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MMed Part III (Minor Dissertation)

Treatment outcomes of Her-2 positive Breast Cancer in the absence of Her-2 targeting agents at Groote Schuur Hospital, Cape Town, South Africa. Are we doing enough?

by

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DECLARATION

I, Dr Aqeela Sirkhotte, declare that the work on this study is my original work except where acknowledgements are indicated. This is an unsponsored study and was carried out for educational purposes only and towards a postgraduate MMed degree. I therefore declare no conflict of interest. I empower the University of Cape Town and the College of Medicine South Africa to reproduce this document as a whole or in part, if necessary, for research or educational purposes. This dissertation has been sent in to the Turnitin module, and I can attest that my supervisor has seen the report and that we have addressed any issues raised.

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Dr Aqeela Sirkhotte

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PUBLICATION-READY FORMAT

This dissertation is being submitted as a publication-ready manuscript according to the SA Journal of Oncology submission guidelines. This article has not been submitted for publication.

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Part A: Abstract

Treatment outcomes of Her-2 positive Breast Cancer in the absence of Her-2 targeting agents at Groote Schuur Hospital, Cape Town, South Africa. Are we doing enough?

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ABSTRACT

Purpose: Breast Cancer has an estimated 2.3 billion cases globally. It is the second most common cause of cancer-related mortality in Sub-Saharan Africa. The addition of anti- Her-2 targeted therapy (Trastuzumab) to either adjuvant or neo-adjuvant treatment significantly improves disease-free survival and overall survival of women with early-stage *Her-2* positive breast cancer. However, at Groote Schuur Hospital anti-*Her-2* targeted treatment is not available. We have therefore evaluated to what extent our patients may be disadvantaged. The primary aims of this study were to evaluate the 3 and 5-year overall survival (OS) and recurrence free survival (RFS) of patients treated for *Her-2* positive breast cancer at Groote Schuur Hospital. Secondary outcomes were to compare the OS and RFS of patients with hormone- receptor positive (HR+) and hormone-receptor negative (HR-) *Her-2* positive (*Her-2+*) breast cancer.

Methods: A retrospective folder review was conducted of all patients treated for *Her-2* positive breast cancer between January 2016 and December 2016 at the breast oncology clinic at Groote Schuur Hospital, Cape Town. All patients with histologically proven *Her-2* 2+ and 3+ breast cancer stage I to III between 25 and 70 years old were included. Of the 561 patients on the hospital's electronic patient registry (EPR), 112 fulfilled the inclusion criteria and of these 87 patients were included for formal analysis due to missing folders. OS and RFS were calculated using Kaplan Meier analysis. A multivariate analysis using the Cox regression model and log rank testing was used to determine any association between OS or RFS and age, hormone receptors, or clinical stage. A p-value of <0.05 was used to determine statistical significance.

Results: The cumulative OS and RFS at 3 years were 87% and 80%, respectively. The 5-year cumulative OS were 78% (*Her-2* 2+) and 73% (*Her-2* 3+) respectively. The 5-year RFS was 73%. The OS and RFS for patients with HR+ tumours were statistically significantly greater than those of HR- tumours (p=0.006 and p=0.024 respectively). The 3-year OS for HR+ and HR- was 92% and 77%. The 5-year OS for HR+ and HR- tumours was 89% and 55%, respectively. The RFS at 3 years for HR+ and HR- was 84 % and 69 % respectively, and 82% and 55%, respectively at 5 years. The Cox regression analysis showed stage and HR status had a contribution to RFS. The log rank testing showed HR+ has a significant contribution to both OS and RFS.

Conclusion: The 5-year OS and RFS rates of our study were similar to those reported in long term prospective trials published in the pre-Trastuzumab era. In comparison to data published with the addition of Trastuzumab to treatment, our results were inferior. In addition, *Her-2* positive HR+ tumors had superior outcomes to *Her-2* positive HR- breast cancer tumors. Twenty-five years since the advent of Trastuzumab, at Groote Schuur Hospital does not have access. In a resource limited setting where *Her-2* targeted agents for all indicated patients may not be possible, a case may be made for provision to patients with *Her-2* HR- disease.

Part B-Publication ready Manuscript

According to the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), 2020 breast cancer has surpassed lung cancer as the most common cancer worldwide, with an estimated 2.3 million new cases globally (1,2). Breast cancer is one of the leading causes of cancer deaths in over 100 countries, and the second most common cause of cancer-related mortality in Sub-Saharan Africa (3). The global burden of breast cancer is reported to be an age standardized incidence rate of 43.3 per 100,000 women per year and an age standardized mortality rate of 12.9 per 100,000 women per year (1,2,3). In South Africa, breast cancer accounts for 27.1% of all new cancers diagnosed in women of all ages (1,4). The mortality rate of breast cancer is higher in less developed countries, with women in transitioning countries having a 17% higher mortality rate compared to women in transitioned countries (3). This may be due to multiple factors such as lack of screening, patient education, late presentation and the availability of effective treatments like the Her-2 targeting agent, Trastuzumab(3). Human epidermal growth factor receptor Her-2 is overexpressed in approximately 15-20% of newly diagnosed breast cancer tumors (5). The overexpression of the Her-2 receptor in breast cancer is associated with a more aggressive disease, a higher recurrence rate and a poorer prognosis (5,6).

Her-2 Pathophysiology

The Her-2 receptor is part of the epidermal growth factor family, and the Her-2 gene is located on the long arm of chromosome 17 (6). Her-2 overexpression is caused by the amplification of the Her-2 gene, which results in increased levels of the Her-2 receptor on the tumor cell surface. Upon ligand binding to the extracellular domain of the Her-2 receptor, Her-2 proteins are dimerized, and transphosphorylation of their intracellular domains occurs (7). Hetero or homodimerization results in a cascade of intracellular signaling pathways, namely RAS, Raf, Phosphatidylinositol 3-kinase, Mammalian target Rapamycin, and Mitogen activated protein kinase (MAPK), promoting cell growth, proliferation and cell survival (6–9). The overexpression of the Her-2 receptor has also been shown to dysregulate both intrinsic and extrinsic pathways of apoptosis (9). Thus, Her-2 targeting has become an important part of modern breast cancer treatment in patients with Her-2 overexpression.

