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Thesis

Clinical Characteristics of Patients Diagnosed with Triple Negative Breast Cancer in Cyprus: A Retrospective Analysis

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Abstract

Trible Negative Breast Cancer (TNBC) is a distinct subtype of Breast Cancer (BC) defined by the absence of Hormone and HER2 receptors on the cell surface of cancer cell. TNBC absence of hormone receptor suggests lack of target specific treatment and chemotherapy remained as the major therapeutic option for systemic treatment until recently. TNBC is connected with worse prognosis and high rate of recurrence during the first 5 years after diagnosis. In this retrospective study an analysis of clinical characteristics of patients diagnosed with TNBC and treated in Bank of Cyprus Oncology Center in the decade 2008-2017 was conducted. Data was collected from electronic registry and medical documents of the patients.

330 women were included in TNBC cohort and 25 women in weak HR positive cohort. Patients median Age of Diagnosis was 58 years old. Only 0,92% of the patients were diagnosed with de novo metastatic disease. 5-year OS rate was evaluated at 75,41% and median OS did not reach at the end of follow-up. The Median Ki67 score was 60%. 10,95% of the examined patients had germline BRCA1 (5,84%) or BRCA2 (5,11%) mutations. Stage (HR 1,5; p<0,0001) and Ki67% were poor prognostic factors whereas Anthracycline based chemotherapy regiment on adjuvant or neoadjuvant setting was connected with favorable prognosis compared to non-Anthracycline based chemotherapy regiments (HR 0,51; p<0,0001). 69 patients (21,1%) presented relapse (local or systemic) during follow-up period. Median OS after recurrence was 23 months. Patients with systemic relapse had shorter OS (HR 3,52, p=0,004). Most common site of metastasis was lower respiratory tract (59,42%). 36,23% of patients that recurrence occurred presented CNS involvement (brain metastasis or leptomeningeal disease).

Management of recurrent and metastatic TNBC remains challenging. Better understanding of TNBC, which presents genetic and genomic variability, is needed for the development of novel therapeutic agents and specific biomarkers based on precision medicine.

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Chapter 1

Introduction

1.1 Epidemiology

BC cancer has become the most common cancer diagnosed globally and it accounts for 1 out 8 cancer diagnosis. 2.3 million new cases of BC were diagnosed in both sexes in 2020 worldwide and it represents 25% of cancer cases in females.[1,2] BC is the leading cancer diagnosis in women in 157 out 185 countries providing data to Global Cancer Observatory.[3] According to data extraction from GLOBOCAN 2020, a database gathering information from 185 countries worldwide, 2.3 million cases worldwide of female BC were diagnosed. De novo metastatic disease accounts for 3-6% in high income countries and 10-30% in countries with lower income countries.[4]

The highest incidence rates, reaching more than 80 cased in 100.000 females, were observed in developed countries (Australia, New Zealand, Northern America, Western and Northern Europe). Whereas the lowest incidence of newly diagnosed BC was observed in developing regions (Central America, South Africa, Middle Africa and South-Central Asia), with the rates being lower than 40 cases every 100.000 women. Disparities in treatment and early diagnosis of BC are evident by the fact the mortality rates are much higher in transition countries than in countries with high socioeconomic status.[5] Differences between coverage rates of organized screening programs according to European Commission Initiative evident even between European countries. Cyprus has one of the lowest rates of biannually examination in patients in ages between 50-69 years. In year 2017, only 35.1% of women took part in organized screening program however, the total coverage of women population went to 63.4% due to opportunistic examination.[6]

It is estimated that in 2040 the number of annual numbers of BC diagnosis will be increased by 40% reaching 3 million cases globally. Whereas the deaths of BC are

estimated to present rise by 50% reaching 1 million deaths every year, while in 2020 685000 deaths were noted. The main factors corresponding for the estimated increase are the aging population and global population growth.[5]

