the basal membrane. Although untreated DCIS progresses to invasive cancer in many cases, there are no reliable markers to distinguish this from non-progressive lesions, with the result that certain patients are at risk of being overtreated.[1]

1.7.1.2 Lobular Carcinoma in Situ

Lobular carcinoma in situ (LCIS), also termed lobular neoplasia, is the counterpart to DCIS in the lobules. In contrast to DCIS, LCIS is not considered a malignant lesion with the potential for progression in itself, but is rather a premalignant condition which leads to an increase in breast cancer risk ipsilateral as well as contralateral.[1]

1.7.2 Invasive Disease

The following classification is a clinically defined, treatment related division of subtypes of invasive breast cancer based on breast biomarkers. The different subtypes vary substantially in terms of their response to therapy, patterns of metastases, and genomic profile as well as with regard to other factors.[10]

1.7.2.1 Luminal-Like

The luminal like subtype can be divided into two categories – luminal A-like and luminal B-like - both of which are HER2 negative. While the luminal A-like subtype is characterized by high expression of hormone receptors and low proliferation rate, the luminal B-like has lower hormone receptor levels and a high proliferation rate. Furthermore, luminal A-like is generally histological grade 1 or 2, whereas luminal B-like is generally histological grade 3. All of these factors result in a favorable and an unfavorable prognosis, respectively.[10]

1.7.2.2 HER2-Like

Both subgroups of HER2-like subtype are defined as HER2 positive and are mostly histological grade 3, but one has high levels of hormone receptors and the other is hormone receptor-negative.[10]

1.7.2.3 Basal-Like

Basal-like is a subtype with negative hormone receptors and an absence of HER2 expression. Additionally, the basal-like subtype is usually associated with higher grading.[10] Although triple-negative breast cancer is similar to basal-like subtype due to negative hormone receptors and a lack of HER2 expression, the two types of breast cancer remain distinct.

1.8 TNM Staging

As in other carcinomas, the extent of breast carcinoma is defined by tumor, node, metastasis (TNM) staging. T denotes primary tumor size, N describes infiltration of regional lymph nodes, and M stands for presence of distant metastases. Different prefixes, for example c, p, and yp, define the circumstances of TNM staging, i.e. clinical, pathological, and pathological following neoadjuvant therapy, respectively. TNM staging taken together with grading, hormone receptors, and HER2 status allows for a classification of patients in clinical prognostic stage groups and pathological prognostic stage groups. The first group applies to all patients with breast cancer, the second to patients who have been treated with surgery as initial treatment.[10]

1.9 Metastases

Apart from spreading in local surroundings such as the breast parenchyma, nipple, fascia, muscle, and chest wall, breast cancer can also disseminate via the lymphatic system and blood vessels.[9] Invasion of regional lymph nodes, i.e. ipsilateral axillary, ipsilateral supraclavicular, and ipsilateral internal mammary (Fig. 1), has to be distinguished from invasion of any other lymph nodes, such as cervical, contralateral internal mammary, or contralateral axillary. Spread to the latter is classified as occurrence of distant metastases.[10] Common metastatic sites caused by blood spread include the skeletal system, lungs, liver, spleen, ovary, adrenal glands, and central nervous system, as well as a tendency for the gastrointestinal tract, ovary, and serosal surfaces in invasive lobular carcinoma.[9] Diagnostical work-up to detect metastases after primary diagnosis, if indicated, includes thoracic and abdominal computed tomography and scintigraphy of the skeletal system.

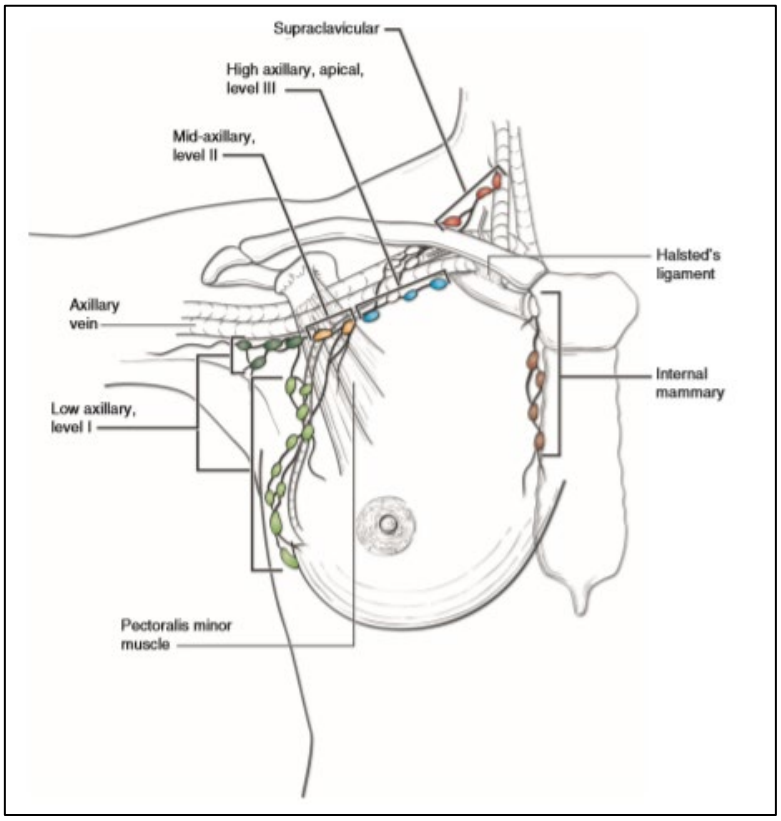

Figure 1. *Schematic diagram of the breast and regional lymph nodes. Used with the permission of the American College of Surgeons. Amin, M.B., Edge, S.B., Greene, F.L., et al. (Eds.) AJCC Cancer Staging Manual, 8th Ed. Springer New York, 2017.*

1.10 Diagnosis

The diagnosis of breast cancer involves reviewing the patient's history, carrying out a physical examination, using imaging techniques, and performing a biopsy. Taking the patient's history serves to evaluate risk factors, family history, and personal history.[4] Some features of palpable breast masses, such as the absence of pain, irregularity, fixation to the chest wall, and hard appearance, indicate invasivity.[1] However, about a fourth of all invasive cancers is not palpable, thus physical examination and imaging methods have to be considered simultaneously.[5] Imaging methods include mammography, magnetic resonance mammography, breast ultrasound, and other methods such as single photon emission computed tomography and positron emission tomography, with the latter two playing a minor role in breast cancer diagnosis.

Especially in younger women, breast ultrasound and magnetic resonance imaging are important due to higher sensitivity in dense tissue as well as the absence of the risks of

ionizing radiation. However, because of low specificity, the latter should only be considered in high risk groups or as additional investigation.[22]

Mammography as a screening tool has been a matter of controversial discussions. As shown in a review by Gøtzsche PC and Jørgensen KJ[23], the resulting reduced breast cancer mortality rates are counterbalanced by increased levels of overdiagnosis, which causes unnecessary treatment and psychological distress.

To make an ultimate diagnosis of the type of breast cancer and guide treatment decision regarding breast cancer biomarkers, a biopsy is essential. Methods include core needle biopsy, fine needle aspiration, and open surgical biopsy, guided by ultrasound or mammography.[5]

1.11 Treatment

Treatments for breast cancer can principally be divided into curative and palliative therapy, with the purpose to cure the disease or to palliate symptoms and prevent further disease progression into advanced disease, respectively. Curative therapy includes neoadjuvant therapy, i.e. therapy before surgery, adjuvant therapy, i.e. therapy after surgery, surgical intervention at the primary site and regional lymph nodes, and radiation therapy. Palliative therapy consists of pharmacologic therapy, radiotherapy, and surgical intervention both at the primary site and at metastatic sites.

The following chapters will shortly describe different aspects of both curative and palliative therapy with a special focus on HER2-targeted therapy. Surgical intervention at metastatic sites as well as neoadjuvant, intraoperative, and palliative radiotherapy will not be discussed further.

1.11.1 Primary Tumor Surgery

The surgical approach aims to preserve breast tissue, i.e. breast conserving surgery, or remove it, i.e. different types of mastectomy. Breast conserving therapy refers to a combination of breast conserving surgery and radiation therapy. Contraindications for breast conserving therapy include inflammatory carcinoma, most cases of multicentric disease, contraindications for radiation therapy, e.g. prior radiation therapy or pregnancy, positive margins after several attempts at resection, patient decision, and ineligibility due to tumor size in relation to breast size.[24]

It is not only the size of the tumor which influences the decisions as to whether to undertake breast conserving surgery or a mastectomy, but also the anticipated cosmetic result as well

as the ability to achieve margin control and undergo adjuvant radiation.[25] Furthermore, neoadjuvant therapy can decrease tumor size in locally advanced disease and increase eligibility for breast conserving strategies.[26,27] Consequently, a large primary tumor itself is no absolute contraindication for breast conserving therapy.

Margins of resection play a substantial role in breast-conserving surgery, with negative margins resulting in low rates of local recurrence,[28,29] whereas positive margins result in higher rates.[28–31]

Breast conserving therapy in inflammatory breast cancer is highly controversial, but an inflammatory component does not seem to be an absolute contraindication.[32,33]

Different types of mastectomy include radical mastectomy, modified radical mastectomy, simple mastectomy, skin-sparing mastectomy, and nipple-areolar sparing mastectomy. The choice of the type of mastectomy must be made individually for every patient.[24]

In patients with mutations in BRCA1 or BRCA2 gene, mastectomy is used as a prophylaxis for breast cancer.[34,35]

Apart from primary tumor surgery, surgical management includes the management of axillary lymph nodes. Clinically positive axilla, which is, for example, confirmed by preoperative sonography, is treated with axillary dissection, whereas clinically negative axilla leads to sentinel node biopsy, which may result in axillary dissection in case of a positive sentinel node.[24]

1.11.2 Adjuvant Radiotherapy

As shown in a meta-analysis by the Early Breast Cancer Trialists' Collaborative Group, whole-breast radiation therapy after breast conserving therapy reduces both the recurrence rate and the risk of death from breast.[36] Similarly, postmastectomy radiation therapy leads to a decrease in the locoregional recurrence rate and increased survival rates in specific patient populations.[37–39] Locoregional lymph nodes can also be irradiated.[24] Several specific topics - such as different types of schedules, i.e. conventional and hypofractionated, radiation therapy boost to the tumor bed, and accelerated partial-breast irradiation - will not be discussed here.

1.11.3 Neoadjuvant Pharmacologic Therapy

Neoadjuvant therapy refers to therapy given prior to surgery. Apart from allowing for early evaluation of the effectiveness of systemic therapy, it is used to downstage locally advanced tumors and therefore increase rates of breast conserving therapy as well as decrease rates of

axillary dissection and their subsequent complications such as lymphedema.[40,41] Pathological complete response (pCR) at surgery after neoadjuvant therapy results in significantly higher rates of overall survival and disease free survival compared to patients with residual invasive disease.[42–44]

1.11.3.1 Neoadjuvant HER2 Negative Patients

Common chemotherapy regimens for patients with HER2 negative breast cancer can be anthracycline-based or anthracycline-free, such as doxorubicin plus cyclophosphamide with a taxane (e.g. docetaxel)[45] or docetaxel and cyclophosphamide[46], respectively. A decision should be made based on both risk setting and comorbidities, such as cardiac diseases. In triple-negative breast cancer, the addition of carboplatin to neoadjuvant regimens has been shown to improve rates of pCR.[47,48] Since adverse events are more common and there is no strong data suggesting a benefit in event free and overall survival, its use remains controversial. However, in recent years, it has become a standard for high risk higher stage triple negative breast cancer to include carboplatin in the neoadjuvant treatment and thereby improve outcome, with few trials showing benefit. Based on these trials, Sankt Gallen consensus meeting has implemented the use of carboplatin in the neoadjuvant setting.[49]

1.11.3.2 Neoadjuvant Hormone Receptor Positive Patients

Neoadjuvant endocrine therapy is a possible approach in patients who have high expression of hormone receptors and for whom chemotherapy is not suitable. In a phase II study from Spain, premenopausal women showed worse response rates with neoadjuvant endocrine therapy than with chemotherapy.[50] In postmenopausal women with hormone receptor positive breast cancer, studies showed similar rates of response and possibility to undergo breast conserving surgery in patients treated with neoadjuvant endocrine therapy compared to chemotherapy.[50–53] Therefore, neoadjuvant endocrine therapy poses a possible approach in HER2 negative, strongly hormone receptor positive, postmenopausal breast cancer patients.

1.11.3.3 Neoadjuvant HER2 Positive Patients

In patients with HER2 positive breast cancer who have an indication for neoadjuvant therapy, HER2-targeted therapy is a mainstay of treatment as it increases pCR. Consequently, the combination of HER2-targeted therapy and chemotherapy improves long term outcome measures compared to chemotherapy alone.[42,54,55] Agents targeting HER2

include the humanized monoclonal antibody trastuzumab as well as pertuzumab, lapatinib, neratinib, and trastuzumab-emtansine (T-DM1).[56] Using trastuzumab in a neoadjuvant setting is well established.[42,54,55] Adding pertuzumab to a combination of docetaxel and trastuzumab has been shown to improve pCR rates compared to using only docetaxel and trastuzumab, without substantially increasing adverse events, apart from diarrhea.[57] Other approaches include lapatinib or neratinib by themselves or in combination with trastuzumab, subcutaneous trastuzumab, T-DM1 by itself or in combination with trastuzumab, and trastuzumab biosimilars.

Apart from HER2-targeted therapy, chemotherapy still plays a substantial role in HER2 positive breast cancer patients in the neoadjuvant setting. Various regimens, which may or may not be anthracycline-based, are possible. One of them consists of fluoruracil, epirubucin, and cyclophosphamide administered every three weeks for four cycles followed by paclitaxel weekly in combination with trastuzumab for 12 cycles, resulting in a pCR rate of about 55%[58]. Another regimen consists of doxorubicin and cyclophosphamide every three weeks for four cycles followed by paclitaxel plus trastuzumab weekly, resulting in a similar pCR rate.[59] A trial comparing an anthracycline-based, dual HER2 blockade regimen to nine cycles of paclitaxel and carboplatin every three weeks with concurrent trastuzumab and pertuzumab showed similar pCR rates in both groups, however, febrile neutropenia occurred more frequently in the anthracycline-group.[60] Regarding taxanes, docetaxel poses an alternative for paclitaxel and nabpaclitaxel can be administered in case of contraindication for standard paclitaxel, such as allergic reactions.

1.11.4 Adjuvant Pharmacologic Therapy

Adjuvant therapy represents therapy administered after surgery and includes chemotherapy, endocrine therapy, and other types such of therapy as HER2-targeted therapy. It aims at preventing local recurrence and the development of metastases and therefore reducing cancer-specific mortality.

1.11.4.1 Adjuvant Chemotherapy

The choice to administer adjuvant chemotherapy is based on a risk assessment and an evaluation of expected benefits and the potential adverse events and toxicity caused by the therapy. Apart from taking hormone receptor and HER2 expression into account, there are also various biomarker assays to guide clinical decision-making, such as the Breast Cancer Index® or EndoPredict®.[61] Chemotherapeutic regimens can vary by country, hospital, and

attending physician. A regimen based on anthracycline and taxane, such as doxorubicin and cyclophosphamide followed by paclitaxel, is an approach that is chosen often, because it reduces recurrence risk, breast cancer mortality, and overall mortality compared to other regimens.[62] Paclitaxel administered weekly is superior regarding disease free and overall survival compared to paclitaxel every three weeks.[63] In lower risk settings as well as in the context of comorbidities, a combination of docetaxel and cyclophosphamide administered every three weeks for four cycles poses an alternative to anthracycline-based regimens.[64,65] The interval times between cycles should be shortened, which is referred to as dose-dense and results in prolonged disease free and overall survival without increasing adverse events due to the therapy.[66,67]

1.11.4.2 Adjuvant Endocrine Therapy

In hormone receptor positive patients, the choice of adjuvant endocrine therapy depends largely on the menopausal status.

In postmenopausal women, aromatase inhibitors such as letrozole, anastrozole, and exemestane are the preferred therapy, because they have shown lower recurrence rates during treatment and lower 10-year breast cancer mortality rates than tamoxifen.[68] In case of short term treatment-related adverse events, i.e. especially musculoskeletal symptoms[69–71], switching to a different aromatase inhibitor is a possible approach.[72] Other adverse events include sexual dysfunction[73] as well as cognitive problems, hot flashes, declining physical health scores[74], and bone loss due to estrogen deficiency[75]. Common approaches in targeting bone loss include calcium and vitamin D supplementation as well as pharmacological therapy, i.e. bisphosphonates and denosumab. In patients who are for any reason not eligible for aromatase inhibitors, tamoxifen is the preferred therapy.[76] Duration of treatment is a minimum of five years, however, for high risk patients also an extended duration has been evaluated and has become partly established over years.