Her-2 Testing

There is no gold standard for Her-2 testing; however, immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) tests are the most commonly used techniques (6,10). The IHC score is on a scale of 0-3+ with 0 and 1+ being considered negative, 2+ considered equivocal and 3+ considered positive (10,11). FISH testing is often performed to verify equivocal Her-2 2+ results. The American Society of Oncology guidelines recommend repeat testing for IHC Her-2 2+ by an independent observer or with a FISH test. Confirmatory FISH tests or repeat IHC testing ensure that all patients requiring Trastuzumab are accurately

identified. However, poorly resourced centres may not always have the capacity to perform confirmatory tests on all equivocal results, and FISH testing may not be prioritized, particularly if Her-2 targeted agents are unavailable.

Trastuzumab

In September 1998 the Food and Drug Administration (FDA) approved the use of Trastuzumab in Her-2 positive metastatic breast cancer (12,13). Trastuzumab is a recombinant humanized monoclonal antibody that selectively targets the extracellular domain of the Her-2 receptor. Since 1998, numerous large randomized clinical trials have demonstrated the efficacy of Trastuzumab in the adjuvant setting (7). The Herceptin adjuvant trial (HERA), National Surgical Adjuvant breast and Bowel Project (NSABP-31), Central Cancer Treatment Group (NCCTG) N9831 and the Breast Cancer International Research (BCIRG), were four major trials that confirmed Trastuzumab to be an effective adjuvant treatment (13,14). The trials included more than 13000 women with early-stage Her-2 positive breast cancer. The above mentioned trials showed that Trastuzumab reduces the 3-year risk of recurrence by approximately 50 percent (14–16). The benefit was similar across the trials despite differences in patient population, chemotherapy regimen and sequencing of treatment (14,15). The HERA trial in its final analysis including 11 years of follow-up concluded that the disease-free survival was improved by 6.3% at three years (17). In addition, the trial concluded that one year of adjuvant Trastuzumab was sufficient to significantly improve overall survival and disease-free survival. However, extending treatment with Trastuzumab to two years showed no added benefit (17). In addition, subgroup analysis highlighted the importance of tumor HR status (17). Despite Her-2 receptor overexpression, HR status remains a significant determinant of disease outcome, with more recurrences and deaths in women with HR- breast cancer (14,15). With the advent of Trastuzumab, the prognostic landscape for Her-2 positive breast cancer has improved substantially (15). The addition of Trastuzumab to either adjuvant or neo-adjuvant treatment significantly improves DFS and OS of women with early-stage *Her-2* positive breast cancer, by as much as 50% and therefore is the standard of care for the last 25 years (12,13,15,17).

While the efficacy of Trastuzumab has been established for many years, many centres in resource limited settings still cannot afford this drug. Indeed, State health facilities in the Western Cape, including Groote Schuur Hospital, do not have access to Trastuzumab. The aim of this retrospective study is to provide tangible information on the outcomes of patients treated for Her-2 positive breast cancer at Groote Schuur Hospital. These findings will thus allow evaluation of real-world data in a population of Her-2 positive patients in the absence of Her-2 targeting treatment. Comparison with previous published data may allow an estimation of the effect that the absence of Trastuzumab may have and may thus inform recommendations within the Western Cape's public health sector.

Methods

Study aims and Objectives.

The purpose of this study was to evaluate the treatment outcomes for patients treated for Her-2 positive breast cancer at Groote Schuur Hospital.

The primary objectives:

- 1) To determine the 3 and 5-year overall survival (OS) and recurrence free survival (RFS) rates for patients treated for Her-2 positive breast cancer.

The secondary objectives:

- 1) To compare OS and RFS of patients with HR+ and HR- Her-2 positive breast cancer.
- 2) To evaluate if HR, clinical stage, or age had an impact on outcomes.
- 3) To document patient and tumor characteristics.

Study design

A retrospective folder review was conducted of all patients treated for Her-2 positive breast cancer between January 2016 and December 2016 at the breast oncology clinic at Groote Schuur Hospital, Cape Town. All patients with histologically proven Her-2 positive breast cancer stage I to III between 25 and 70 years old were included. Patients with stage IV disease or whose treatment was initiated at an institution other than Groote Schuur Hospital were excluded from the study. Ethical approval was obtained from the University of Cape Town's Human Research Ethics Committee (HREC approval number 643/2021).

Her-2 positivity was determined from IHC, which was performed on all breast biopsy specimens. Tumours were deemed to be definitively Her-2 positive if scored as Her-2 3+ on IHC. Equivocal (Her-2 2+) results confirmed positive using FISH were deemed to be Her-2 positive. Due to cost, nationally remote testing, and lengthy testing turnaround times, not all Her-2 2+ samples were sent for FISH confirmation. Such Her-2 2+ samples in the absence of FISH were assumed to be Her-2 positive so as not to undertreat.

Treatment:

All patients were treated with surgery and chemotherapy. Radiotherapy and hormonal therapy were initiated only if indicated. All patients were reviewed at the breast multidisciplinary team meeting prior to initiating treatment. The decision for radical treatment was based on various patient characteristics such as Eastern Co-operative Oncology Group (ECOG) performance status, age, medical co-morbidities, clinical stage, and extent of the tumor. Neoadjuvant chemotherapy was offered to reduce the tumor size, allowing for wide local excision (WLE), or rendering an inoperable tumor operable. If no neoadjuvant chemotherapy was initiated, then adjuvant chemotherapy was offered depending on post-operative histological findings.

The type of surgical resection was based on a clinical and radiological assessment of the tumor, and the probability of obtaining a complete tumor resection as determined by the breast surgeon. The surgery was WLE, or a total mastectomy as determined by the multidisciplinary clinic in consultation with the patient. The lymph nodes were addressed by either sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND). A SLNB was performed if the patient was clinically and radiologically N0. All patients with clinically palpable nodes or nodes visible on radiological imaging were offered an ALND.

Adjuvant whole breast radiotherapy was offered to all patients who had WLE. Radiotherapy was also given to patients who had a mastectomy with high-risk pathological features.