TNBC accounts for approximately 10-20% of all invasive Breast Cancer Cases. The incidence is higher in premenopausal, African American women and females under 40 years old. Additionally, TNBC is the most common BC subtype in patients with germline BRCA mutations.[7,8,9]

1.2 Classification of Breast Cancer

Intrinsic classification of BC was determined by Perou et al. in 2000 based on microarrays analysis of 8100 genes surgical specimens of BC. Through this analysis the phenotypic diversity of tumors was associated with gene expression diversity that could be proven by microarrays analysis. By analyzing gene expression patterns, a "molecular portrait" of tumors was obtained that could be interpreted into the biological behavior of a tumor. Variation in growth rate, in the activity of specific signaling pathways and in the cellular composition of the tumors were all reflected in the corresponding variation in the expression of specific subsets of genes. BC was divided in four intrinsic subgroups according to type of epithelial cells (Basal or Luminal cells) and expression of hormonal receptors or overexpression of HER2 receptor. The four types were divided as Luminal A/B, HER2 overexpressed and Basal Like. Interestingly, the classification provided a significant predictive and prognostic tool for the treatment of BC.[10]

Intrinsic subtypes were adapted by St Gallen Consensus in 2011. The international consensus that is gathered to address important clinical problems and dilemmas about the treatment of Breast Cancer. The consensus appreciated the classification of BC as prognostic and predictive panel. More precisely BC could be characterized as Luminal A (Hormone Receptor +, HER2 -, Ki67<14%), Luminal B (Hormone Receptor +, HER 2 or Hormone Receptor +, HER2 -, Ki67>14%), HER2 amplified (Hormone Receptor -

, HER2 -) or Triple Negative (Hormone Receptor -, HER 2 -). The expert panel consensus suggested as surrogate method of classification the use of immunochemistry (ICH) in the place of microarrays analysis which presented similar efficacy with a reduction of financial cost. The term “Basal Like” was redefined as “Triple Negative Breast Cancer” (TNBC) referring to the absence of expression of any of the receptors of clinical interest. An overlap of 80% was noticed between the two terms according to PAM50 assay, however TNBC includes special histologic subtypes such as medullary and adenoid cystic types.[11] This classification manages to guide the treatment decisions about systematic therapy in adjuvant a palliative setting. However, Ki67 score measurement presented deviation between laboratories and at the meantime there was a debate about the proper value cut off point for the definition of “Ki67 low” or “Ki67 high” status. In St Gallen panel consensus in 2013 the cutoff point was redefined from 14% to 20%. Also, the Panel stressed the need for standardization, and that laboratories should participate in quality assurance programs.[12] In 2015 the panel expressed again that the results of gene expression assays such as PAM50 can be achieved with less expensive methods such as ICH. However, a lower analytical validity in the Ki67 measurement method was noticed.[13]

Luminal A and Luminal B BC present sensitivity to hormonal treatment whereas BC with amplification of HER2 receptors present sensitivity to anti-HER2 targeted therapy. On the other hand, Triple Negative subtype is refractory to Hormonal and anti-HER2 treatments, and the only available treatment option for decade was chemotherapy.[14] Every intrinsic subtype can be characterized as a different disease entity with different prognosis workup and management.

1.3 Pathology of Triple Negative Breast Cancer

As it was described before TNBC is characterized by the lack of expression of Hormonal and HER2 receptors. However, the simplistic classification of TNBC only by its immunochemical features lacks in ability of understanding biologic features of a

disease that in reality it presents complex and heterogenous behavior. TNBC can be described as an umbrella term covering a variety of entities with marked genetic, transcriptional, histologic, and clinical differences.[15,16]