In premenopausal women, approaches include ovarian function suppression combined with endocrine therapy and endocrine therapy alone. Ovarian function suppression can be achieved pharmacologically, i.e. with gonadotrophin-releasing hormone agonists such as goserelin, or non-pharmacologically, i.e. with oophorectomy and ovarian irradiation. In patients who were at high risk for recurrence, ovarian function suppression plus exemestane showed improved disease outcomes, but patients presented with adverse events such as musculoskeletal symptoms, hot flashes, and osteoporosis.[77–79] In lower risk settings, tamoxifen is a reasonable choice.[76]

1.11.4.3 Adjuvant HER2-targeted therapy

Adding trastuzumab to adjuvant chemotherapy in HER2 positive patients significantly increases overall survival and disease-free survival rates.[80] The duration of treatment is one year, since an extension to two years showed no additional benefit.[81] Dual HER2 blockade in high-risk patients, for example with trastuzumab and pertuzumab, increases disease-free survival rates, as has been shown in the phase III APHINITY trial.[82] In patients with residual invasive disease after neoadjuvant therapy containing a taxane (with or without anthracycline), T-DM1 has been shown to reduce the risk of recurrent disease or death.[83] Cardiatoxicity, i.e. decline in left ventricular ejection fraction, congestive heart failure, and cardiomyopathy, is a potential adverse event associated with adjuvant HER2-targeted therapy[80,84] and therefore cardiac function has to be closely monitored.

1.11.5 Palliative Pharmacologic Therapy

As soon as metastases occur in breast cancer, the disease is unlikely to be cured and the patient is in a palliative setting. Some patients are in a palliative setting due to locally advanced disease, comorbidities, and/or age, also meaning that it is unlikely that the disease will be cured with feasible therapies. The goal of palliative therapy is to delay disease progression, palliate tumor-associated symptoms, and preserve and restore quality of life.
Median survival in metastatic breast cancer has improved from 1990 to 2010 in recurrent as well as in de-novo stage IV disease.[85]

1.11.5.1 Palliative HER2 Positive Patients

In metastatic, HER2 positive breast cancer, HER2-directed therapy is a mainstay of treatment, regardless of hormone receptor status. As has been shown in the CLEOPATRA trial, a dual HER2 blockade with trastuzumab and pertuzumab plus docetaxel is superior to trastuzumab and placebo plus docetaxel in terms of overall survival, progression-free survival, and duration of response.[86,87] The PERUSE trial suggests that docetaxel can be replaced with paclitaxel, which results in different rates of adverse events, i.e. more neuropathy but less febrile neutropenia and mucositis.[88] In patients with hormone receptor positive disease, chemotherapy is typically followed by endocrine therapy. Alternatively, first line HER2 blockade plus endocrine therapy - such as lapatinib plus letrozole,[89] or trastuzumab plus pertuzumab plus an aromatase inhibitor[90] - poses a possible approach in this setting. In a subset of patients who had been previously treated with trastuzumab and a

taxane, T-DM1 led to longer overall and progression-free survival rates, while resulting in fewer adverse events compared to lapatinib plus capecitabine.[91]

There are various approaches to targeting progressive disease in the context of HER2 positive, metastatic breast cancer, the description of which would exceed the limitations of this thesis. Therefore, only trastuzumab deruxtecan and tucatinib will be discussed briefly here. Trastuzumab deruxtecan is an antibody-drug conjugate, which showed durable antitumor activity in an open-label, phase 2 study involving patients with a median of six previous treatments. Adverse events included nausea, myelosuppression, and interstitial lung disease.[92] In the HER2CLIMB trial, the HER2 tyrosine kinase inhibitor tucatinib showed superior overall and progression-free survival in combination with trastuzumab and capecitabine compared to placebo in heavily pretreated patients. The antitumor activity in a subset of patients with brain metastases is especially notable. In this patient population, the progression-free survival at 1 year was 24.9% with tucatinib plus trastuzumab and capecitabine compared to 0% with placebo plus trastuzumab and capecitabine, and the median progression-free survival was 7.5 and 5.4 months, respectively.[93]

1.11.5.2 Palliative Triple-Negative Patients

Since neither HER2-targeted therapy nor endocrine therapy are viable options in triple-negative breast cancer patients, chemotherapy plays a substantial role. The choice of chemotherapy is driven by tumor burden, health status, prior treatment and toxicities, and patient preferences. Therapy can consist of one single agent, such as paclitaxel weekly,[94] or a combination of different agents, such as doxorubicin plus paclitaxel.[95] In a phase III trial, the latter resulted in superior response rates and an increase in the time until treatment failure compared to doxorubicin or paclitaxel alone, but did not improve survival.[95] The possibility of enhancing response rates with polychemotherapeutic regimens has to be balanced with the possibility of a higher rate of adverse events compared to single agent therapy.

1.11.5.3 Palliative Hormone Receptor Positive Patients

In hormone receptor positive, HER2 negative breast cancer patients, therapeutic approaches consist of endocrine therapy, targeted therapy, and chemotherapy. Targeted therapies include cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, such as palbociclib and ribociclib; phosphoinositide 3-kinase (PI3) inhibitors, such as alpelisib; and mechanistic target of rapamycin (mTOR) inhibitors, such as everolimus. PI3 inhibitors are used in the case of phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha mutations. A

systematic review and network meta-analysis has shown the superiority of targeted therapy plus hormone therapy compared to anastrozole alone as well as the superiority of CDK 4/6 inhibitors plus hormone therapy compared to chemotherapy with or without targeted therapy in terms of progression-free survival in the first-line or second-line setting.[96] In terms of hormone therapy, possible options include aromatase inhibitors, tamoxifen, fulvestrant, and gonadotropin-realeasing hormone agonists, with menopausal status being the deciding factor. In the case of progressive disease, chemotherapy should be considered.[24]

1.11.5.4 BRCA Mutated Palliative Patients

In patients with germline BRCA mutation and advanced breast cancer, polyadenosine diphosphate-ribose polymerase (PARP) inhibitors as a single agent are a therapeutic approach, since both talazoparib and olaparib resulted in longer progression-free survival times compared to standard therapy.[97,98]

1.11.6 Immunotherapy in Breast Cancer

Although immunotherapy has already changed the treatment of certain types of cancer substantially, breast cancer patients do not yet seem to benefit significantly from these developments. The most promising data come from triple-negative breast cancer trials. In the IMPassion 130 trial, the combination of nab-paclitaxel with atezolizumab, a humanized monoclonal programmed death ligand 1 (PD-L1) antibody, in patients with previously untreated, metastatic, triple-negative breast cancer led to prolonged progression-free survival in both the intention-to-treat population and the PD-L1 positive subgroup compared to nab-paclitaxel plus placebo.[99] This trial led to approval of this combination for metastatic or unresectable locally advanced, PD-L1 expressing, triple-negative breast cancer by the US Food and Drugs Administration. Other trials are investigating immunotherapy in early triple-negative breast cancer patients. In a first interim analysis of the KEYNOTE-522 trial, the addition of pembrolizumab, a humanized, monoclonal PD-1 antibody, to neoadjuvant chemotherapy with paclitaxel and carboplatin in previously untreated stage II or stage III triple-negative breast cancer patients led to a significantly higher proportion of patients with a pathological complete response compared to placebo plus chemotherapy.[100]

These results give rise to hope for better treatment options in triple-negative breast cancer patients. More clinical trials addressing other subtypes of breast cancer are warranted.

2 Materials and Methods

2.1 Trial Design

We conducted a single-center, retrospective data analysis at the Department of Internal Medicine, Division of Oncology at the Medical University Graz, Austria. Ethics committee of the Medical University Graz approved the study (ethics committee vote number 31-212 ex 18/19). Data collection was carried out with the electronic health records database Medocs, which captures data from public hospitals of the Steiermärkische Krankenanstaltengesellschaft m.b.H., and Oracle, an intern database of the Division of Oncology at the State Hospital in Graz. Data was recorded with the web application Research Electronic Data Capture (REDCap) and queries consisted of demographics; primary tumor data, i.e. histological subtype, grading, hormone receptors, HER2 IHC score, and HER2 FISH results, if applicable; therapy, i.e. surgery, neoadjuvant therapy, and adjuvant therapy; and events, i.e. local recurrence, distant recurrence, second breast carcinoma, second non-mammary carcinoma, and death.

2.2 Patients

Patients were eligible if they had received a diagnosis of invasive breast cancer with a HER2 IHC score of 2+ or 3+ confirmed either by histological examination of biopsy specimen if placed in neoadjuvant or palliative setting, or by excision specimen of the primary tumor if placed in adjuvant setting. Furthermore, patients were included if diagnosed with - or treated for - breast cancer at the Medical University Graz between 2006 and 2016, allowing for sufficient follow-up time, defined by a patient identification number between 20060000 and 20169999. Exclusion criteria consisted of non-invasive breast cancer, e.g. ductal carcinoma in situ, and cases where the patients had never personally attended an appointment at the Division of Oncology. Patients to be included were selected from the total number of 3300 breast cancer patients identified at the Division of Oncology, Department of Internal medicine between 2006 and 2018.

2.3 End Points

The aim of this thesis was to assess the composition of patients with a HER2 IHC score of 2+ or 3+ in terms of molecular subtypes and treatment setting. The co-primary end point was the clinical outcome, i.e. overall survival (OS), invasive disease-free survival (iDFS), distant disease-free survival (DDFS), and recurrence-free survival (RFS), in the total study

population, in (neo)adjuvant setting, in HER2 positive patients stratified by receipt of HER2 targeted therapy, by HER2 IHC score, and by hormone receptor status, and in HER2 negative patients stratified by hormone receptor status. End points were defined according to the standardized definition for efficacy end points (STEEP) criteria, i.e. time from registration to death from breast cancer, death from a non-breast cancer cause, or death from unknown cause for OS; time from registration to first event, which includes invasive ipsilateral breast tumor recurrence, local or regional invasive recurrence, distant recurrence, death from breast cancer, death from a non-breast cancer cause, death from unknown cause, invasive contralateral breast cancer, or second nonbreast primary invasive cancer for iDFS; time from registration to either distant recurrence, death from breast cancer, death from a non-breast cancer cause, death from unknown cause, or second nonbreast primary invasive cancer for DDFS; and time from registration to either invasive ipsilateral breast tumor recurrence, local or regional invasive recurrence, distant recurrence, death from breast cancer, death from a non-breast cancer cause, or death from unknown cause for RFS.[101] Our secondary end point was to assess how many patients in which subgroups were affected by the change in guidelines regarding HER2 positivity in FISH testing and whether this element influenced therapeutic decision and led to measurable changes in outcome.

2.4 Statistical Analysis

All statistical analyses were performed with Stata 16.1 (Stata Corp., Houston, TX, USA). Continuous variables were reported as medians [25th-75th percentile], and count data as absolute frequencies (%). The distribution of continuous variables between two groups was compared with rank sum-tests, whereas the association between two categorical variables was investigated with Pearson Chi-square and Fisher's exact tests. Median follow-up was estimated with the reverse Kaplan-Meier method according to Schemper & Smith.[102] Follow-up data were truncated at 10 years. Kaplan-Meier estimates were used to compute clinical outcomes, i.e. OS, iDFS, DDFS, and RFS, and survival functions between two or more groups were compared with log-rank tests.

3 Results

3.1 Patients and Demographics

Of the 3300 patients identified as breast cancer patients at the Division of Oncology between 2006 and 2018, 2794 were selected for assessment of eligibility due to their identification numbers between 20060000 and 20169999. Of these, 2014 were excluded based on their HER2 IHC score of 0 and 1+. Of the remaining 780 patients, 44 did not attend their appointment or declined therapy, 9 had non-invasive breast cancer, 6 were treated in Graz because of a different carcinoma and presented with breast cancer as a comorbidity, and 9 had missing data. Another 101 patients were excluded because of a HER2 IHC score of 2+ or 3+ only in an excision specimen while being in a neoadjuvant or palliative setting, or because of a HER2 IHC score of 2+ or 3+ only in a biopsy specimen while being in an adjuvant setting. The final cohort consisted of 611 patients, of which 264 (43.21%) were HER2 negative, 343 (56.14%) HER2 positive, and four (0.65%) had unknown HER2 status. The HER2 positive group encompassed 258 patients with a HER2 IHC score of 3+ and 85 patients with a HER2 IHC score of 2+ and positive FISH results, i.e. a HER2/CEP17 ratio of 2.0 and more in FISH. (Fig. 2)

Of the 546 patients who were treated with a curative intention, 310 (56.78%) were HER2 positive, 232 (42.49%) were HER2 negative, and 4 had unknown HER2 status. The first group encompassed 142 (45.81%) neoadjuvant and 168 (54.19%) adjuvant cases, while the HER2 negative group encompassed 77 (33.18%) neoadjuvant and 155 (67.10%) adjuvant cases. Three patients in the HER2 negative, adjuvant group were male (0.55% of all patients treated in curative intention, 1.29% of HER2 negative patients). The median age at diagnosis was 58.0 years [25^{th} to 75^{th} percentiles: 49.2 – 67.9] in the HER2 positive group and 60.3 years [25^{th} to 75^{th} percentiles: 50.1 – 69.1] in the HER2 negative group. (Table 1)

Of the 65 patients who were placed in palliative setting, 49, i.e. 8.02% of the total cohort, were primary metastatic at diagnosis. The remaining 16 patients did not have metastases and were in a palliative setting due to comorbidities and locally advanced disease, or unspecified reason. The palliative group was evenly divided between HER2 positive patients (33 cases, 50.77%) and HER2 negative patients (32 cases, 49.23%).

3.2 Primary Tumor Data

Histological data of primary tumor was either assessed by biopsy in neoadjuvant setting or by primary tumor surgery in adjuvant setting. Palliative cases are not described.

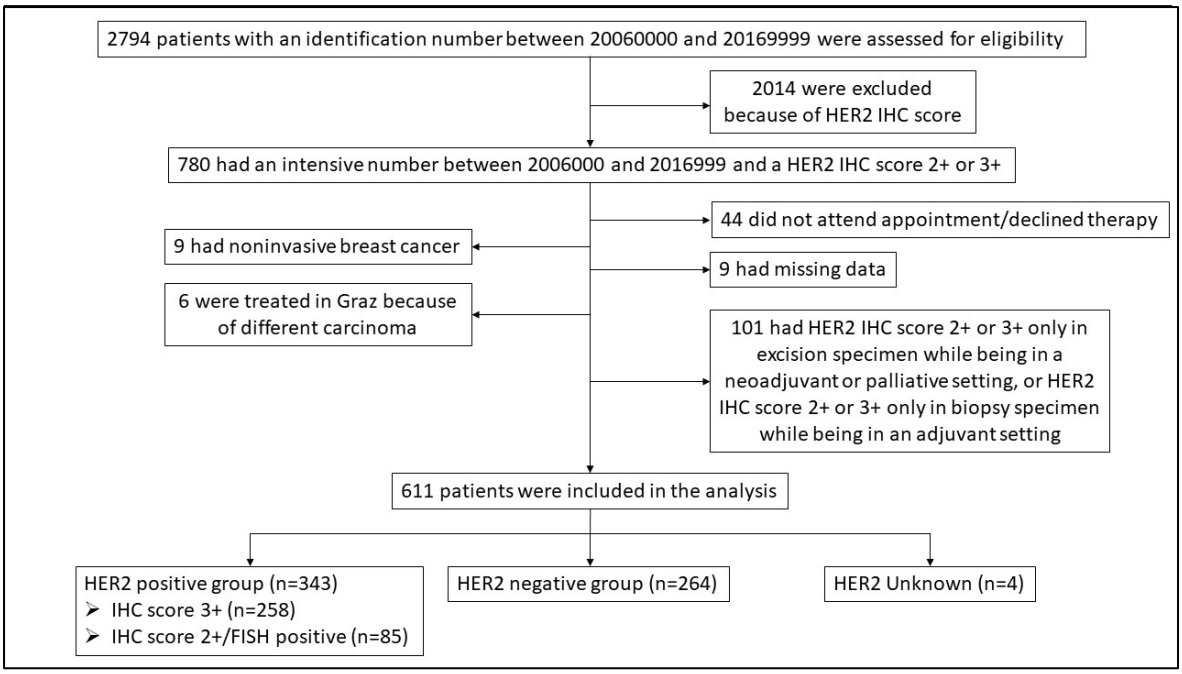

Figure 2. *Consort diagram of patient enrollment.*

The HER2 positive group (n=310) encompassed 233 (75.16%) patients with a HER2 IHC score of 3+, and 77 (24.84%) patients with a HER2 IHC score of 2+ and positive FISH results, i.e. a HER2/CEP17 ratio of 2.0 and above. Histological subtypes included IDC not otherwise specified in 261 (84.19%) cases; mixed IDC and ILC in 18 (5.81%); ILC in 12 (3.87%); mucinous carcinoma in 5 (1.61%); micropapillary carcinoma in 4 (1.29%); and other histological subtypes in 10 (3.23%). The most common gradings were G3 in 183 (59.03%) cases and G2 in 118 (38.06%) cases. In terms of hormone receptor status, 221 (71.29%) patients presented with positive hormone receptors, of which 160 had positive estrogen and progesterone receptors, while 61 had either of the two. In 89 (28.71%) patients, hormone receptors were not expressed. (Table 1)

In the HER2 negative group (n=232), 186 (80.17%) patients had IDC not otherwise specified; 20 (8.62%) had ILC; 7 (3.02%) had mixed IDC and ILC; 7 (3.02%) had mucinous carcinoma; 3 (1.29%) had micropapillary carcinoma; and 9 (3.88%) had a different histological subtype. The most common gradings were G2 in 130 (56.03%) cases and G3 in 81 (34.91%) cases. In terms of hormone receptor status, 202 (87.07%) patients presented with positive hormone receptors, of which 178 had positive estrogen and progesterone receptors, while 24 had either of the two. Triple negative disease was present in 30 (12.93%) patients. (Table 1)

Table 1. Demographics, Treatment Setting, and Primary Tumor Data of Patients Treated with a Curative Intention (n=546)*		
	HER2 Positive (n=310)	**HER2 Negative (n=232)**
Demographics		
Age		
Median (25th to 75th percentiles) – yr	58.0 (49.2-67.9)	60.3 (50.1-69.1)
Sex – no (%)		
Female	310 (100)	229 (98.71)
Male	0 (0)	3 (1.29)
Treatment setting – no (%)		
Neoadjuvant Setting	142 (45.81)	77 (33.18)
Adjuvant Setting	168 (54.19)	155 (67.10)
Tumor Data		
HER2 IHC/FISH – no (%)		
IHC 3+	233 (75.16)	na
IHC 2+, FISH positive	77 (24.84)	na
Histological Subtype – no (%)		
IDC	261 (84.19)	186 (80.17)
Mixed IDC and ILC	18 (5.81)	7 (3.02)
ILC	12 (3.87)	20 (8.62)
Mucinous	5 (1.61)	7 (3.02)
Micropapillary	4 (1.29)	3 (1.29)
Other	10 (3.23)	9 (3.88)
Grading – no (%)		
G1	4 (1.29)	20 (8.62)
G2	118 (38.06)	130 (56.03)
G3	183 (59.03)	81 (34.91)
Unknown	5 (1.61)	1 (0.43)
HR Status – no (%)		
HR Positive	221 (71.29)	202 (87.07)
HR Negative	89 (28.71)	30 (12.93)

Table 1. *Demographics, treatment setting, and primary tumor data of patients treated with a curative intention.*
**Four patients treated in curative had unknown HER2 status and are therefore not described in this table. IHC denotes immunohistochemistry, FISH denotes fluorescence in situ hybridization, HR denotes hormone receptor.*

3.3 Surgical Therapy

In patients treated with curative intention, the extent of surgical therapy was similar in HER2 positive (n=310) and HER2 negative (n=232) cases. In the first group, 208 (67.10%) patients underwent breast conserving surgery, whereas 100 (32.26%) underwent mastectomy.