All HR+ patients were offered hormone therapy. Tamoxifen (a selective estrogen receptor modulator) was initiated in both pre- and post-menopausal patients with HR+ breast cancer. Anastrozole (an aromatase inhibitor) was only initiated in post-menopausal patients with contra-indications or intolerable side effects from Tamoxifen.

Follow-up and evaluation of Her-2 positive breast cancer patients after radical treatment at Groote Schuur Hospital involves six monthly follow-up visits for the first year, then annually for a total of three years. Follow-up visits include a physical examination, to assess for local recurrence or distant metastases. Mammography is performed annually; other radiological and blood investigations are performed only if there is a clinical suspicion of local recurrence or distant metastases. At the end of three years, all patients in clinical remission are discharged and referred to their primary health care facility for follow-up.

Statistical analysis

Survival outcomes were analysed using Kaplan-Meier analysis. OS was calculated from the date of diagnosis until death from any cause. RFS was calculated from the date of treatment completion until the time of recurrence or death. A log rank test was used to compare survival between patients with Her-2 positive HR+ and Her-2 positive HR- breast cancer. Other variables collected were age, performance status, medical co-morbidities, menopausal status, histology, staging radiology, clinical and pathological staging, tumor characteristics, and the treatment modality. The variables were documented and described using simple percentages and descriptive statistics. A multivariate analysis using the Cox regression model was used to determine any association between survival outcomes and age, HR status, and clinical stage. The data was analyzed using SPSS software (version 29.0 IBM, USA). A p-value of <0.05 was used to determine statistical significance.

Results

Of the 561 patients diagnosed with breast cancer in 2016, 112 patients had Her-2 positive breast cancer and fulfilled the criteria to be included in the retrospective folder review. There were 25 missing folders, therefore only 87 patients were included for formal analysis.

The patients ranged in age from 25 to 70, with most of the patients being over 40 years [Table1]. All patients presented with symptoms. The majority (77%) of the patients had an ECOG (Eastern Co-operative Oncology Group) performance status of 1. Of the patients who had co-morbidities, hypertension was the most common. Four patients tested positive for

HIV at cancer diagnosis and were subsequently initiated on anti-retroviral treatment [Table 1].

All the patients had infiltrative ductal carcinoma except for 1 patient with infiltrating lobular carcinoma. Within the cohort, 77% of the patients were Her-2 3+ and 23% were Her-2 2+. The FISH test was run as a confirmatory test in 8% of the patients with Her-2 2+ breast cancer. All the results revealed Her-2 overexpression [Table 1]. In addition, estrogen receptor positivity was found in 70%, and most patients showed high positivity with 67.2% with a score of 7-8/8. A score of <3 was regarded as negative. A Ki-67 test was only done on 6.9% of the patients; of these patients, 4.6% had a score of greater than 40% and 2.3% (n=2) between 20-40%. Progesterone receptors were tested in 33% with 20% having a positive score of 7-8/8. Most of the patients were post-menopausal and 31% were pre-menopausal [Table1].

Clinical staging was by means of the American Joint Committee on Cancer (AJCC). Of the patients, 60% had cT2 and 17.2% had cT3 breast cancer. Clinical N1 nodes were palpable in 33.3% of the patients. Most patients had clinical stage IIA breast cancer, which was followed by stage IIB breast cancer [Table1]. All patients underwent staging mammography and chest x-rays. [Table1].

[Table 1]

Patient and tumour characteristics

| Characteristics | Number (n=87) | Percent |
|--------------------------|---------------|---------|
| Age | | |
| 25-40 | 14 | 16.1 |
| 41-55 | 38 | 43.0 |
| 56-70 | 35 | 40.0 |
| ECOG status | | |
| PS 0- asymptomatic | 0 | 0 |
| PS 1-symptomatic | 67 | 77.0 |
| PS 2- in bed <50% of day | 20 | 23.0 |
| PS 3- in bed >50% of day | 0 | 0 |
| Ps-4 bedbound | 0 | 0 |
| Mammogram | | |
| Screening | 0 | |
| Symptomatic | 87 | 100.0 |
| Co-morbidities | | |
| Hypertension | 40 | 46.0 |
| Diabetes | 9 | 10.3 |
| Ischemic Heart Disease | 4 | 4.6 |
| COAD/Asthma | 4 | 4.6 |
| Hypercholesterolemia | 8 | 9.1 |

| | | |
|--|----|-------|
| Other | 5 | 5.5 |
| None | 40 | 45.0 |
| Menopause | | |
| Pre-menopausal | 31 | 35.6 |
| Post-menopausal | 56 | 64.4 |
| HIV status | | |
| Negative | 83 | |
| positive | 4 | 4.5 |
| Staging Investigations | | |
| Mammogram | 87 | 100.0 |
| CXR | 87 | 100.0 |
| Abdominal ultrasound | 38 | 43.7 |
| Bone scan | 30 | 34.5 |
| CT-chest/abdomen/pelvis | 4 | 4.6 |
| cT-Stage | | |
| T1 | 13 | 14.9 |
| T2 | 53 | 60.2 |
| T3 | 15 | 17.2 |
| T4 | 6 | 6.8 |
| cN-stage | | |
| N0 | 58 | 66.6 |
| N1 | 29 | 33.3 |
| N2 | 0 | 0.0 |
| N3 | 0 | 0.0 |
| AJCC 8th edition staging | | |
| IA | 11 | 12.6 |
| IIA | 32 | 36.8 |
| IIB | 27 | 31.0 |
| IIIA | 7 | 8.0 |
| IIIB | 8 | 9.2 |
| IIIC | 2 | 2.3 |
| Histology | | |
| IDC | 86 | 99.0 |
| Lobular Carcinoma | 1 | 1.0 |
| Her-2 expression | | |
| 2+ | 20 | 23.0 |
| 3+ | 67 | 77.0 |
| FISH-performed | | |
| No | 80 | 92.0 |
| yes | 7 | 8.0 |
| FISH-result | | |
| Positive | 7 | 100.0 |
| negative | 0 | 0.0 |
| Estrogen receptors | | |
| Positive | 61 | 70.1 |
| Negative | 26 | 29.9 |

| | | |
|--|----|------|
| Estrogen receptor score if positive | | |
| 7-8/8 | 41 | 67.2 |
| 4-6/8 | 14 | 23.0 |
| 3/8 | 6 | 9.8 |
| Progesterone receptors performed | | |
| Yes | 35 | 40.0 |
| Not done | 52 | 60.0 |
| Progesterone receptors | | |
| Positive | 6 | 7.0 |
| negative | 29 | 33.3 |
| Ki-67 | | |
| Not done | 81 | 93.1 |
| Score 20-40% | 2 | 2.3 |
| Score >40% | 4 | 4.6 |