Compared to that, 147 (63.36%) and 81 (34.91%) underwent the respective surgical therapy in the HER2 negative group. Four patients in the HER2 negative group and two patients in the HER2 positive group were not operated on due to different reasons, e.g. death or patient decision.

In the palliative setting, ten patients underwent a surgical intervention at their primary tumor site.

3.4 Pharmacological Therapy

3.4.1 Curative Intention in HER2 Positive Patients

In the HER2 positive cohort with curative treatment intention, which encompassed 233 patients with a HER2 IHC score of 3+ and 77 patients with a HER2 IHC score of 2+ and positive FISH results, 142 of 310 (45.81%) patients were treated in the neoadjuvant plus adjuvant setting and 168 (54.19%) in the adjuvant setting.

In the neoadjuvant plus adjuvant treatment group (n=142), 139 (97.89%) patients received chemotherapy with or without HER2 targeted therapy, i.e. anthracyclines and taxanes in 126 cases and anthracyclines or taxanes in 13 cases. Two patients received HER2 targeted therapy without chemotherapy. Taken together, 139 (97.89%) patients received HER2 targeted therapy, of which 131 received it neoadjuvantly and adjuvantly, five only neoadjuvantly, and three only adjuvantly. Of these patients, 96 received trastuzumab only and 43 received dual HER2 blockade with trastuzumab and pertuzumab. Three patients did not receive HER2 targeted therapy despite positive HER2 status. Pathological complete response, i.e. ypT0 or ypTis and ypN0, was achieved in 65 (45.77%) patients, whereas 74 (52.11%) did not have pathological complete response. Three patients were not assessed because they were not operated on. In patients with positive hormone receptors (n=90), 73 (81.11%) received hormone therapy.

In the adjuvant treatment group (n=168), 95 (56.55%) patients received chemotherapy with or without HER2 targeted therapy, i.e. anthracyclines and taxanes in 53 cases, anthracyclines or taxanes in 40 cases, and cyclophosphamide, methotrexate and fluoruracil in two cases. 32 patients received HER2 targeted therapy without chemotherapy. Taken together, 122 (83.56%) patients received HER2 targeted therapy, of which 120 received trastuzumab only and 2 received dual HER2 blockade with trastuzumab and pertuzumab. 46 patients did not receive HER2 targeted therapy despite positive HER2 status. In patients with positive hormone receptors (n=131), 123 (93.89%) received hormone therapy. Ten patients in this

group received no pharmacological treatment at all. Reasons for these patients not receiving therapy include patient decision (n=5), comorbidities (n=3), progression before treatment initiation (n=1), and unknown reason (n=1).

All in all, 49 HER2 positive patients treated with a curative intention, of which 46 were in adjuvant setting and three in neoadjuvant plus adjuvant setting, did not receive HER2 targeted therapy despite their positive HER2 status. Reasons for this range from patient decision to comorbidities and unavailable therapy. A description of all reasons is given in table 2.

3.4.2 Curative Intention in HER2 Negative Patients

In the HER2 negative cohort with curative treatment intention, which encompasses patients with a HER2 IHC score of 2+ and negative FISH results, 77 of 232 (33.19%) patients were treated in neoadjuvant plus adjuvant setting and 155 (66.81%) in adjuvant setting.

In the neoadjuvant plus adjuvant treatment group (n=77), 61 (79.22%) patients received chemotherapy, i.e. anthracyclines and taxanes in 56 cases, anthracyclines or taxanes in 4 cases, and vinorelbine in 1 case. Pathological complete response, i.e. ypT0 or ypTis and ypN0, was achieved in 9 patients (14.75%), while 49 (80.33%) had residual invasive cancer or positive lymph nodes. Three patients were not assessed because they did not undergo surgery. In patients with positive hormone receptors (n=54), 53 (98.15%) received hormone therapy.

In the adjuvant treatment group (n=155), 29 (18.71%) patients received chemotherapy, i.e. anthracyclines and taxanes in 15 cases and anthracyclines in 14 cases. In patients with positive hormone receptors (n=148), 141 (95.27%) received hormone therapy. Nine patients in this group received no pharmacological treatment at all. Reasons for these patients not receiving therapy included patient decision (n=5), comorbidities (n=2), physician decision (n=1), and loss to follow-up (n=1).

3.5 Events and Clinical Outcome

The median follow-up time was 7.4 years (95% CI, 6.7 to 7.87) and three quarters of the patients underwent follow-up for at least five years (25^{th} percentile, 5.0). Data is truncated at 10 years.

Table 2. Reasons why patients did not receive HER2 targeted therapy in (neo)adjuvant setting despite positive HER2 status	
Reasons	Patients (n=49)
Patient decision – no. (%)	12 (24.49)
Physician decision – no. (%)	10 (20.41)
Comorbidities – no. (%)	9 (18.37)
Other reasons – no (%)*	8 (16.33)
Unknown – no. (%)	10 (20.41)

Table 2. Reasons why patients did not receive HER2 targeted therapy in (neo)adjuvant setting despite positive HER2 status.
**Other reasons include discrepant information in five patients and unavailable therapy in three patients who received their initial diagnosis before approval of HER2 targeted therapy in their setting.*

3.5.1 Events in Total Study Population

After 10 years, 147 patients in the total study population had died, of which 93 were in (neo)adjuvant setting and 54 in palliative setting. The overall survival rates at 5 and 10 years in the first group were 88.54% (95% CI, 85.44 to 91.02) and 76.57% (95% CI, 71.51 to 80.85), respectively, whereas overall survival rates at 5 years in the latter group were 23.66% (95% CI, 13.72 to 35.14) (Fig. 3).

Invasive disease-free survival rates in (neo)adjuvant setting at 5 and 10 years were 80.18% (95% CI, 76.50 to 83.34) and 65.27% (95%CI, 59.92 to 70.10), respectively. Of the 148 events which occurred, 74 (50.00%) were distant relapses, 25 (16.89%) were deaths, 22 (14.86%) were local relapses, 22 (14.86%) were secondary non-breast carcinomas, and 5 (3.38%) were secondary breast carcinomas. Regarding distant disease-free survival in this setting, 116 events occurred, most of which were distant relapses (85, 73.28%), resulting in distant disease-free survival rates of 83.68% (95% CI, 80.17 to 86.62) and 70.84% (95% CI, 65.40 to 75.59) at 5 and 10 years, respectively. Recurrence-free survival rates were 81.34% (95% CI, 77.66 to 84.47) and 66.69% (95% CI, 61.10 to 71.66) at 5 and 10 years, respectively, stemming from 133 events of which 79 (59.40%) were distant relapses, 30 (22.56%) were deaths, and 24 (18.05%) were local relapses. (Fig. 4)

Most of the events occurred in the first 10 years of the follow-up period (Table 3).

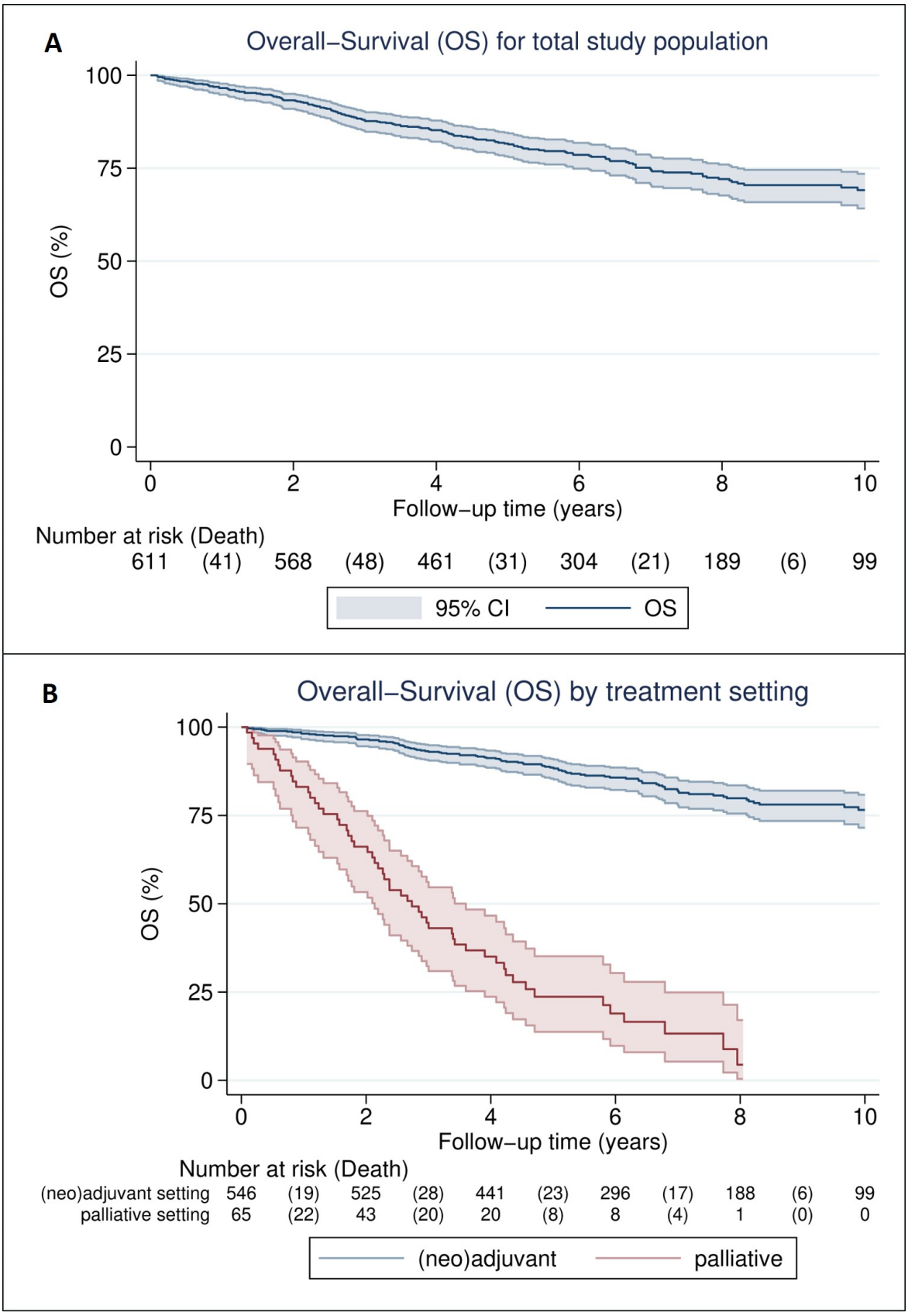

Figure 3. *Overall survival rates in the total study population.*
Panel A shows overall survival for the total study population (n=611). Panel B shows overall survival for the total study population stratified by treatment setting, i.e. (neo)adjuvant (n=546) and palliative (n=65).

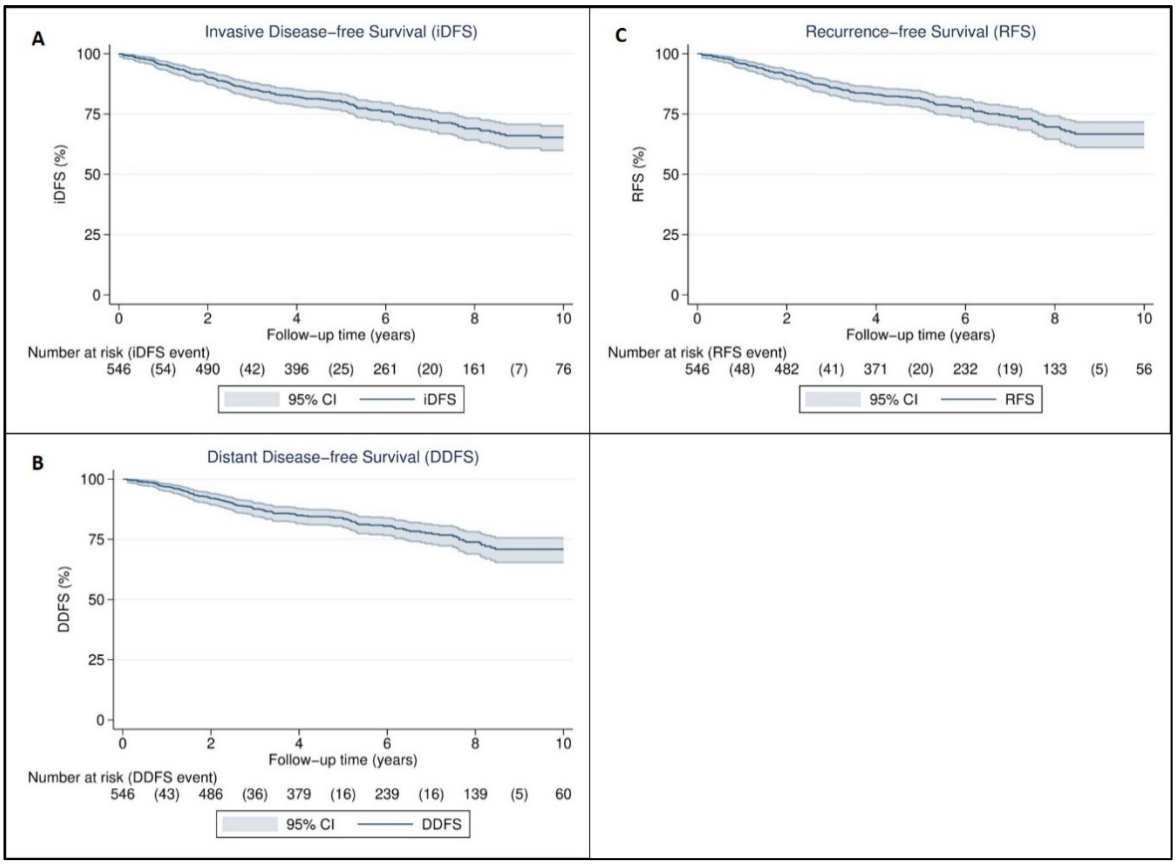

Figure 4. *Clinical outcome in (neo)adjuvant setting (n=546).*
Panel A relates to invasive disease-free survival, panel B to distant disease-free survival, and panel C to recurrence-free survival.

Table 3. Number and types of events in (neo)adjuvant setting truncated at 10 years and in entire follow-up period	
Truncated at 10 years (n=546)	Entire follow-up period (n=546)
Deaths – no. (%)	Deaths – no. (%)
93 (17.03)	102 (18.68)
Distant relapse – no. (%)	Distant relapse – no. (%)
85 (15.57)	86 (15.75)
Local relapse – no. (%)	Local relapse – no. (%)
28 (5.13)	30 (5.49)
Secondary breast carcinoma – no. (%)	Secondary breast carcinoma – no. (%)
9 (1.65)	9 (1.65)
Secondary non-breast carcinoma – no. (%)	Secondary non-breast carcinoma – no. (%)
23 (4.21)	26 (4.76)

Table 3. *Total and relative numbers of events in (neo)adjuvant treatment setting.*
The left column displays data truncated at 10 years, whereas the right column refers to the entire follow up period.

3.5.2 Survival in HER2 Positive Cases

In HER2 positive cases in (neo)adjuvant setting (n=310), 51 deaths, 83 invasive disease events, 65 distant disease events, and 76 recurrence events occurred during the first 10 years of follow-up. Outcomes were significantly better in patients receiving HER2 targeted therapy than in patients without HER2 targeted therapy in terms of overall survival, invasive disease-free survival, distant disease-free survival, and recurrence-free survival (corresponding p-values were <0.005, <0.001, <0.001, and <0.001, respectively). The overall survival rate for the first group at 10 years was 81.38% (95% CI, 74.20 to 86.73), whereas the overall survival rate for the latter group was 63.48% (95% CI, 46.84 to 76.16) (Fig.5). Corresponding rates for invasive disease-free survival were 72.82% (95% CI, 65.58 to 78.78) and 45.26% (95% CI, 29.91 to 59.41), respectively, for distant disease-free survival 79.30% (95% CI, 72.92 to 84.35) and 49.62% (95% CI, 31.79 to 65.15), respectively, and for recurrence free survival 76.08% (95% CI, 69.33 to 81.54) and 41.03% (95% CI, 25.05 to 56.37), respectively (Fig. S1-S3).

In patients with positive HER2 status who did receive HER2 targeted therapy, overall survival rates did not differ significantly between IHC score 2+/FISH positive and IHC score 3+ cases (p=0.826; Fig. 6).

The outcomes of HER2 positive cases stratified by hormone receptor status did not differ significantly at ten years of follow-up but showed differences at five years with a trend towards better survival in hormone receptor positive patients. In said patients, the overall survival rate at five years was 90.31% (95% CI, 85.35 to 93.66), whereas patients with negative hormone receptors had a rate of 82.12% (95% CI, 71.99 to 88.86). Overall survival rates at ten years were 78.49% (95% CI, 70.21 to 84.71) and 76.65% (95% CI, 65.05 to 84.84), respectively, resulting in a p-value of 0.240 (Fig. 7). Corresponding rates for further clinical outcomes were 69.50% (95% CI, 61.40 to 76.23) and 62.65% (95% CI, 49.38 to 73.36), respectively, for invasive disease-free survival (p=0.134); 72.72% (95% CI, 64.05 to 79.62) and 74.17% (95% CI, 62.75 to 82.57), respectively, for distant disease-free survival (p=0.374); and 69.35% (95% CI, 60.58 to 76.54) and 67.63% (95% CI, 55.79 to 76.94), respectively, for recurrence-free survival (p=0.173) (Fig. S4-S6).

3.5.3 Survival in HER2 Negative Cases

In HER2 negative cases in (neo)adjuvant setting (n=232), 40 deaths, 63 invasive disease events, 49 distant disease events, and 55 recurrence events occurred during the first 10 years of follow-up. Overall survival rates did not differ significantly between hormone receptor

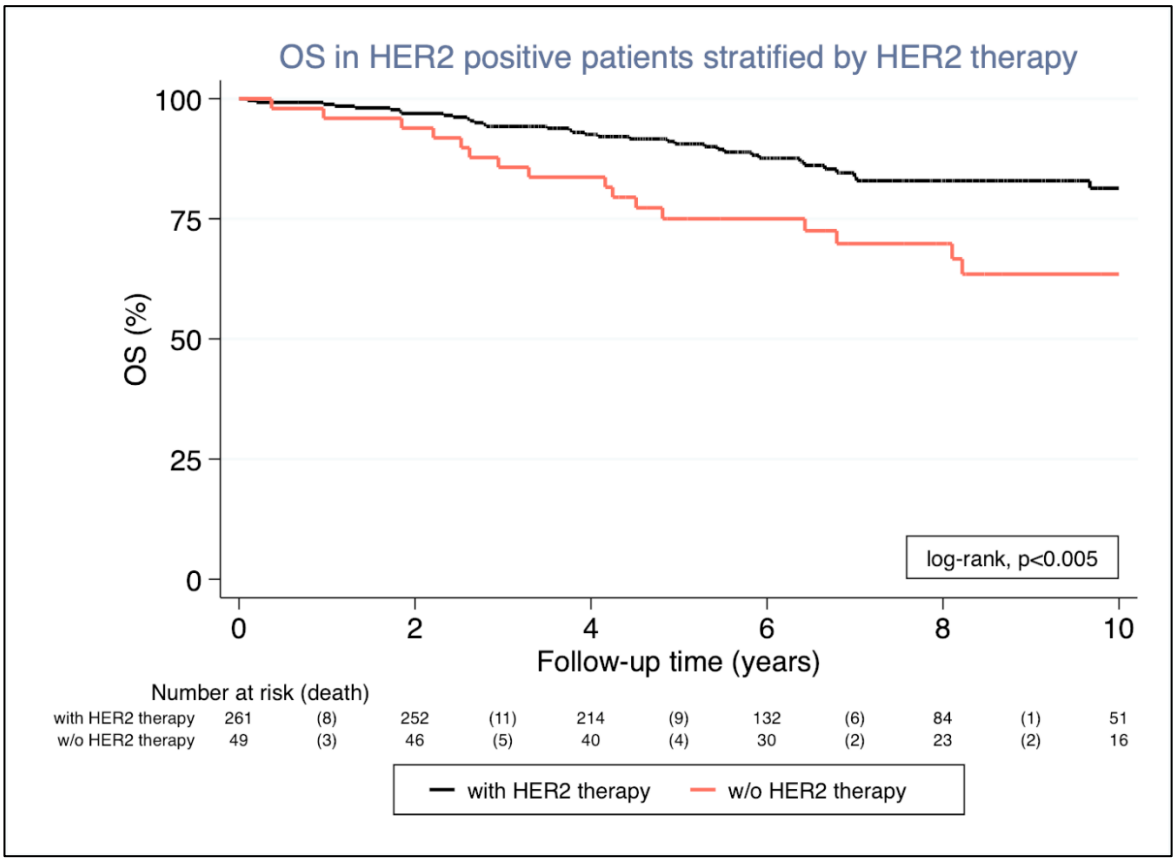

Figure 5. *Overall survival rates for HER2 positive patients in (neo)adjuvant setting.*
The black graph relates to patients who did receive HER2 targeted therapy, whereas the red graph relates to patients who did not receive HER2 targeted therapy.

positive and hormone receptor negative cases (p=0.080), but there was a notable difference at five years of follow-up with overall survival rates of 92.06% (95% CI, 87.13 to 95.15) and 72.35% (95% CI, 52.14 to 85.14), respectively, showing a trend towards better survival in hormone receptor positive patients. Overall survival rates at 10 years were 75.32% (95% CI, 64.89 to 83.05) and 72.35% (95% CI, 52.14 to 85.14), respectively (Fig. 8). Corresponding rates for further clinical outcomes were 63.19% (95% CI, 53.36 to 71.50) and 60.31% (95% CI, 36.56 to 77.56), respectively, for invasive disease-free survival (p=0.283); 69.32% (95% CI, 59.17 to 77.42) and 62.83% (95% CI, 37.33 to 80.32), respectively, for distant disease-free survival (p=0.120); and 64.42% (95% CI, 53.97 to 73.08) and 62.83% (95% CI 37.33 to 80.32), respectively, for recurrence free survival (p=0.265) (Fig. S7-S9).

3.6 Association of HER2 Status with Hormone Receptors

The following chapter describes data regardless of treatment setting. Of 353 patients with a HER2 IHC score of 2+, 264 (74.79%) had negative FISH results, 85 (24.08%) positive FISH

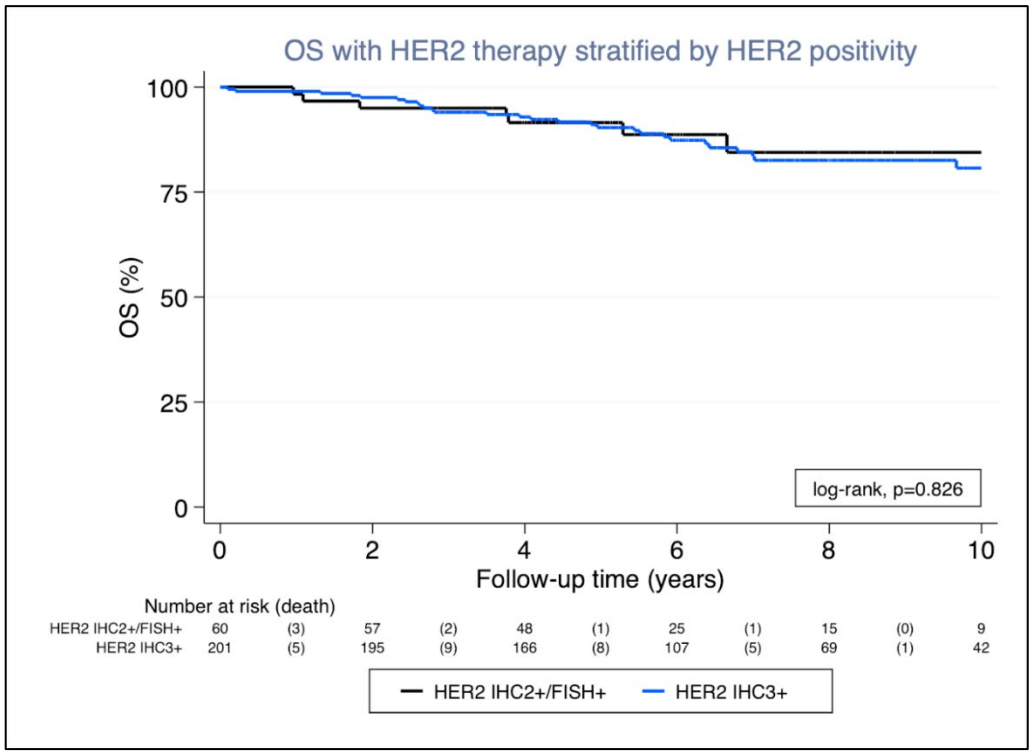

Figure 6. *Overall survival rates for HER2 positive patients in (neo)adjuvant setting receiving HER2 targeted therapy.*
The black graph displays HER2 IHC 2+/FISH positive cases, whereas the blue one displays HER2 IHC 3+ cases.

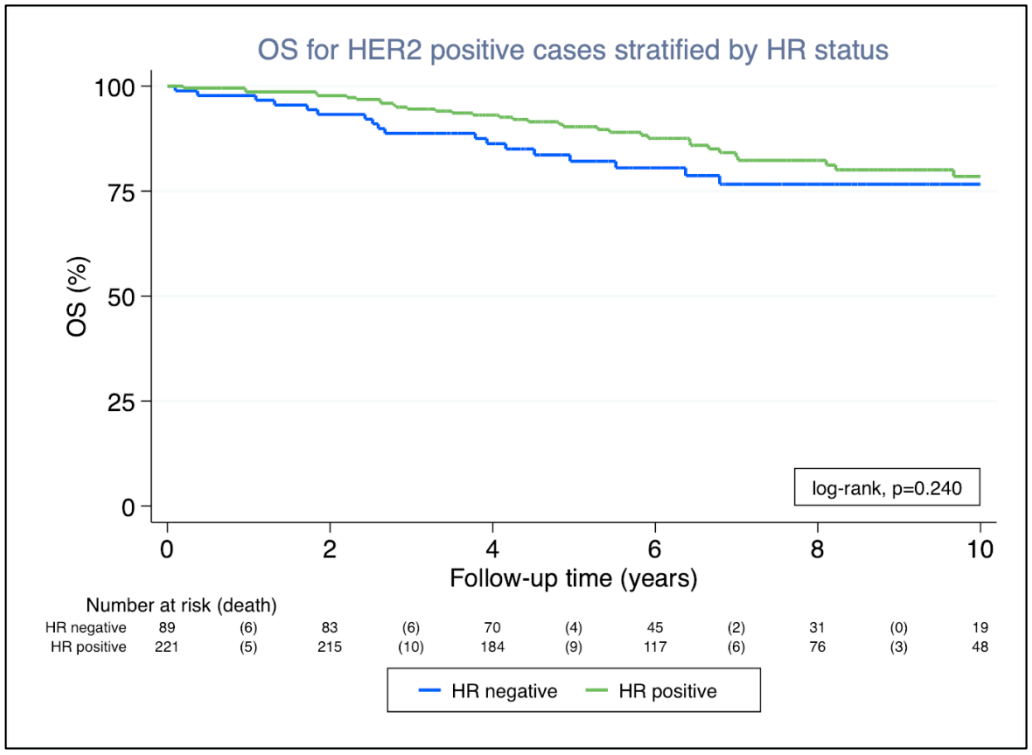

Figure 7. *Overall survival rates for HER2 positive patients in (neo)adjuvant setting stratified by hormone receptor status.*
The blue graph display hormone receptor negative cases, whereas the green graph displays hormone receptor positive cases.

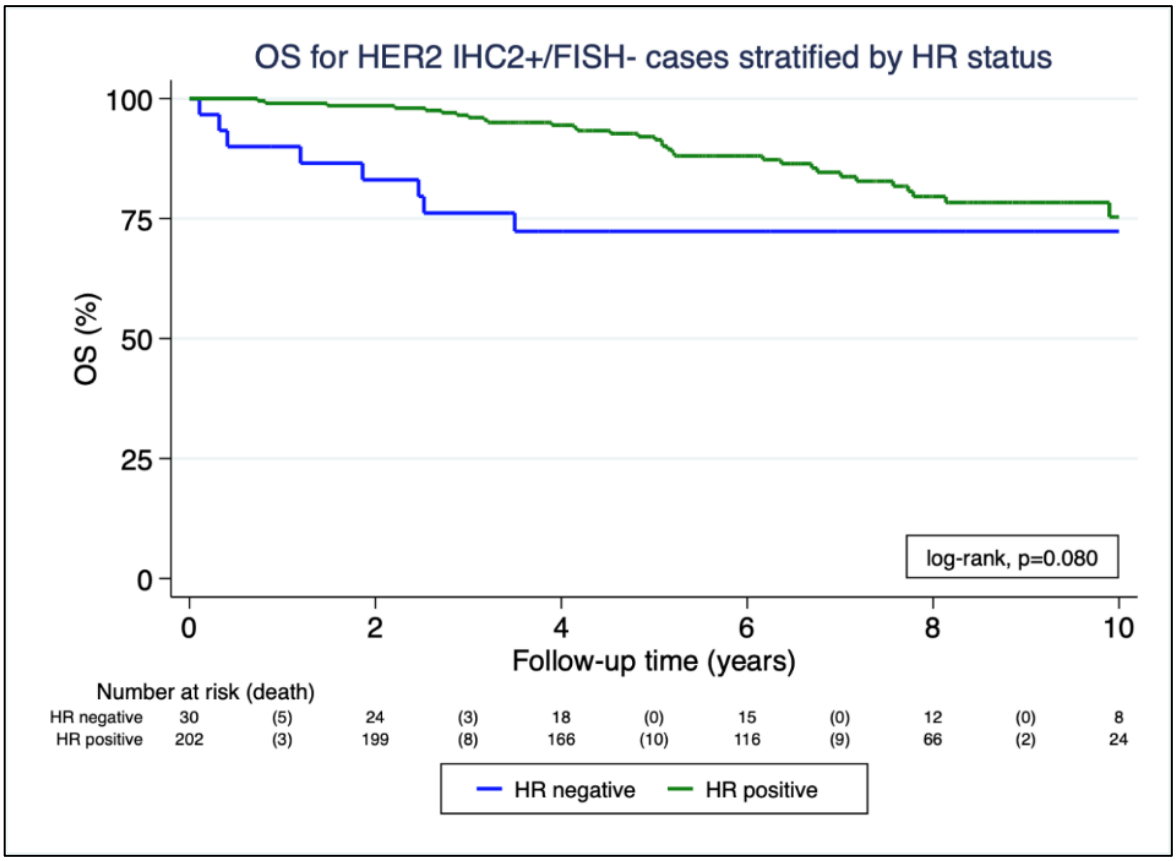

Figure 8. *Overall survival rates for HER2 negative patients in (neo)adjuvant setting stratified by hormone receptor status.*
The blue graph displays hormone receptor negative cases, i.e. triple-negative breast cancer, whereas the green one displays hormone receptor positive cases, i,e, luminal like molecular subtype.

results, and 4 (1.13%) unavailable FISH results. Of 264 patients with a HER2 IHC score of 2+ and negative FISH results, 230 (87.12%) had positive hormone receptors correspondent to luminal-like molecular subtype and 34 (12.88%) had negative hormone receptors correspondent to triple-negative breast cancer. In HER2 positive cases (n=343), which were defined as either having a HER2 IHC score of 2+ and positive FISH results or a HER2 IHC score of 3+, 99 (28.86%) patients had negative hormone receptors, while 244 (71.14%) had positive hormone receptors. The proportion of hormone receptor positive cases was significantly higher in HER2 IHC score 2+/FISH positive cases than in HER2 IHC score 3+ cases, which was 83.53% compared to 67.05%, respectively (Pearson Chi-square 32.42, $p<0.001$; Fig. 9). It can be concluded that there was a similar proportion of hormone receptor positive cases in HER2 IHC score 2+/FISH positive patients and in HER2 IHC score 2+/FISH negative patients (Fig. 9). In HER2 IHC score 2+/FISH positive cases, the median HER2/CEP17 ratio in 71 hormone receptor positive cases was 2.6 (25^{th} to 75^{th} percentiles:

2.37 – 3.63) compared to 3.16 (25^{th} to 75^{th} percentiles: 2.42 – 5.2) in 14 hormone receptor negative cases (p=0.115, Wilcoxon-rank-sum-test).

3.7 Inconclusive HER2 Results

The following chapter describes data regardless of treatment setting.