IDC: Infiltrative ductal carcinoma

Surgery is a requirement for the radical treatment of breast cancer. Of the patients in our study, 74.7% had a mastectomy and ALND, and 10.3% received a mastectomy and SLNB. Only 15 % of the patients had breast conserving surgery, while 6% had a WLE and SLNB and 8% WLE and ALND [Table2]

Post-operative histology showed that 20% of the patients had a complete pathological response to neoadjuvant chemotherapy [Table 2]. Approximately half of the patients had a pT2 stage. Half of the patients had a grade 3 histology, 33% of the patients had LVSI, and 14.9% of the patients had multifocality. Half of the patients had positive pathological nodes, with the majority displaying extracapsular extension. [Table2]

[Table 2]

Surgery and Pathology

| Treatment | Number | Percent |
|---------------------------|---------------|---------|
| Surgery | (n=87) | |
| Mastectomy + ALND | 65 | 74.7 |
| Mastectomy + SLNB | 9 | 10.3 |
| WLE +ALND | 7 | 8.0 |
| WLE +SLNB | 6 | 6.0 |
| Pathological stage | (n=87) | |
| pT1 | 32 | 36.8 |
| pT2 | 40 | 46.0 |
| pT3 | 10 | 11.5 |
| CPR | 5 | 20.0 |
| Tumor grade | | |
| Grade 1 | 14 | 16.0 |
| Grade 2 | 27 | 31.0 |
| Grade3 | 44 | 50.6 |

| | | |
|-----------------------------|---------------|------|
| LVSI | | |
| No | 58 | 66.7 |
| Yes | 29 | 33.3 |
| Multifocality | | |
| Yes | 13 | 14.9 |
| No | 74 | 85 |
| Nodes positive | | |
| No | 42 | 48.3 |
| Yes | 45 | 51.7 |
| ECE | | |
| Positive nodes, ECE? | (n=45) | |
| No | 18 | 40.0 |
| yes | 27 | 60.0 |

ALND: axillary lymph node dissection, SLNB: sentinel lymph node biopsy, WLE: wide local excision

ECE: extracapsular extension, LVSI: lymphovascular invasion

Neoadjuvant chemotherapy was administered to a quarter of the patients, of whom 60% received ACP, 36% received ECP, and one patient received TC. Adjuvant chemotherapy was offered to 65.5% of the patients, while five patients declined adjuvant chemotherapy. ECP was the most frequently utilized adjuvant chemotherapy regimen, with 55% of patients receiving this treatment regime. [Table3].

While 26% of the patients did not receive adjuvant radiotherapy. Of the patients that received a mastectomy 47.1% received chest wall and supraclavicular radiotherapy and only 11.5% required chest wall radiotherapy alone. Thirteen patients had a WLE, 100% received adjuvant radiotherapy [Table 3].

All HR+ patients received endocrine treatment. Tamoxifen was the most frequently prescribed hormonal treatment, with 78.6% prescribed Tamoxifen and only 14.7% were prescribed Anastrozole. During the follow up period it was found that 6.5% of patients defaulted their hormonal treatment [Table 3].

[Table 3]

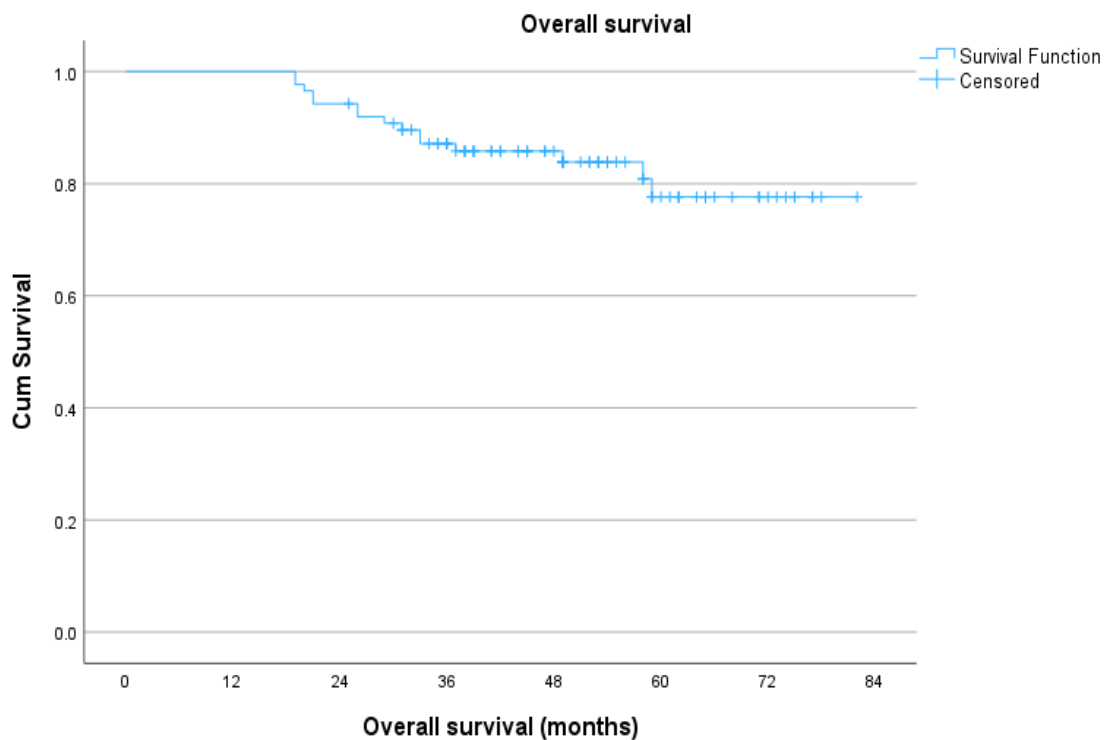
Treatment

| | | |
|------------------------------------|---------------|------|
| Neoadjuvant chemotherapy | | |
| Yes | 25 | 28.7 |
| No | 62 | 71.3 |
| NACT administered | (n=25) | |
| ACP | 15 | 60.0 |
| ECP | 9 | 36.0 |
| TC | 1 | 4.0 |
| Adjuvant chemotherapy | (n=57) | |
| Declined | 5 | 5.7 |
| Yes | 57 | 65.5 |
| Chemotherapy administered | | |
| ACP | 20 | 35.1 |
| ECP | 31 | 54.4 |
| TC | 6 | 10.5 |
| Hormonal Therapy Applicable | | |