Some patients with a HER2 IHC score of 3+ received additional evaluation with FISH, which resulted in three patients having inconclusive results, i.e. a HER2/CEP17 ratio of less than 2.0. In one patient, the HER2 signals per cell were greater than 6.0 and the patient received HER2 targeted therapy. The other patients, however, had signals of less than 4.0 and one of them received HER2 targeted therapy while the other did not.

There were twelve cases in different settings with a HER2 IHC score of 2+ and a HER2/CEP17 ratio between 2.0 and 2.2. Most of these cases had positive hormone receptors (91.67%). In one of these cases, the ratio was evaluated twice, resulting in one result of 1.84 and one result of 2.17, and the patient did not receive HER2 targeted therapy. Another patient had multifocal carcinoma, one focus with a HER2/CEP17 ratio of 2.052, the other with 1.44. This patient received HER2 targeted therapy. Of the remaining 10 patients, 7 received HER2 targeted therapy in different settings, whereas 3 did not receive HER2 targeted therapy despite a HER2/CEP17 ratio greater than 2.0. Overall survival in eight patients from this patient group, i.e. patients in (neo)adjuvant setting, did not differ significantly to cases with a HER2/CEP17 ratio greater than 2.2 (p=0.268; Fig. 10). However, these results must be interpreted with caution due to the small number of cases.

Of the 327 patients in an adjuvant setting who were defined as having a HER2 IHC score of 2+ or 3+ by analysis of an excision specimen, 6 (1.8%) and 55 (16.82%) had a HER2 IHC score of 0 and 1+ in biopsy, respectively. In 85 cases, the HER2 IHC score of biopsies was not available. Most of the other cases had the same scores for biopsy and excision specimens, with some proportion changing from 2+ to 3+ and vice versa. (Fig. 11)

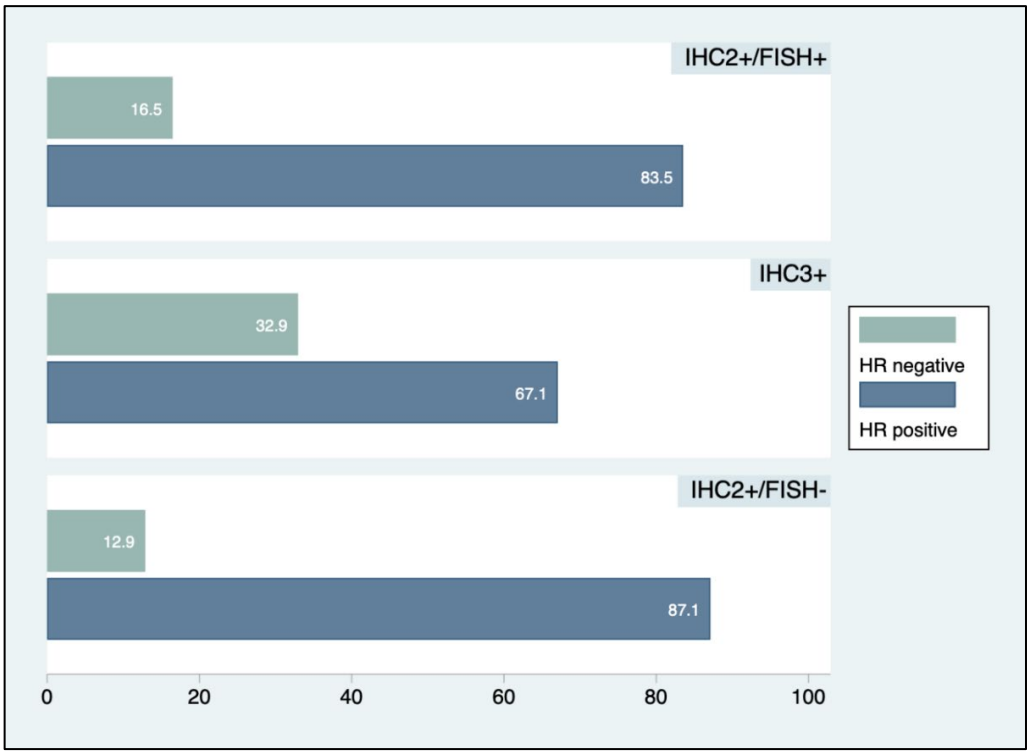

Figure 9. *Proportion (in percent) of HR negative and positive cases.*
The upper panel relates to IHC score 2+/FISH positive cases, the panel in the middle to IHC3+, and the lower panel to IHC2+/FISH negative patients (Pearson Chi-square 32.42, p<0.001).

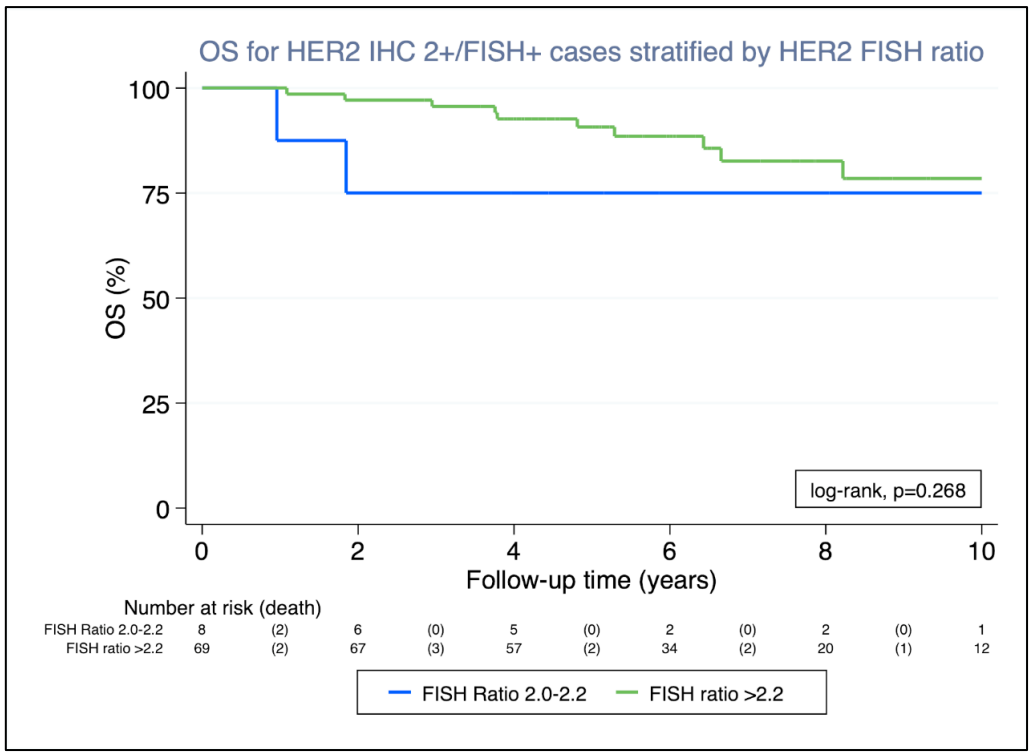

Figure 10. *Overall survival rates for patients in (neo)adjuvant setting with a HER2/CEP17 ratio between 2.0 and 2.2 compared to patients in a (neo)adjuvant setting with a ratio above 2.2.*
The blue graph refers to the former, whereas the green graph refers to the

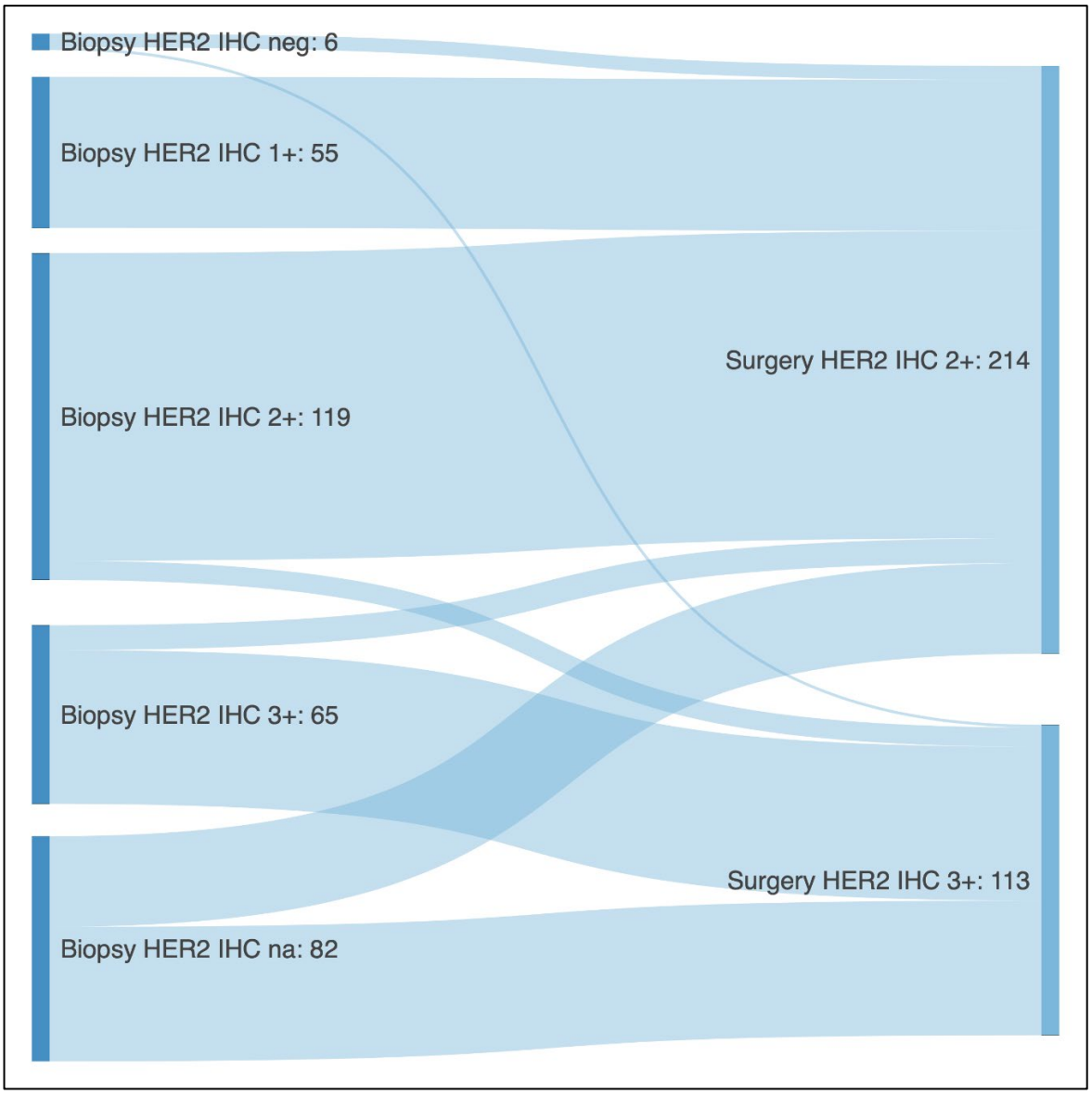

Figure 11. *HER2 IHC scores in biopsy in relation to surgery in adjuvant treatment setting. The left side refers to the former, the right side refers to the latter.*

4 Discussion

As shown in this retrospective study, breast cancer patients with a HER2 IHC score of 2+ and 3+ represent a substantially heterogenous patient group consisting of all molecular subgroups of breast cancer, i.e. luminal like, basal like, and HER2 like, in different settings, i.e. neoadjuvant, adjuvant, and palliative. Of the 611 patients, 353 had a HER2 IHC score of 2+ and 258 had an IHC score of 3+, resulting in 343 HER2 positive cases, 264 HER2 negative cases, i.e. 230 luminal-like and 34 triple-negative subtypes, and 4 cases in which the HER2 status was unknown. In terms of HER2 positive patients, 168 were in adjuvant setting, 142 in neoadjuvant setting, and 33 in palliative setting, whereas 155, 77, and 32 HER2 negative patients were in these settings, respectively.

Overall survival rates at 10 years in (neo)adjuvant setting in the total study population were more than 75%, whereas overall survival rates at 5 years in palliative setting were less than 25%. Invasive disease-free survival rates, distant disease-free survival rates, and recurrence-free survival rates in (neo)adjuvant setting at 10 years were all similar, with values ranging from 65 to 70%. The results of our study population in total are nearly impossible to compare to other studies, since our set of patients is so heterogenous and comprises all three molecular subtypes in different treatment settings. Therefore, only subgroups can be compared with published outcomes, and even then, it is known that clinical studies mostly have a bias towards selecting patients with better performance status for inclusion. Our results may be compared to population-based registries, but there is a lack of high-quality real time data.

In HER2 positive patients in (neo)adjuvant setting, HER2 targeted therapy had a substantial impact on outcomes, leading to significantly better invasive disease-free survival rates, distant disease-free survival rates, recurrence-free survival rates, and, most importantly, better overall survival rates in patients receiving HER2 targeted therapy compared to patients not receiving HER2 targeted therapy. These results align with a Cochrane review evaluating the benefits of regimens containing trastuzumab in early breast cancer compared to chemotherapy alone or no therapy, which showed that regimens involving trasutzumab were associated with statistically significant benefits in terms of overall survival rates and disease-free survival rates.[80] Other studies evaluating HER2 targeted therapy in a neoadjuvant setting[54,55] and dual HER2 blockade in early HER2 positive breast cancer[82] further underscore the importance of adequate HER2 targeted therapy in HER2 positive patients.

However, our results must be interpreted with caution, since patients in both neoadjuvant and adjuvant settings were included. Furthermore, the significantly worse outcomes in patients without HER2 targeted therapy may stem from comorbidities in these patients, who were not sufficiently fit to receive HER2 targeted therapy and may have had a worse outcomes per se. Nevertheless, a substantial proportion of patients did not receive therapy for other reasons, and therefore our results confirm that HER2 targeted therapy in HER2 positive patients is crucial for improving outcomes.

In terms of pathological complete response, HER2 positive patients had a significantly higher rate compared to HER2 negative patients, with rates of 45.77% and 14.75%, respectively ($p<0.001$). The rate in HER2 positive patients is slightly lower than the one in published data[58,59] but still aligns with it, whereas the rate in HER2 negative patients cannot be compared due to the exclusion of patients with a HER2 IHC score of 0 and 1+. Generally, however, a lower pCR rate in HER2 negative patients – due to the high proportion of hormone receptor positive cases - compared to HER2 positive patients poses the expected result.

In HER2 positive patients in (neo)adjuvant setting receiving HER2 targeted therapy, there was no difference in outcome between HER2 IHC 2+/FISH positive cases and HER2 IHC 3+ cases. This leads to the conclusion that the degree of positivity assessed by IHC did not substantially impact the response to HER2 targeted therapy in our study population. Rather, in cases of HER2 positive breast cancer confirmed by any means of assessment, HER2 targeted therapy should be administered.

Nearly three quarters of HER2 positive breast cancer patients had positive hormone receptors. Outcomes in HER2 positive cases with positive hormone receptors did not differ significantly to those with negative hormone receptors, with a 10-year overall survival rate of 78.49% and 76.65%, respectively. However, the overall survival rates at five years showed a trend towards better survival in hormone-receptor positive cases. Compared to a phase III trial with 6273 patients included in the analysis, a smaller proportion of hormone receptor positive cases can be seen, i.e. 57.4%, and an 8-year disease-free survival of 80% in hormone receptor positive cases and of 79% in hormone receptor negative cases.[103] Although these results seem similar, comparisons should be made with caution. Firstly, our study comprised both patients in neoadjuvant and adjuvant settings, whereas the other evaluated patients in adjuvant setting. Furthermore, results in other categories used to measure outcomes, such as invasive disease-free survival rates, were lower in our cohort. Moreover, we reported rates at 10 years, while the other study reported disease-free survival

rates at 8 years. Of interest is the significant difference between hormone receptor positive and negative patients in terms of the distribution of first disease-free survival events in years 6 to 8, and the type of first distant recurrence shown in the other study. These measures were not evaluated in our study and therefore cannot be reproduced.

HER2 negative patients in our cohort comprised hormone receptor positive breast cancer patients, i.e. luminal like, and triple-negative breast cancer patients. Outcome measures in (neo)adjuvant setting did not differ significantly between these two groups, with a 10-year overall survival rate of 72.35% and 75.32%, respectively. However, outcomes in the two groups diverged substantially in the first few years and converged in years eight to ten, displaying a clear trend towards better survival rates in hormone receptor positive patients, with a 5-year overall survival rate of 92.06% compared to 72.35% in hormone receptor negative patients. These results align with the results from a study evaluating data from more than 150,000 patients, which showed worse overall survival rates and cause-specific survival rates in triple-negative breast cancer patients compared to non-triple-negative breast cancer patients.[104] This said, our results for HER2 negative patients should nonetheless be interpreted with caution, given that we only assessed the small proportion of triple-negative and luminal-like patients who had a HER2 IHC score of 2+. Therefore, a substantial number of patients with score 1+ and 0 are not included in our analysis, which may have an impact on the results. We will address this in future, when data for the whole cohort of breast cancer patients at the Medical University Graz becomes available.

The association between hormone receptors and HER2 status was also notable. In our study, the proportion of hormone receptor positive cases was higher in IHC 2+/FISH positive cases than in IHC 3+ cases. Additionally, the median HER2/CEP17 ratio in IHC 2+/FISH positive cases was higher when hormone receptors were negative. This could lead to the conclusion that hormone receptor negativity may be linked to a higher degree of HER2 positivity, which could result in a better response to HER2 targeted therapy. However, only 14 patients had IHC 2+/FISH positive breast cancer with negative hormone receptors and the difference was not significant. Furthermore, responses to HER2 targeted therapy and outcome measures were not compared between these groups and therefore a possible association cannot be validated.

Regarding HER2 heterogeneity, Figure 11 is particularly interesting, since it shows that an appreciable share of patients had different IHC scores in biopsy and surgery despite

receiving no therapy at this time. Furthermore, one patient underwent a FISH assessment of the same specimen twice, each resulting in different HER2/CEP17 ratios - one greater than and one less than 2.0 - and this patient did not receive HER2 targeted therapy. This gives rise to a question as to whether these inconclusive results are due to HER2 heterogeneity and how to deal with such patients. Regarding HER2 status assessment and the translation into clinical practice, there are some notable discrepancies to the ASCO and CAP recommendations. Some patients with an IHC score 3+ received an evaluation with FISH, despite being classified as positive by ASCO/CAP. This resulted in three patients having inconclusive test results, i.e. IHC 3+ and a HER2/CEP17 ratio of less than 2.0. In one patient, the HER2 signals per cell were greater than 6.0 and the patient received HER2 targeted therapy. The other patients, however, had signals of less than 4.0 and one of them received HER2 targeted therapy while the other did not.

Of 353 patients with a HER2 IHC score of 2+, only 12 patients had a HER2/CEP17 ratio of less than 2.2 and greater than 2.0, with positive hormone receptors in nearly all patients. Of these patients, four did not receive HER2 targeted therapy, while the eight other patients did receive HER2 targeted therapy in different settings. Of the patients receiving HER2 targeted therapy, one had a multifocal carcinoma, with one focus with a ratio of less than 2.0 and the other with a ratio greater than 2.0. On the other hand, one patient's ratio was evaluated twice, resulting in one value of less than 2.0 and one greater than 2.0, and the patient did not receive HER2 targeted therapy. Overall survival rates of eight of these patients, i.e. patients in (neo)adjuvant setting, were similar to outcomes in patients with a HER2/CEP17 ratio greater than 2.2. However, the case number is too low to draw conclusions about statistical significance, hence these results are anecdotal evidence. Furthermore, the groups compared were distinguished solely on the basis of their HER2/CEP17 ratios, irrespective of treatment setting, receipt of HER2 targeted therapy, or hormone receptor expression.

There is seemingly no broad consensus on how to deal with inconclusive results in terms of HER2 status. Furthermore, this data draws into question the consistent conversion of ASCO and CAP recommendations on HER2 assessment into clinical practice. The change in the threshold in the guidelines defining HER2 positivity via the HER2/CEP17 ratio - from 2.2 to 2.0 – gives rise to a question of clinical importance arises. Out of 353 patients with a HER2 IHC score of 2+, only 12 had a HER2/CEP17 ratio between 2.0 and 2.2 and were therefore affected by the change. Due to the low number of patients with these results in our study population, it is not possible to make valid statements about differences in outcomes

for patients in this situation stratified by receipt of HER2 targeted therapy. This means that the clinical importance change of threshold cannot be determined here. However, this topic warrants thorough evaluation in further studies, since withdrawing HER2 targeted therapy from patients marked as HER2 negative while they would still benefit from HER2 targeted treatment may be considered highly unethical. On the other hand, these patients may be exposed to the unnecessary toxicity of HER2 targeted therapy, which might be avoided if therapy is not needed. A promising development in this area is the HER2 targeted product trastuzumab deruxtecan, an antibody-drug conjugate approved for metastatic or unresectable HER2 positive breast cancer. Apart from its antitumor activity in HER2 positive breast cancer, trastuzumab deruxtecan demonstrated antitumor activity against breast cancer patient derived xenograft models with low HER2 expression.[105] Furthermore, it showed promising antitumor activity in humans as has been shown in a phase I study of this therapy in HER2 low expressing breast cancer, including a subgroup of HER2 IHC 1+ breast cancer.[106] Phase II and III studies evaluating the benefit of this treatment in breast cancer patients with inconclusive HER2 results as well as in HER2 low-expressing breast cancer and HER2 IHC 1+ breast cancer are ongoing and will provide important results. This development could substantially change treatment options for these patient groups.

Our study has various limitations. Firstly, the study was conducted retrospectively, which substantially elevates the risk of bias. Moreover, the study population was extraordinarily heterogenous, which limits its informative value for specific patient groups. In times of highly personalized medicine, this represents a major problem. Furthermore, the data collected may be incomplete, since there is no unique electronic health record in Austria in which patient data from all hospitals and physicians in private practice is captured. Instead, only internal data of the Division of Oncology at the Medical University in Graz and electronic health records from the hospitals of the Steiermärkische Krankenanstaltengesellschaft m.b.H. were available.

5 Conclusion

As shown in this study, breast cancer patients with a HER2 IHC score of 2+ or 3+ are a diverse patient population in different settings. Overall survival rates at ten years in (neo)adjuvant setting were more than 75%, whereas overall survival rates at five years in palliative setting were less than 25%. In HER2 positive patients in our study, HER2 targeted treatment was a crucial component resulting in better outcomes. The rate of pathological complete response in our patient population was significantly better in HER2 positive patients than in HER2 negative patients and aligns with published data. Clinical outcomes did not differ significantly between HER2 positive, hormone receptor positive and HER2 positive, hormone receptor negative patients in our study, but there was a trend towards better outcomes in the first group. In our patient cohort, there was a clear tendency towards better survival rates in HER2 negative patients with positive hormone receptors compared to HER2 negative patients with negative hormone receptors. However, outcomes converged again in years eight to ten of follow-up and therefore there was no significant difference in survival. Only a very minor group of patients in our study had a HER2/CEP17 ratio between 2.0 and 2.2. Therefore, the clinical importance of the threshold change of the ASCO/CAP guidelines for HER2 positivity from 2.2 in 2006 to 2.0 in 2013 could not be determined. HER2 heterogeneity and inconclusive HER2 results represent a difficulty for clinical decision-making in the area HER2 targeted therapy, given the need to counterbalance possible improvements in outcomes with the possible adverse events of the therapy. This difficulty is illustrated by the inconsistent approaches to dealing with patients in this situation which we encountered in this study. A promising development in this area is trastuzumab deruxtecan, which might substantially change treatment options for these patients.

6 Bibliography

1. Hayes DF, Lippmann ME. Breast Cancer. In: *Harrison's Prinicples of Internal Medicne*. Vol 1. 20th ed. McGraw-Hill Education; 2018:555-566.

2. Tavassoli FA, Devilee P, eds. *Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Vol 4. 3rd ed. IARC Press; 2003.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34. doi:10.3322/caac.21551

4. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. *Biol Res*. 2017;50(1):33. doi:10.1186/s40659-017-0140-9

5. Carey LA. Breast Cancer. In: *Netter's Internal Medicine*. 2nd ed. Saunders Elsevier; 2009:572-577.

6. Shield KD, Soerjomataram I, Rehm J. Alcohol Use and Breast Cancer: A Critical Review. *Alcohol Clin Exp Res*. 2016;40(6):1166-1181. doi:10.1111/acer.13071

7. Macacu A, Autier P, Boniol M, Boyle P. Active and passive smoking and risk of breast cancer: a meta-analysis. *Breast Cancer Res Treat*. 2015;154(2):213-224. doi:10.1007/s10549-015-3628-4

8. Moinfar F. *Essentials of Diagnostic Breast Pathology*. Springer; 2007.

9. Gudi M, Stamp GWH. Breast. In: *The Cancer Handbook*. Vol 1. 2nd ed. Jon Wiley and Sons Ltd.; 2007.

10. Hortobagyi GN, Connolly JL, D'Orsi CJ, et al. Breast. In: *AJCC Cancer Staging Manual*. 8th ed. Springer; 2017:589-633.

11. Hammond MEH, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *J Clin Oncol*. 2010;28(16):2784-2795. doi:10.1200/JCO.2009.25.6529

12. Pathmanathan N, Balleine RL. Ki67 and proliferation in breast cancer. *J Clin Pathol*. 2013;66(6):512-516. doi:10.1136/jclinpath-2012-201085

13. Penault-Llorca F, Radosevic-Robin N. Ki67 assessment in breast cancer: an update. *Pathology (Phila)*. 2017;49(2):166-171. doi:10.1016/j.pathol.2016.11.006

14. Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *J Clin Oncol*. 2006;25(1):118-145. doi:10.1200/JCO.2006.09.2775

15. Jafari SH, Saadatpour Z, Salmaninejad A, et al. Breast cancer diagnosis: Imaging techniques and biochemical markers. *J Cell Physiol*. 2018;233(7):5200-5213. doi:10.1002/jcp.26379

16. Wolff AC, Hammond MEH, Schwartz JN, et al. 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*. 2007;131(1):18-43. doi:10.1043/1543-2165(2007)131[18:ASOCCO]2.0.CO;2

17. Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol*. 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984

18. Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *Arch Pathol Lab Med*. 2013;138(2):241-256. doi:10.5858/arpa.2013-0953-SA

19. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738

20. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med*. 2018;142(11):1364-1382. doi:10.5858/arpa.2018-0902-SA

21. Press MF, Sauter G, Buyse M, et al. HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials. *J Clin Oncol Off J Am Soc Clin Oncol*. 2016;34(29):3518-3528. doi:10.1200/JCO.2016.66.6693

22. Rossi L, Mazzara C, Pagani O. Diagnosis and Treatment of Breast Cancer in Young Women. *Curr Treat Options Oncol*. 2019;20(12):86. doi:10.1007/s11864-019-0685-7

23. Gøtzsche P, Jørgensen K. Screening for breast cancer with mammography. *Cochrane Database Syst Rev*. 2013;(6). doi:10.1002/14651858.CD001877.pub5

24. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF). S3-Leitlinie Früherkennung, Diagnose, Therapie und Nachsorge des Mammakarzinoms, Version 4.3, 2020, AWMF Registernummer: 032-045OL, http://www.leitlinienprogramm-onkologie.de/leitlinien/mammakarzinom/ (abgerufen am 08.07.2020).

25. McLaughlin SA. Surgical management of the breast: breast conservation therapy and mastectomy. *Surg Clin North Am*. 2013;93(2):411-428. doi:10.1016/j.suc.2012.12.006

26. Alm El-Din MA, Taghian AG. Breast conservation therapy for patients with locally advanced breast cancer. *Semin Radiat Oncol*. 2009;19(4):229-235. doi:10.1016/j.semradonc.2009.05.005

27. Newman LA. Decision Making in the Surgical Management of Invasive Breast Cancer-Part 2: Expanded Applications for Breast-Conserving Surgery. *Oncol Williston Park N*. 2017;31(5):415-420.

28. Park CC, Mitsumori M, Nixon A, et al. Outcome at 8 years after breast-conserving surgery and radiation therapy for invasive breast cancer: influence of margin status and systemic therapy on local recurrence. *J Clin Oncol Off J Am Soc Clin Oncol*. 2000;18(8):1668-1675. doi:10.1200/JCO.2000.18.8.1668

29. Cowen D, Houvenaeghel G, Bardou V, et al. Local and distant failures after limited surgery with positive margins and radiotherapy for node-negative breast cancer. *Int J Radiat Oncol Biol Phys*. 2000;47(2):305-312. doi:10.1016/s0360-3016(99)00553-2

30. Bodilsen A, Bjerre K, Offersen BV, et al. Importance of margin width in breast-conserving treatment of early breast cancer. *J Surg Oncol*. 2016;113(6):609-615. doi:10.1002/jso.24224

31. Singletary SE. Surgical margins in patients with early-stage breast cancer treated with breast conservation therapy. *Am J Surg*. 2002;184(5):383-393. doi:10.1016/s0002-9610(02)01012-7

32. Brzezinska M, Williams LJ, Thomas J, Michael Dixon J. Outcomes of patients with inflammatory breast cancer treated by breast-conserving surgery. *Breast Cancer Res Treat*. 2016;160(3):387-391. doi:10.1007/s10549-016-4017-3

33. Brzezinska M, Dixon JM. Inflammatory breast cancer: no longer an absolute contraindication for breast conservation surgery following good response to neoadjuvant therapy. *Gland Surg*. 2018;7(6):520-524. doi:10.21037/gs.2018.08.04

34. Hartmann LC, Sellers TA, Schaid DJ, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. *J Natl Cancer Inst*. 2001;93(21):1633-1637. doi:10.1093/jnci/93.21.1633

35. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. *J Clin Oncol Off J Am Soc Clin Oncol*. 2004;22(6):1055-1062. doi:10.1200/JCO.2004.04.188

36. Darby S, McGale P, Correa C, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and. *Lancet Lond Engl*. 2011;378(9804):1707-1716. doi:10.1016/S0140-6736(11)61629-2

37. Clarke M, Collins R, Darby S, et al. 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 Lond Engl*. 2005;366(9503):2087-2106. doi:10.1016/S0140-6736(05)67887-7

38. Ragaz J, Olivotto IA, Spinelli JJ, et al. Locoregional radiation therapy in patients with high-risk breast cancer receiving adjuvant chemotherapy: 20-year results of the British Columbia randomized trial. *J Natl Cancer Inst*. 2005;97(2):116-126. doi:10.1093/jnci/djh297

39. Nielsen HM, Overgaard M, Grau C, Jensen AR, Overgaard J. Study of failure pattern among high-risk breast cancer patients with or without postmastectomy radiotherapy in addition to adjuvant systemic therapy: long-term results from the Danish Breast Cancer Cooperative Group DBCG 82 b and c randomized studies. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(15):2268-2275. doi:10.1200/JCO.2005.02.8738

40. Mamtani A, Barrio AV, King TA, et al. How Often Does Neoadjuvant Chemotherapy Avoid Axillary Dissection in Patients With Histologically Confirmed Nodal Metastases? Results of a Prospective Study. *Ann Surg Oncol*. 2016;23(11):3467-3474. doi:10.1245/s10434-016-5246-8

41. Gralow JR, Burstein HJ, Wood W, et al. Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(5):814-819. doi:10.1200/JCO.2007.15.3510

42. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet Lond Engl*. 2014;384(9938):164-172. doi:10.1016/S0140-6736(13)62422-8

43. Mieog JSD, van der Hage JA, van de Velde CJH. Preoperative chemotherapy for women with operable breast cancer. *Cochrane Database Syst Rev*. 2007;(2):CD005002. doi:10.1002/14651858.CD005002.pub2

44. Symmans WF, Wei C, Gould R, et al. Long-Term Prognostic Risk After Neoadjuvant Chemotherapy Associated With Residual Cancer Burden and Breast Cancer Subtype. *J Clin Oncol Off J Am Soc Clin Oncol*. 2017;35(10):1049-1060. doi:10.1200/JCO.2015.63.1010

45. Rastogi P, Anderson SJ, Bear HD, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26(5):778-785. doi:10.1200/JCO.2007.15.0235

46. Nakatsukasa K, Koyama H, Oouchi Y, et al. Docetaxel and cyclophosphamide as neoadjuvant chemotherapy in HER2-negative primary breast cancer. *Breast Cancer Tokyo Jpn*. 2017;24(1):63-68. doi:10.1007/s12282-016-0666-7

47. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol Off J Am Soc Clin Oncol*. 2015;33(1):13-21. doi:10.1200/JCO.2014.57.0572

48. von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. *Lancet Oncol*. 2014;15(7):747-756. doi:10.1016/S1470-2045(14)70160-3

49. Balic M, Thomssen C, Wurstlein R, Gnant M, Harbeck N. St. Gallen/Vienna 2019: A Brief Summary of the Consensus Discussion on the Optimal Primary Breast Cancer Treatment. *Breast Care Basel Switz*. 2019;14(2):103-110. doi:10.1159/000499931

50. Alba E, Calvo L, Albanell J, et al. Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. *Ann Oncol Off J Eur Soc Med Oncol*. 2012;23(12):3069-3074. doi:10.1093/annonc/mds132

51. Palmieri C, Cleator S, Kilburn LS, et al. NEOCENT: a randomised feasibility and translational study comparing neoadjuvant endocrine therapy with chemotherapy in ER-rich postmenopausal primary breast cancer. *Breast Cancer Res Treat*. 2014;148(3):581-590. doi:10.1007/s10549-014-3183-4

52. Spring LM, Gupta A, Reynolds KL, et al. Neoadjuvant Endocrine Therapy for Estrogen Receptor-Positive Breast Cancer: A Systematic Review and Meta-analysis. *JAMA Oncol*. 2016;2(11):1477-1486. doi:10.1001/jamaoncol.2016.1897

53. Semiglazov VF, Semiglazov VV, Dashyan GA, et al. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. *Cancer*. 2007;110(2):244-254. doi:10.1002/cncr.22789

54. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet Lond Engl*. 2010;375(9712):377-384. doi:10.1016/S0140-6736(09)61964-4

55. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet Oncol*. 2014;15(6):640-647. doi:10.1016/S1470-2045(14)70080-4

56. Hayes DF. Further Progress for Patients with Breast Cancer. *N Engl J Med*. 2019;380(7):676-677. doi:10.1056/NEJMe1816059

57. Gianni L, Pienkowski T, Im Y-H, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2012;13(1):25-32. doi:10.1016/S1470-2045(11)70336-9