| | | |
|------------------------------------|---------------|------|
| Yes | 61 | |
| No | 26 | |
| Hormonal Therapy prescribed | (n=61) | |
| Tamoxifen | 48 | 78.6 |
| Anastrozole | 9 | 14.7 |
| Defaulted | 4 | 6.5 |

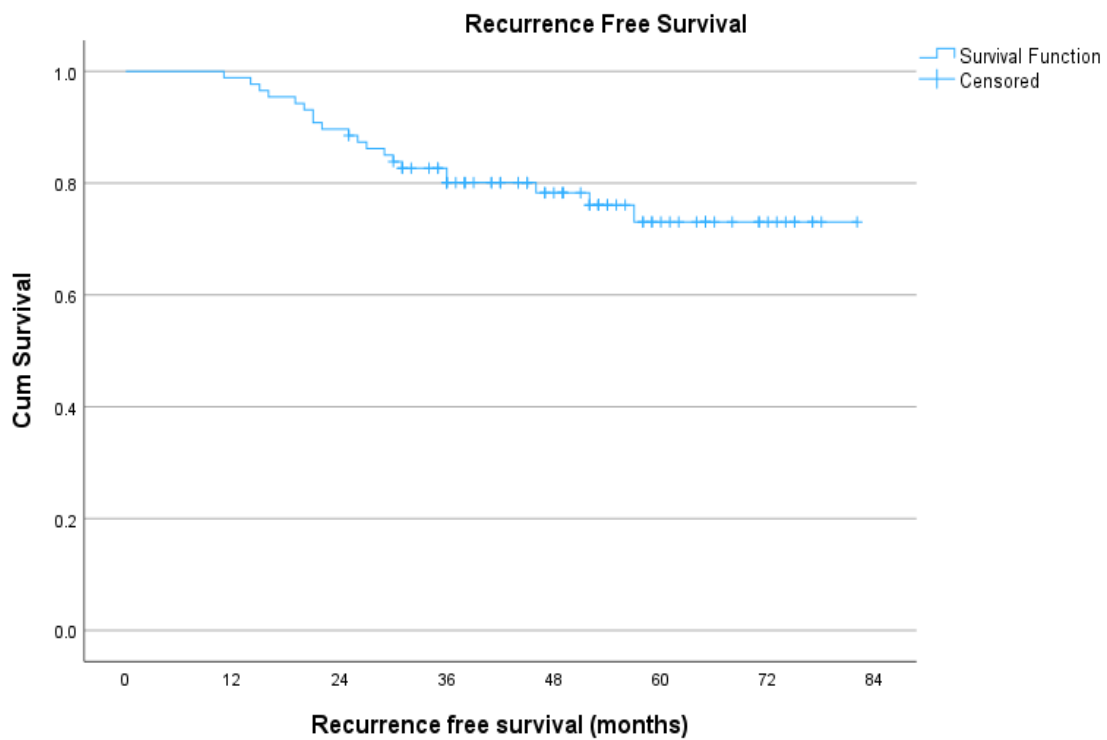
ACP: Adriamycin/Cyclophosphamide/Paclitaxel, ECP: Epirubicin/ Cyclophosphamide/Paclitaxel, TC: Taxotere/Cyclophosphamide

The 3-year OS for the cohort is displayed in Figure 1. Of the 87 patients included, 17% had died. The OS at 3 years after the date of diagnosis was 87%. The OS at 5 years for the entire cohort was 78% for Her-2 2+ and 70% for the Her-2 3+ patients. The mean OS was 72 months (95% CI; 67.44-76.58).