58. Buzdar AU, Suman VJ, Meric-Bernstam F, et al. Fluorouracil, epirubicin, and cyclophosphamide (FEC-75) followed by paclitaxel plus trastuzumab versus paclitaxel plus trastuzumab followed by FEC-75 plus trastuzumab as neoadjuvant treatment for patients with HER2-positive breast cancer (Z1041): a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2013;14(13):1317-1325. doi:10.1016/S1470-2045(13)70502-3

59. Robidoux A, Tang G, Rastogi P, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2013;14(12):1183-1192. doi:10.1016/S1470-2045(13)70411-X

60. van Ramshorst MS, van der Voort A, van Werkhoven ED, et al. Neoadjuvant chemotherapy with or without anthracyclines in the presence of dual HER2 blockade for HER2-positive breast cancer (TRAIN-2): a multicentre, open-label, randomised,

phase 3 trial. *Lancet Oncol*. 2018;19(12):1630-1640. doi:10.1016/S1470-2045(18)30570-9

61. Harris LN, Ismaila N, McShane LM, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol Off J Am Soc Clin Oncol*. 2016;34(10):1134-1150. doi:10.1200/JCO.2015.65.2289

62. Peto R, Davies C, Godwin J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet Lond Engl*. 2012;379(9814):432-444. doi:10.1016/S0140-6736(11)61625-5

63. Sparano JA, Wang M, Martino S, et al. Weekly paclitaxel in the adjuvant treatment of breast cancer. *N Engl J Med*. 2008;358(16):1663-1671. doi:10.1056/NEJMoa0707056

64. Blum JL, Flynn PJ, Yothers G, et al. Anthracyclines in Early Breast Cancer: The ABC Trials-USOR 06-090, NSABP. *J Clin Oncol Off J Am Soc Clin Oncol*. 2017;35(23):2647-2655. doi:10.1200/JCO.2016.71.4147

65. Jones SE, Savin MA, Holmes FA, et al. Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(34):5381-5387. doi:10.1200/JCO.2006.06.5391

66. Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. *Lancet Lond Engl*. 2019;393(10179):1440-1452. doi:10.1016/S0140-6736(18)33137-4

67. Bonilla L, Ben-Aharon I, Vidal L, Gafter-Gvili A, Leibovici L, Stemmer SM. Dose-dense chemotherapy in nonmetastatic breast cancer: a systematic review and meta-analysis of randomized controlled trials. *J Natl Cancer Inst*. 2010;102(24):1845-1854. doi:10.1093/jnci/djq409

68. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet Lond Engl*. 2015;386(10001):1341-1352. doi:10.1016/S0140-6736(15)61074-1

69. Henry NL, Giles JT, Ang D, et al. Prospective characterization of musculoskeletal symptoms in early stage breast cancer patients treated with aromatase inhibitors. *Breast Cancer Res Treat*. 2008;111(2):365-372. doi:10.1007/s10549-007-9774-6

70. Presant CA, Bosserman L, Young T, et al. Aromatase inhibitor-associated arthralgia and/ or bone pain: frequency and characterization in non-clinical trial patients. *Clin Breast Cancer*. 2007;7(10):775-778. doi:10.3816/CBC.2007.n.038

71. Crew KD, Greenlee H, Capodice J, et al. Prevalence of joint symptoms in postmenopausal women taking aromatase inhibitors for early-stage breast cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2007;25(25):3877-3883. doi:10.1200/JCO.2007.10.7573

72. Henry NL, Azzouz F, Desta Z, et al. Predictors of aromatase inhibitor discontinuation as a result of treatment-emergent symptoms in early-stage breast cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2012;30(9):936-942. doi:10.1200/JCO.2011.38.0261

73. Baumgart J, Nilsson K, Evers AS, Kallak TK, Poromaa IS. Sexual dysfunction in women on adjuvant endocrine therapy after breast cancer. *Menopause N Y N*. 2013;20(2):162-168. doi:10.1097/gme.0b013e31826560da

74. Ganz PA, Petersen L, Bower JE, Crespi CM. Impact of Adjuvant Endocrine Therapy on Quality of Life and Symptoms: Observational Data Over 12 Months From the Mind-Body Study. *J Clin Oncol Off J Am Soc Clin Oncol*. 2016;34(8):816-824. doi:10.1200/JCO.2015.64.3866

75. Confavreux CB, Fontana A, Guastalla JP, Munoz F, Brun J, Delmas PD. Estrogen-dependent increase in bone turnover and bone loss in postmenopausal women with breast cancer treated with anastrozole. Prevention with bisphosphonates. *Bone*. 2007;41(3):346-352. doi:10.1016/j.bone.2007.06.004

76. Davies C, Godwin J, Gray R, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet Lond Engl*. 2011;378(9793):771-784. doi:10.1016/S0140-6736(11)60993-8

77. Pagani O, Francis PA, Fleming GF, et al. Absolute Improvements in Freedom From Distant Recurrence to Tailor Adjuvant Endocrine Therapies for Premenopausal Women: Results From TEXT and SOFT. *J Clin Oncol Off J Am Soc Clin Oncol*. Published online October 16, 2019:JCO1801967. doi:10.1200/JCO.18.01967

78. Francis PA, Regan MM, Fleming GF, et al. Adjuvant ovarian suppression in premenopausal breast cancer. *N Engl J Med*. 2015;372(5):436-446. doi:10.1056/NEJMoa1412379

79. Francis PA, Pagani O, Fleming GF, et al. Tailoring Adjuvant Endocrine Therapy for Premenopausal Breast Cancer. *N Engl J Med*. 2018;379(2):122-137. doi:10.1056/NEJMoa1803164

80. Moja L, Tagliabue L, Balduzzi S, et al. Trastuzumab containing regimens for early breast cancer. *Cochrane Database Syst Rev*. 2012;(4):CD006243. doi:10.1002/14651858.CD006243.pub2

81. Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet Lond Engl*. 2017;389(10075):1195-1205. doi:10.1016/S0140-6736(16)32616-2

82. von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. *N Engl J Med*. 2017;377(2):122-131. doi:10.1056/NEJMoa1703643

83. von Minckwitz G, Huang C-S, Mano MS, et al. Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. *N Engl J Med*. 2019;380(7):617-628. doi:10.1056/NEJMoa1814017

84. Bowles EJA, Wellman R, Feigelson HS, et al. Risk of heart failure in breast cancer patients after anthracycline and trastuzumab treatment: a retrospective cohort study. *J Natl Cancer Inst*. 2012;104(17):1293-1305. doi:10.1093/jnci/djs317

85. Caswell-Jin JL, Plevritis SK, Tian L, et al. Change in Survival in Metastatic Breast Cancer with Treatment Advances: Meta-Analysis and Systematic Review. *JNCI Cancer Spectr*. 2018;2(4):pky062. doi:10.1093/jncics/pky062

86. Swain SM, Baselga J, Kim S-B, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med*. 2015;372(8):724-734. doi:10.1056/NEJMoa1413513

87. Baselga J, Cortes J, Kim S-B, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012;366(2):109-119. doi:10.1056/NEJMoa1113216

88. Bachelot T, Ciruelos E, Schneeweiss A, et al. Preliminary safety and efficacy of first-line pertuzumab combined with trastuzumab and taxane therapy for HER2-positive locally recurrent or metastatic breast cancer (PERUSE). *Ann Oncol Off J Eur Soc Med Oncol*. 2019;30(5):766-773. doi:10.1093/annonc/mdz061

89. Johnston S, Pippen JJ, Pivot X, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2009;27(33):5538-5546. doi:10.1200/JCO.2009.23.3734

90. Rimawi M, Ferrero J-M, de la Haba-Rodriguez J, et al. First-Line Trastuzumab Plus an Aromatase Inhibitor, With or Without Pertuzumab, in Human Epidermal Growth Factor Receptor 2-Positive and Hormone Receptor-Positive Metastatic or Locally Advanced Breast Cancer (PERTAIN): A Randomized, Open-Label Phase II Trial. *J Clin Oncol Off J Am Soc Clin Oncol*. 2018;36(28):2826-2835. doi:10.1200/JCO.2017.76.7863

91. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367(19):1783-1791. doi:10.1056/NEJMoa1209124

92. Modi S, Saura C, Yamashita T, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer. *N Engl J Med*. 2020;382(7):610-621. doi:10.1056/NEJMoa1914510

93. Murthy RK, Loi S, Okines A, et al. Tucatinib, Trastuzumab, and Capecitabine for HER2-Positive Metastatic Breast Cancer. *N Engl J Med*. 2020;382(7):597-609. doi:10.1056/NEJMoa1914609

94. Mauri D, Kamposioras K, Tsali L, et al. Overall survival benefit for weekly vs. three-weekly taxanes regimens in advanced breast cancer: A meta-analysis. *Cancer Treat Rev*. 2010;36(1):69-74. doi:10.1016/j.ctrv.2009.10.006

95. Sledge GW, Neuberg D, Bernardo P, et al. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for

metastatic breast cancer: an intergroup trial (E1193). *J Clin Oncol Off J Am Soc Clin Oncol*. 2003;21(4):588-592. doi:10.1200/JCO.2003.08.013

96. Giuliano M, Schettini F, Rognoni C, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis. *Lancet Oncol*. 2019;20(10):1360-1369. doi:10.1016/S1470-2045(19)30420-6

97. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. *N Engl J Med*. 2018;379(8):753-763. doi:10.1056/NEJMoa1802905

98. Robson M, Im S-A, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med*. 2017;377(6):523-533. doi:10.1056/NEJMoa1706450

99. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med*. 2018;379(22):2108-2121. doi:10.1056/NEJMoa1809615

100. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med*. 2020;382(9):810-821. doi:10.1056/NEJMoa1910549

101. Hudis CA, Barlow WE, Costantino JP, et al. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. *J Clin Oncol Off J Am Soc Clin Oncol*. 2007;25(15):2127-2132. doi:10.1200/JCO.2006.10.3523

102. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials*. 1996;17(4):343-346. doi:10.1016/0197-2456(96)00075-x

103. Lambertini M, Campbell C, Gelber RD, et al. Dissecting the effect of hormone receptor status in patients with HER2-positive early breast cancer: exploratory analysis from the ALTTO (BIG 2-06) randomized clinical trial. *Breast Cancer Res Treat*. 2019;177(1):103-114. doi:10.1007/s10549-019-05284-y

104. Li X, Yang J, Peng L, et al. Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer. *Breast Cancer Res Treat*. 2017;161(2):279-287. doi:10.1007/s10549-016-4059-6

105. Ogitani Y, Aida T, Hagihara K, et al. DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor Efficacy with Differentiation from T-DM1. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2016;22(20):5097-5108. doi:10.1158/1078-0432.CCR-15-2822

106. Modi S, Tsurutani J, Tamura K, et al. Trastuzumab deruxtecan (DS-8201a) in subjects with HER2-low expressing breast cancer: Updated results of a large phase 1 study. Poster presented at the: San Antonio Breast Cancer Symposium; December 4, 2018; San Antonio, TX.

Appendix – Supplemental Figures

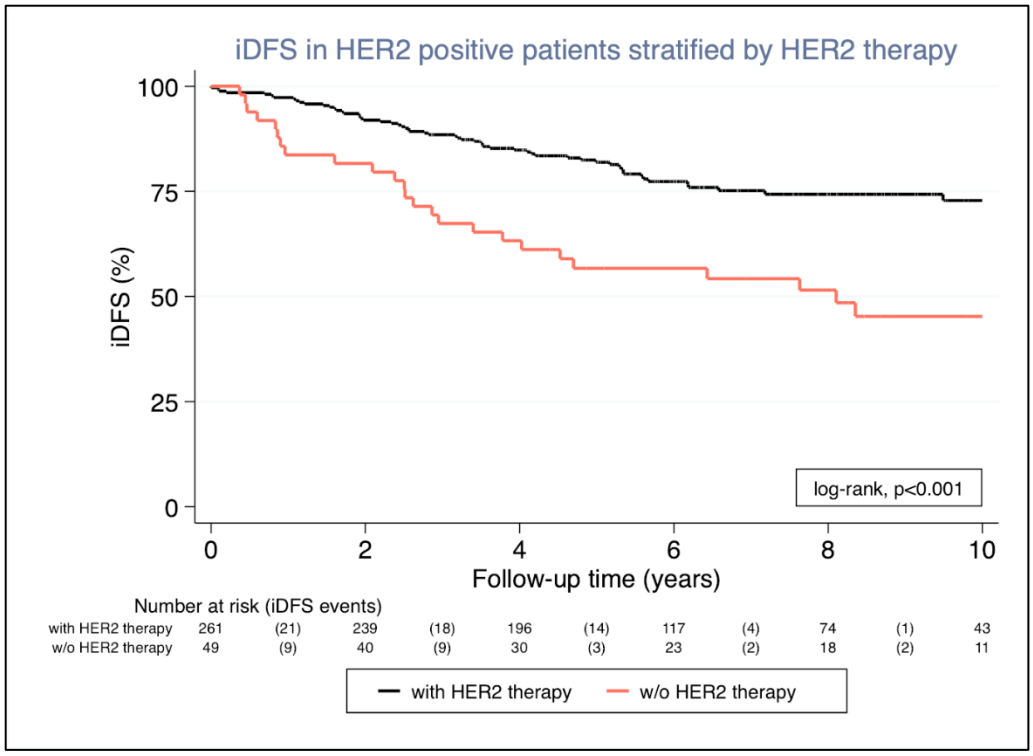

Figure S1. *Invasive disease-free survival rates for HER2 positive patients in (neo)adjuvant setting who did receive (black graph) and who did not receive (red graph) HER2 targeted therapy.*

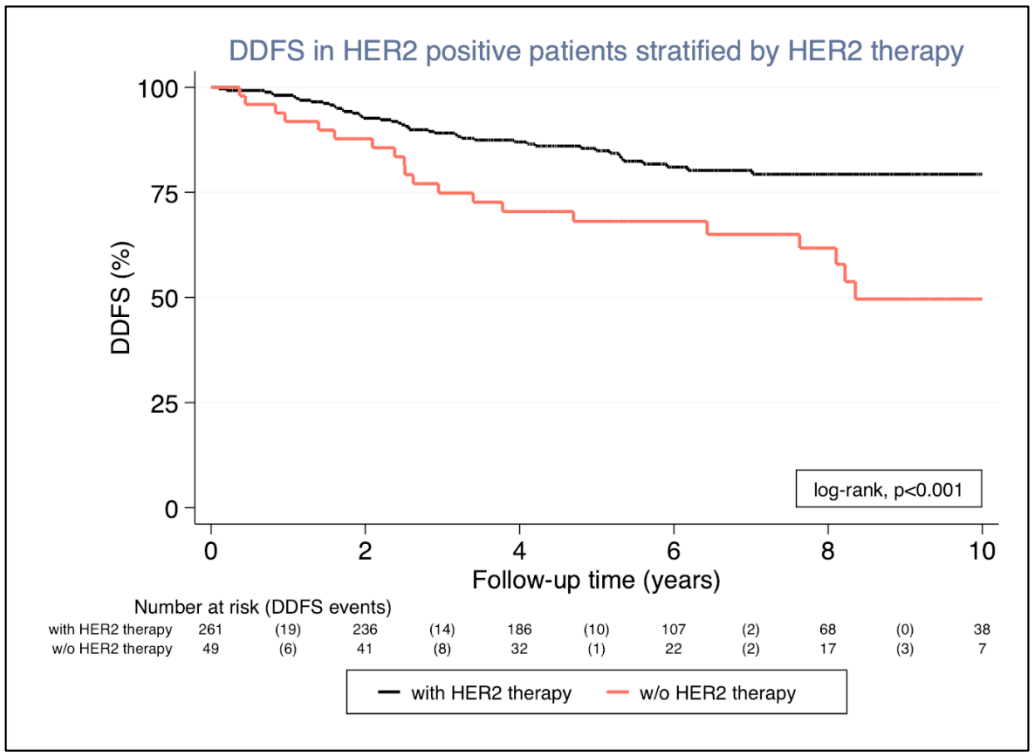

Figure S2. *Distant disease-free survival rates for HER2 positive patients in (neo)adjuvant setting who did receive (black graph) and who did not receive (red graph) HER2 targeted therapy.*

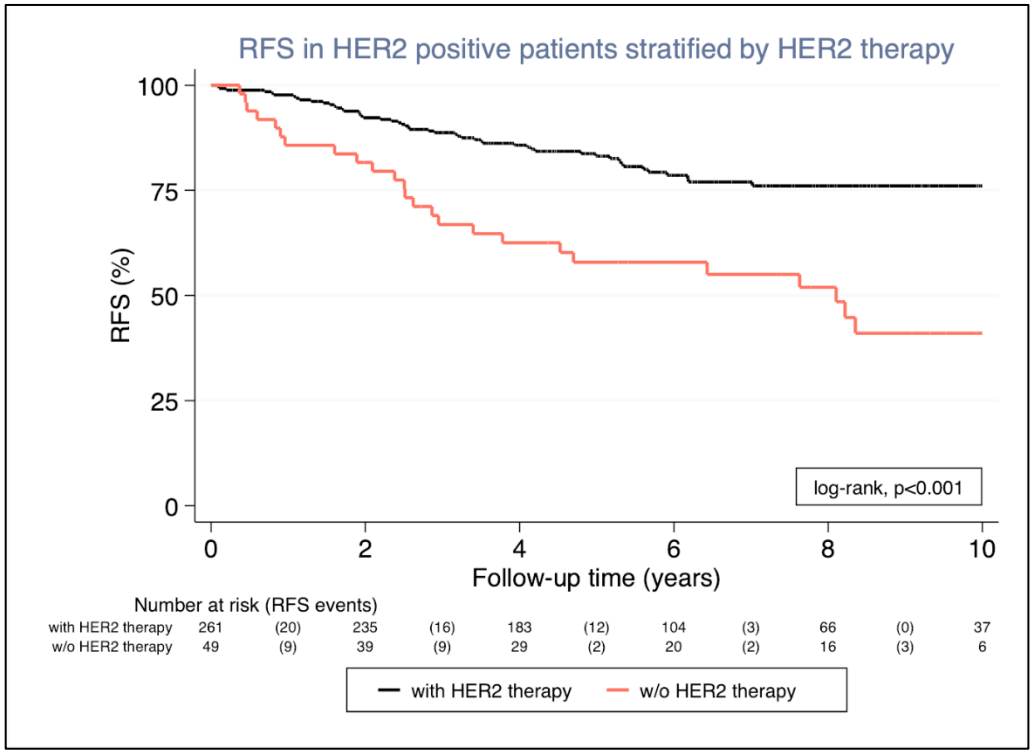

Figure S3. *Recurrence-free survival rates for HER2 positive patients in (neo)adjuvant setting who did receive (black graph) and who did not receive (red graph) HER2 targeted therapy.*

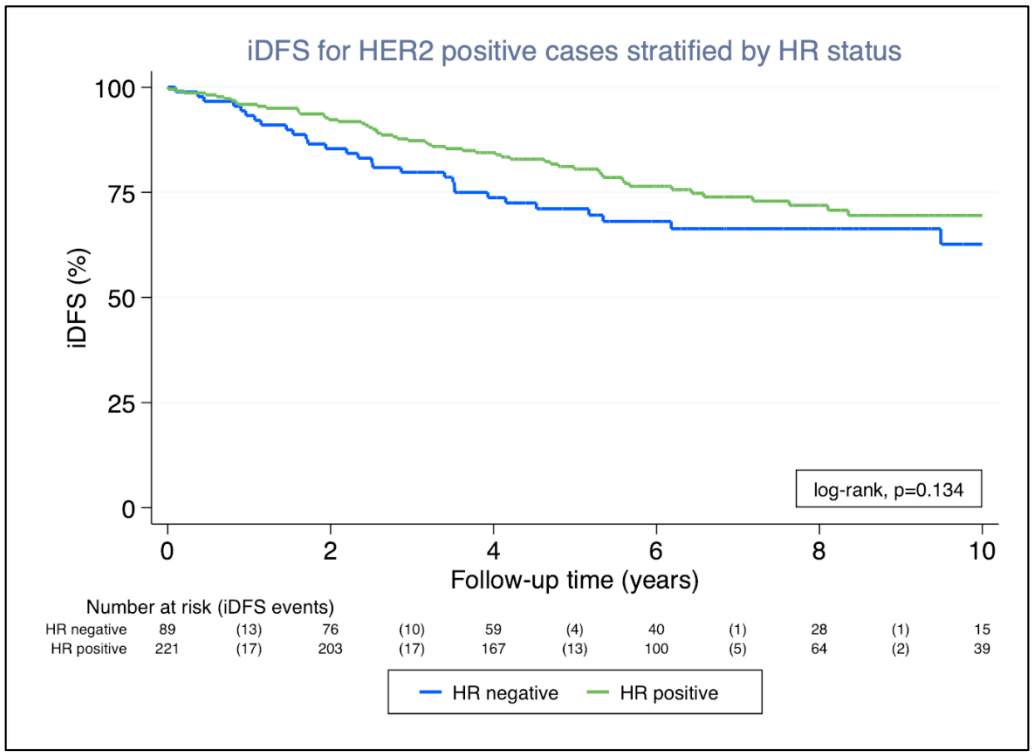

Figure S4. *Invasive disease-free survival rates for HER2 positive patients in (neo)adjuvant setting with positive (green graph) and negative (blue graph) hormone receptors.*

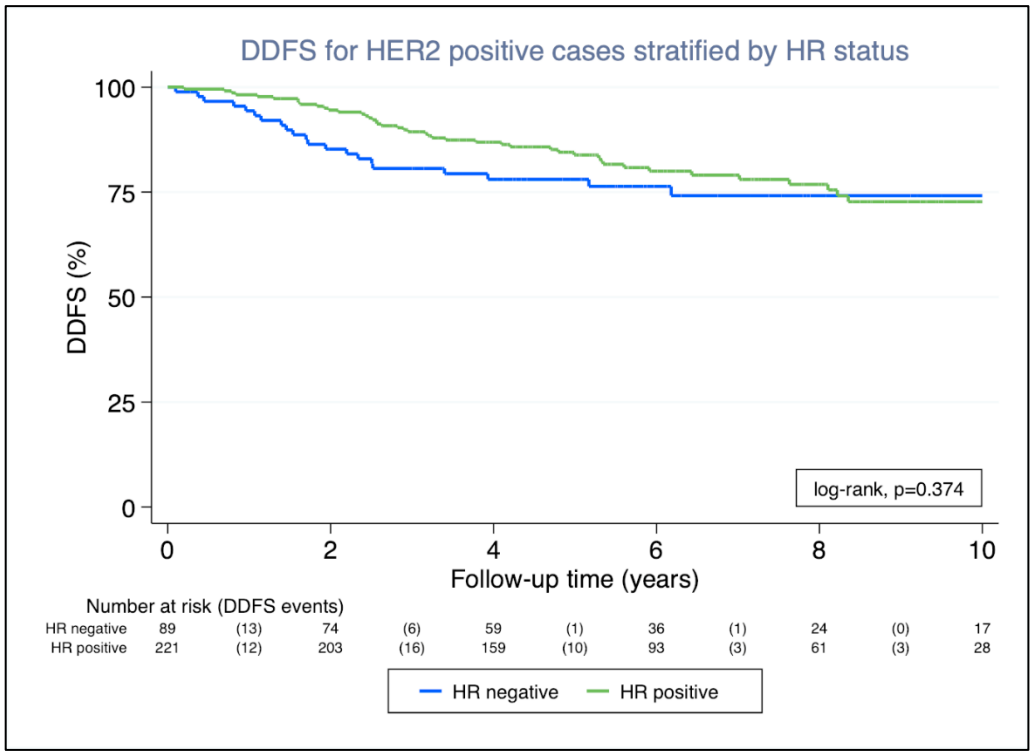

Figure S5. *Distant disease-free survival rates for HER2 positive patients in (neo)adjuvant setting with positive (green graph) and negative (blue graph) hormone receptors.*

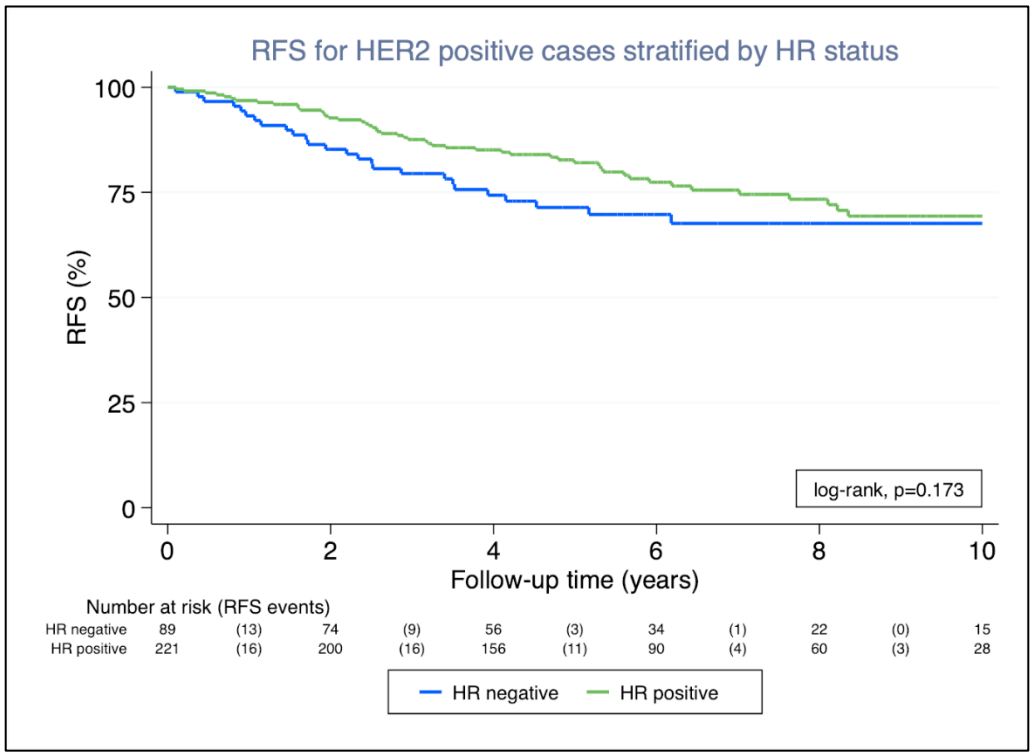

Figure S6. *Recurrence-free survival rates for HER2 positive patients in (neo)adjuvant setting with negative (blue graph) and positive (green graph) hormone receptors.*

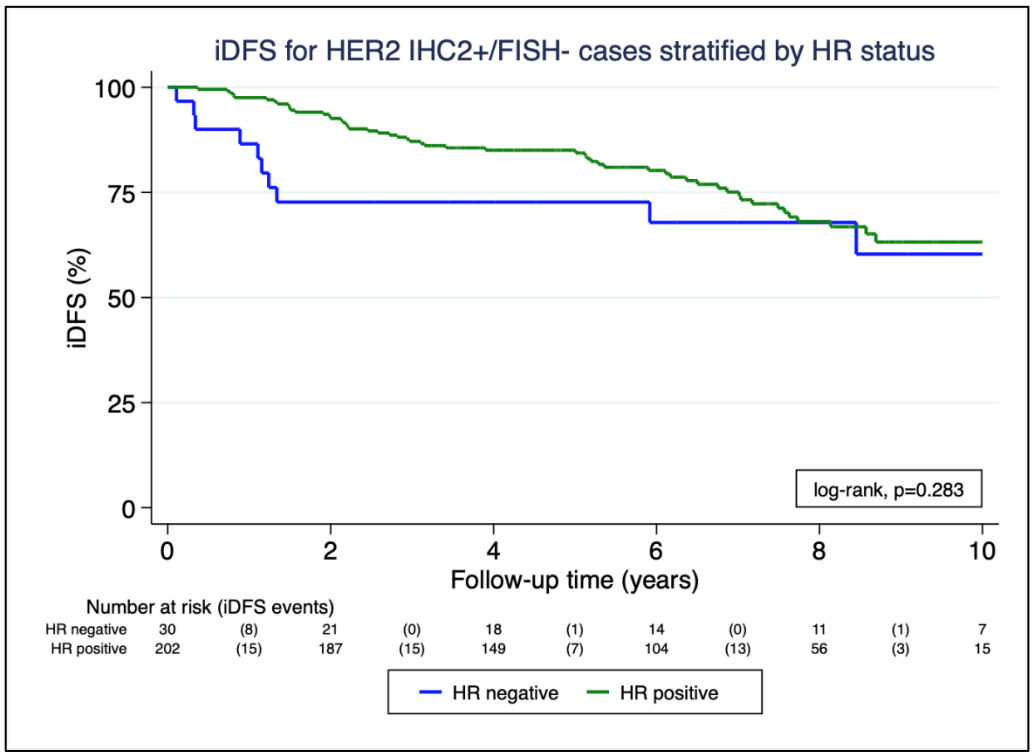

Figure S7. *Invasive disease-free survival rates for HER2 negative patients in (neo)adjuvant setting stratified by hormone receptors. The blue graph displays hormone receptor negative cases, i.e. basal like breast cancer, whereas the green one displays hormone receptor positive cases, i.e. luminal like breast cancer.*

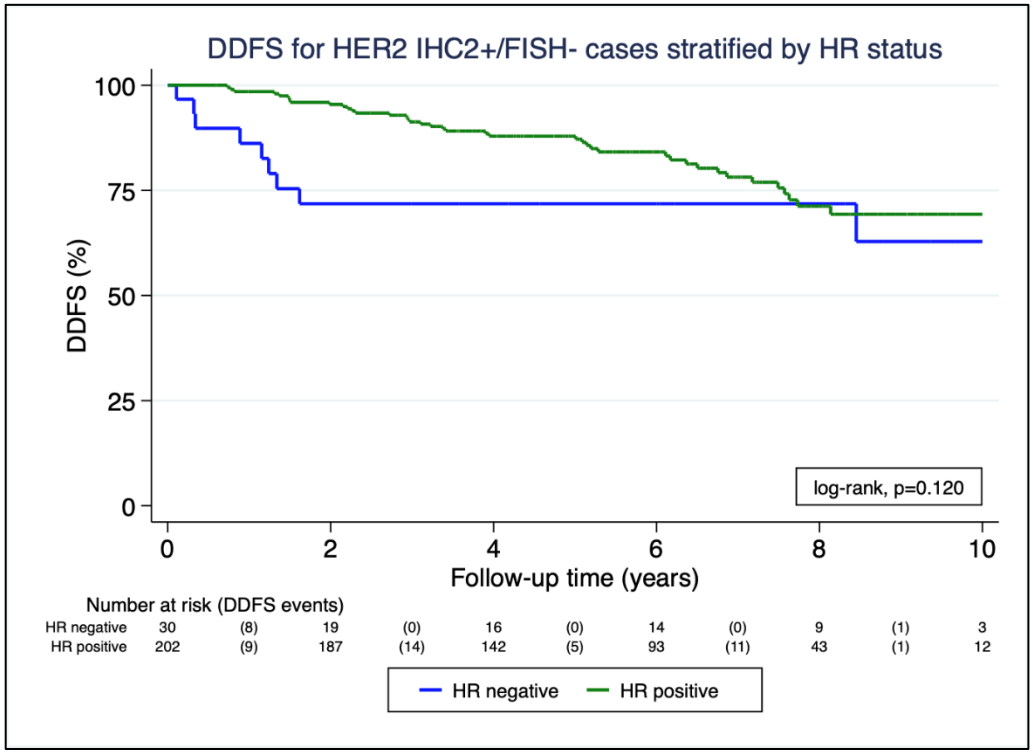

Figure S8. *Distant disease-free survival rates for HER2 negative patients in (neo)adjuvant setting stratified by hormone receptors. The blue graph displays hormone receptor negative cases, i.e. basal like breast cancer, whereas the green one displays hormone receptor positive cases, i.e. luminal like breast cancer.*

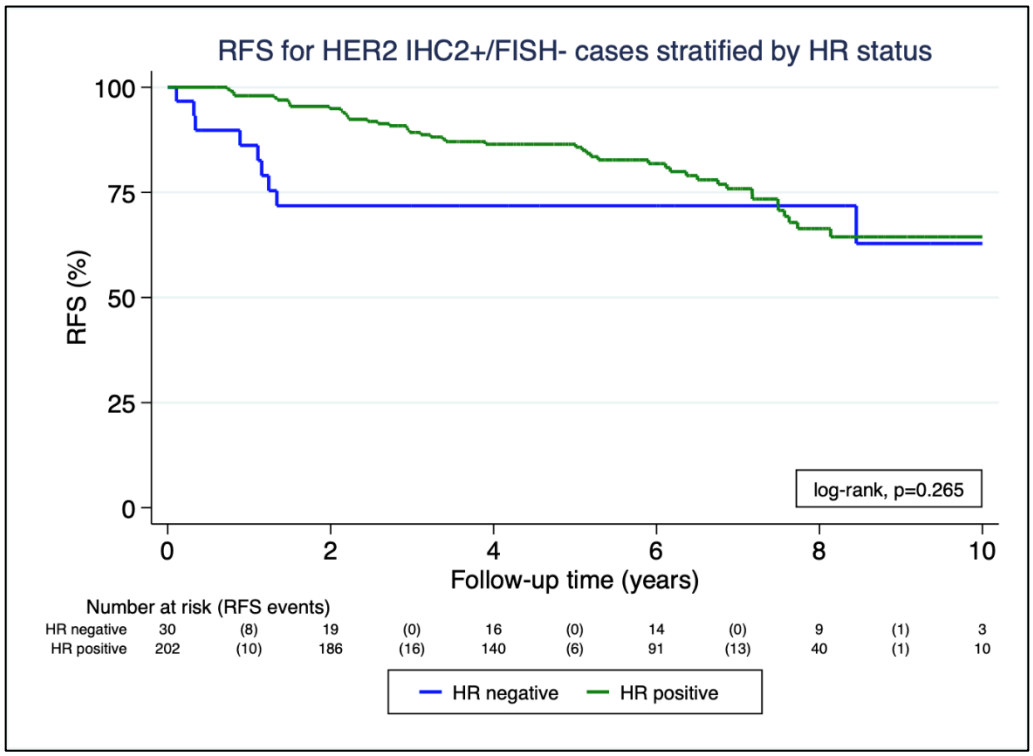

Figure S9. *Recurrence-free survival rates for HER2 negative patients in (neo)adjuvant setting stratified by hormone receptors. The blue graph displays hormone receptor negative cases, i.e. basal like breast cancer, whereas the green one displays hormone receptor positive cases, i.e. luminal like breast cancer.*