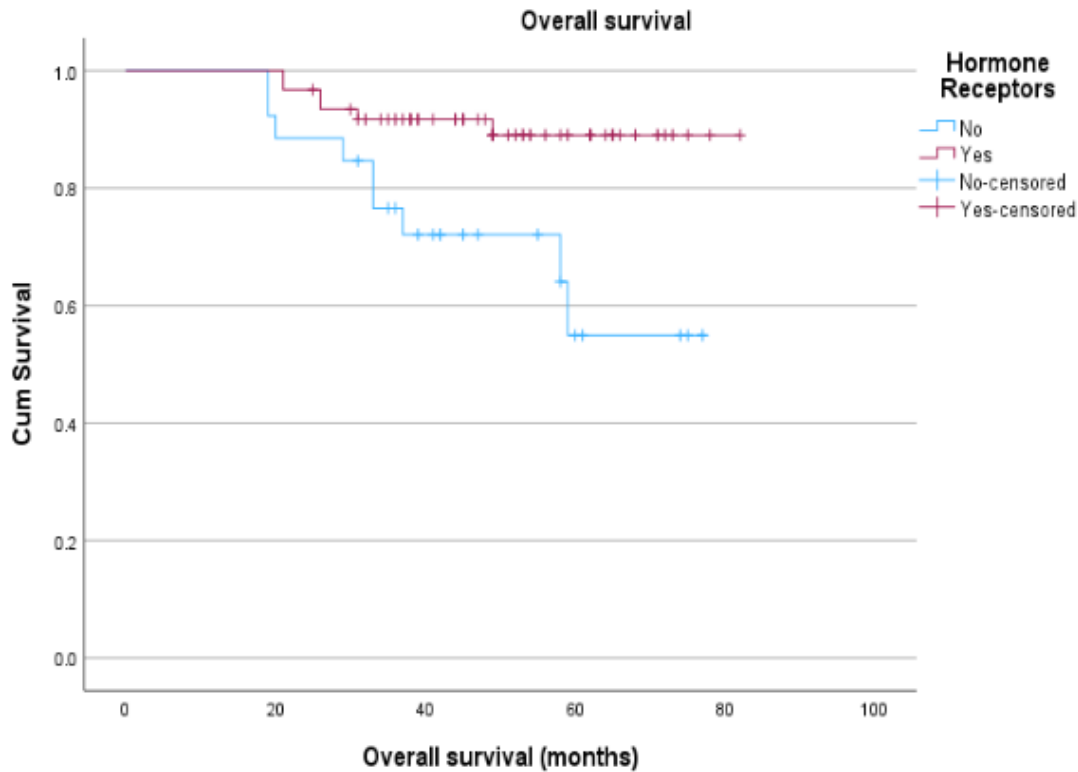
[Figure1] Overall survival

The 3-year RFS for the cohort is displayed in Figure 2. Of the 87 patients, 16 patients recurred. The cumulative RFS at 3 and 5 years was 80% and 73% respectively. The mean RFS was 68.3 months (95% CI; 63.04-73.58).



[Figure2]: Recurrence Free Survival

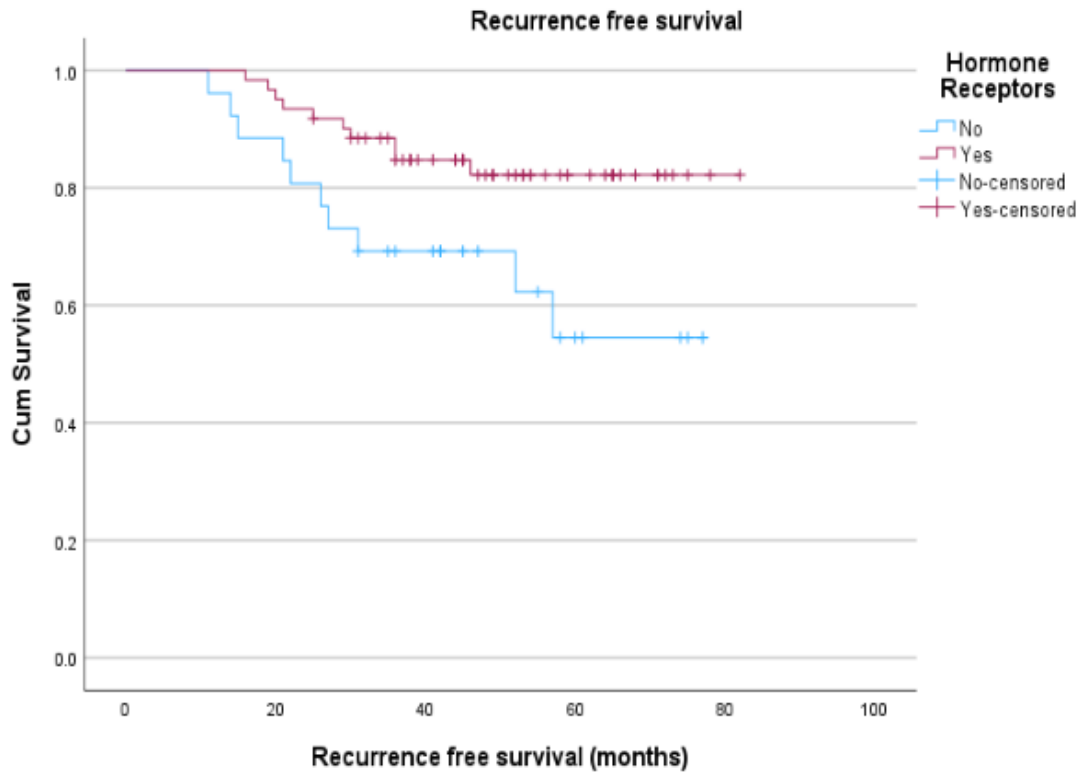
The OS compared between patients with Her-2 positive HR+ and Her-2 positive HR- breast cancer is displayed in figure 3. The OS at 3 years for patients with Her-2 positive HR+ and Her-2positive HR- breast cancer was 92% and 77% respectively. The 5-year OS for Her-2 positive HR+ breast cancer was 89% and 55% for patients with Her-2 positive HR- breast cancer. The mean OS for Her-2 positive HR+ breast cancer was 76.37 months (95% CI;72.10-80.65) and 59.98 months (95% CI;51.05-68.91) for Her-2positive HR- breast cancer. The log rank test showed the OS of patients with Her-2 positive HR+ was superior to that of Her-2 positive HR- breast cancer tumors ($p = 0.006$).



[Figure 3] Overall Survival according to HR status

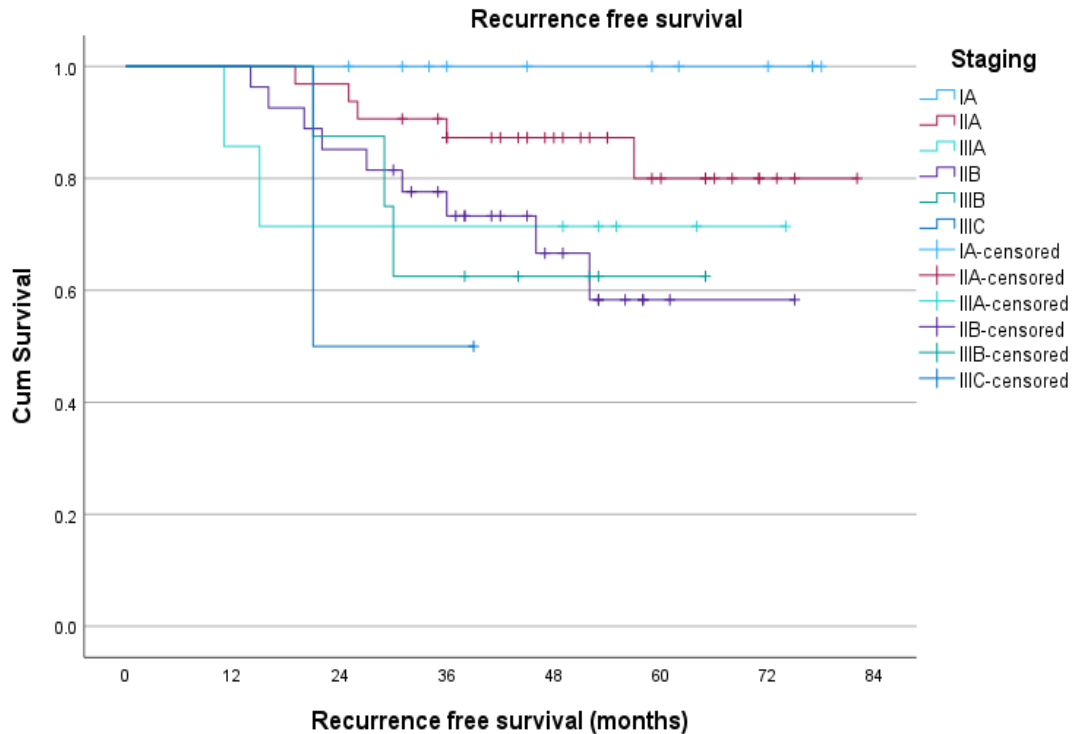
A multivariate cox regression analysis was conducted to determine an association of OS with the variables age, clinical stage, and HR status. The model was statistically significant $\chi^2(3) = 9,981(p=0.019)$. The cox regression analysis showed HR+ to have a better survival (hazard ratio 0.214, 95% CI; 0.069-0.668; $p=0,008$).

The RFS comparing patients with Her-2 positive HR+ and Her-2 positive HR- breast cancer is displayed in figure 4. The RFS at 3 years in patients with Her-2 positive HR+ and Her-2 positive HR- breast cancer was 84 % and 69% respectively. At 5 years the RFS for patients with Her-2 positive HR+ breast cancer was 82% and 55% for patients with Her-2 positive HR- breast cancer. The mean RFS for Her-2 positive HR+ breast cancer was 72.56 months (95% CI;67.19-77.92) and 56.4 months (95% CI;46.36-66.52). The log rank analysis showed HR+ tumours had a superior RFS to HR- tumours ($p=0.024$).



[Figure 4] Recurrence Free Survival according to HR status

The Kaplan Meier curve displayed in figure 5 shows the difference in RFS depending on the clinical stage. The log rank analysis showed that the RFS between stages was not statistically significant. However, there was a notable trend for RFS to decrease with increasing stage.



The following was observed:

- IA:** 11 patients, no recurrence (0%). Recurrence free survival at 3 years (36 months) was 100%.
- IIA:** 32 patients, five recurrence (16%). Recurrence free survival at 3 years (36 months) was 87%.
- IIIA:** Seven patients, two recurrence (29%). Recurrence free survival at 3 years (36 months) was 71%.
- IIB:** 27 patients, nine recurrence (34%). Recurrence free survival at 3 years (36 months) was 73%.
- IIIB:** Eight patients, three recurrence (38%). Recurrence free survival at 3 years (36 months) was 63%.
- IIIC:** Two patients, one recurrence (50%). Recurrence free survival at 3 years (36 months) was 50%.

[Figure 5] Recurrence Free Survival according to Clinical Stage

A multivariate cox regression analysis performed to determine an association between RFS, age, clinical stage, and HR status. The model was statistically significant $\chi^2(3) = 14,611$, ($p=0,002$). The cox regression analysis showed an association between RFS, HR and clinical stage. Both HR and clinical stage had a statistically significant contribution to the RFS (hazard ratio= 0.26, 95%CI; 0.098-0.700, $p=0,008$, hazard ratio=1.44, 95% CI; 1.10-1.89, $p=0,008$) respectively.

Discussion:

Breast Cancer is one of the leading causes of cancer deaths in over 100 countries globally, and the second most common cause of cancer related mortality in Sub-Saharan Africa (3). In our study, in the year 2016 there were 561 newly diagnosed breast cancer patients, with 20% having Her-2 positive breast cancer.

Patients presenting with late-stage breast cancer is more frequent in low to middle income countries, with data showing that 70% of breast cancer in patients in high income countries present with early-stage disease, this includes stages I to IIIA. In low to middle income countries less than 50% of patients diagnosed with breast cancer have early-stage disease (20). In our study 88.6% of the patients had early-stage breast cancer, this is likely attributable to readily available screening and improvement in breast cancer awareness within the Western Cape. In contrast to other provinces in South Africa, a study conducted in the province of Limpopo showed that 76% of patients presented with late stage disease, and the main reason for the delay being a lack of knowledge and public awareness (21). Another study conducted in Soweto, Gauteng showed that limited education, lack of breast cancer knowledge, lack of public awareness as well health system inefficiencies were reasons for the delayed presentation and late-stage breast cancer(22).

From our study patients in the Western Cape the cumulative OS at 3-years was 87%. The 5-year cumulative OS in our study was 78% for Her-2 2+ and 70% for Her-2 3+ patients. Prior to the advent of anti-Her-2 targeted therapy, the prognosis for Her-2 positive breast cancer was much poorer than that of Her-2 negative breast cancer, with 5-year overall survival rates of 63.4% and 83%, respectively (25). Since the adoption of anti-Her-2 targeted therapy the 5-year OS no longer differed, with 88.3% for Her-2 positive and 90.4% for Her-2 negative patients (23). The long-term outcome of patients with early-stage Her-2 positive breast cancer was reviewed in a population based multicenter cohort study conducted in Sweden by Ellegard et. al., who examined the prognostic factors and relapse patterns after the implementation of Trastuzumab. The study found that the 5-year breast cancer specific survival, distant RFS survival, and local RFS were 93.4%, 89.7% and 98.0%, respectively, for Trastuzumab treated patients and 87.4%, 81.6% and 87.4%, respectively, in patients not treated with Trastuzumab (24). The benefit of Trastuzumab was further supported by the 11-year follow-up of the HERA trial (18). The results showed that one year of Trastuzumab significantly reduced the risk of both disease-free survival events and death compared with observation (17). The 5-year OS for our study was comparable to data in the pre Trastuzumab era. Our study found that the cumulative OS at three years was 87%.

However, due to our assumption of Her-2 positivity in Her-2 2+ tumours not confirmed by FISH, it is possible that Her-2 positivity was overestimated in our cohort. In our study, only 7/20 patients with Her-2 2+ had a FISH test to confirm Her-2 overexpression. Thus, approximately 15% of the group deemed her-2 positive were Her-2 2+ unconfirmed by FISH. In a study by Payandeh et al., concordance rates between IHC and FISH testing were 31.1% for IHC Her-2 2+ and 84.1% for IHC Her-2 3+ (25). It is likely therefore that some of the unconfirmed Her-2 2+ would still have been confirmed Her-2 positive on FISH.

In our study, at 3 years the cumulative OS for HR+ and HR- tumors was 92% and 77%, respectively, and at 5 years it was 89% and 55%. In Her-2 positive tumors, hormone receptor status remains a significant determinant of disease outcome, with more recurrences and deaths in women with Her-2 positive HR-breast cancer(14,15). The results from our study echoed this with HR + tumors showing better OS than HR- tumors.

Reported outcomes on the RFS from studies conducted: Gonzalez et al. reported on 965 Her-2 positive T1a, b N0 M0 breast cancers diagnosed between 1990 and 2002 at the M. D. Anderson Cancer Center (MDACC) (28). The findings showed patients with Her-2 positive breast cancer had a worse RFS than those who were Her-2 negative, with 5-year RFS rates of 77.1% and 93.7% respectively ($p < 0.0001$)(26).

Within our study, 23% of the patients had a recurrence, with a RFS at 5-years of 73%, which was in keeping with the above-mentioned study. Romond et al reported on the combined analysis of the NSABP-31 and the NCCTG N9831 trials, which were landmark trials investigating chemotherapy combined with adjuvant trastuzumab (12). Two-year median follow up analysis showed a significantly longer disease-free survival with a 52% lower risk of disease-free event and an absolute difference of 12% in the DFS between the groups at three years. In addition, the risk of distant recurrence was further reduced by 53 % (95% CI, 0.37–0.61; $p < .0001$) and at median two-year follow-up there was also a significant effect on overall survival (12). It is estimated when comparing our results to those of published studies that with the addition of Trastuzumab we would improve the RFS and OS at our institution by up to 50% in early-stage breast cancer.

Gomez et al. reviewed the prognostic effect of hormone receptor status in early Her-2 positive breast cancer. The study showed that Her-2 positive HR- patients were significantly more likely to relapse than Her-2 positive HR+ patients, with 5-year RFS rates of 65.0% and 74.6%, respectively ($P=0.045$) (18). This study also concluded that patients with Her-2+ positive HR- breast cancer have a tendency towards earlier recurrences (18,19). The HERA trial also concluded that one year of adjuvant Trastuzumab after chemotherapy for patients with early-stage Her-2 positive breast cancer significantly improves the long term DFS compared with observation. Chumsri et al. revealed similar findings after performing a multivariate analysis on two groups that included Her-2 positive HR- and Her-2 positive HR+ tumors (29). HR+ tumors were shown to have a better RFS in the first five years (27). Within our study, 70% of the patients had HR+ tumors. The cumulative RFS at 5 years with HR+ and HR- tumors was 89% and 55 % respectively. These results are in keeping with the studies mentioned above. Moreover, Ellegard et al., in a multicenter cohort study showed the main prognostic factors for locoregional recurrence and distant recurrence to be: lymph node status, hormonal status, and trastuzumab treatment (24). We showed Her-2 positive HR+ breast cancer tumors to have superior OS and RFS compared to Her-2 positive HR- breast cancer tumors. Similarly, our study showed HR+ to have an impact on patient outcomes. With Her-2 positive HR- breast tumours showing inferior outcomes, where financial resources are limited, this subgroup should be considered for ant- Her-2 targeted therapy.

The protocol at Groote Schuur Hospital has been amended since 2016; to incorporate Ki- 67 and progesterone receptors being performed routinely on all biopsy specimens. This assists the MDT decision making upon initiating neoadjuvant chemotherapy particularly when Her-2 2+ scores are present. Neoadjuvant chemotherapy is currently administered to more patients, this enables for prognostication, more WLE and SLNB being performed. Anastrozole has also become freely available within the state health system. While these improvements in care will have benefited patients. Unfortunately, due to limited financial resources within the state health system, twenty-five years later Trastuzumab is still not accessible to Her-2 positive breast cancer patients at Groote Schuur Hospital. The results of our study have shown our 5-year OS and RFS is comparable to data pre- Trastuzumab era. It also showed that HR- breast cancer tumors had inferior outcomes compared to HR+ breast cancer tumors, with the addition of Trastuzumab this would improve our outcomes by as much as fifty percent.

Limitations

The following factors may have impacted the outcomes of the study; a larger sample size may have been more representative. The missing folders may have added valuable information to the results of the study. The inclusion criteria were limited to patients younger the 70 years old and an ECOG performance

status of 2 and less. These inclusion criteria may have excluded patients who may have had different outcomes. The limited number confirmatory FISH tests may have potentially created an overestimation of the true number of Her-2+ positive breast cancer patients.

Conclusion

The results of our study have shown our 5-year OS and RFS is comparable to data pre- Trastuzumab era. Our study also showed that Her-2 positive HR- breast cancer tumors had inferior OS and RFS compared to Her-2 positive HR+ breast cancer tumors. Future recommendations within a limited resource constrained health system with the above findings and available data, Trastuzumab should be considered for at least twelve months for early-stage Her-2 positive breast cancer. Our study showed that patients who had HR- had worse outcomes. With limited funding this population would benefit the most from anti-Her-2 targeted therapy.

Ethical considerations:

This study was a retrospective study approved by the University of Cape Town's Human Research Ethics Committee. The retrospective design of this study did not infringe upon the rights or care of patients. Confidentiality was maintained using unique patient folder numbers, therefore patient anonymity maintained.

Competing interests

The authors declare they have no competing interests.

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