TNBC mostly consisted of Invasive Ductal Carcinomas (IDCA), histologically. IDCA are characterized by brisk lymphocyte infiltration and tumor necrosis patterns.[7] Though, TNBC presents also rarer histologic patterns such as medullary pattern, apocrine features carcinoma, secretory carcinoma and metaplastic carcinoma. Lobular carcinomas are not common to present Triple Negative features. Medullary carcinoma presents a good prognosis despite high grade histology and is described to have high lymphocyte infiltration.[17] Apocrine carcinoma may present HER2 overexpression and is characterized by high androgen receptors signaling.[18] Secretory carcinoma has salivary gland histopathologic profile, low proliferation rate and good prognosis.[19] Secretory carcinoma is associated with t(12;15) translocation, which results in an ETV6-NTRK3 fusion gene. Metaplastic carcinomas are described as heterogenous group of tumors with metaplastic differentiation of the neoplastic epithelium to squamous and / or mesenchymal cells. Metaplastic carcinomas present refractoriness to chemotherapy so it exhibits low survival rate.[20]

Lehman and colleagues identified 6 subtypes of TNBC based on gene expression of 21 breast cancer data set that included 587 TNBC cases. The analysis categorized TNBC to 6 gene expression entities namely Basal-like 1 (BL1), Basal-like 2 (BL2), Immunomodulatory (IM), Mesenchymal (M), Mesenchymal stem-like (MSL) and Luminal Androgen Receptor (LAR). The study managed to identify driver mutations and oncogenic pathways of every subtype.

BL1 and BL2 subtypes presented mutations enriching cell cycle and cell division pathways (cell cycle, DNA replication reactome, G2 cell-cycle pathway, RNA polymerase, and G1 to S cell cycle). High proliferation of these types of TNBC has as result high Ki67 expression in immunochemistry assessments. Fast proliferation rate and enriched Ki67 expression suggests that BL1 and BL2 subtypes present sensitivity

to chemotherapy class taxanes. BL2 subtype presents mutations in glycolysis and growth factor signaling pathways.

IM subtype shows high expression of genes taking part in immunogenic response. Expressed genes activate immune cell signaling, cytokine signaling, antigen processing and presentation, and signaling through core immune signal transduction pathways. IM genes taking part in immune response activation and enrichment are found also in medullary histologic type of TNBC.

M and MSL subtypes present high expression of genes that activate pathways taking part in cell motility, ECM receptor interactions and cell differentiation. MSL subtype presents expression of genes that promote processes linked to growth factor signaling pathways that include inositol phosphate metabolism, EGFR, PDGF, calcium signaling, G-protein coupled receptor, and ERK1/2 signaling as well as ABC transporter and adipocytokine signaling. MSL subtype also shows enrichment of genes stimulating angiogenesis.

LAR subtype does not present ER and PR receptors, even though it is heavily enriched in hormonally regulated pathways including steroid synthesis, porphyrin metabolism, and androgen/estrogen metabolism. LAR subtype is strongly related to histologic subtype of apocrine carcinoma. It presents a low proliferation index and longer OS.[21]

In 2016 the 6-type classification was redefined to 4 types, namely BL1, BL2, M and LAR. TNBC subtypes were examined in terms of survival, prognosis, mutation burden metastatic site preference and were genomically analyzed as part of The Cancer Genome Atlas. IM subtype gene expression profile was affected by high concentrations of Tumor Infiltrating Lymphocytes (TIL) in the stromal of BC. TIL concentration is easily assessed by simple Hematoxylin Eosin stain section. High concentration of TILs is connected to increased sensitivity to immunotherapy with check point inhibitors. BL1, BL2, and LAR subtypes presented similar levels of TILs, but M subtype showed low TILs in cancer stroma. MSL gene expression profile was a misconception resulting from the presence of mesenchymal-like stromal cells. Importantly, there was a

significant difference between the efficacy of neoadjuvant chemotherapy between the four subtypes with BL1 presenting the highest pCR rate and LAR subtype the lowest. Different subtypes displayed different clinical features with BL1 subtype showing higher grade, lower stage and increased patient overall and relapse-free survival. Subtypes also presented different metastatic patterns.[22]

1.4 Clinical Characteristics and Treatment of Triple Negative Breast Cancer

As it was derived from the analysis of Perou and colleagues, lack of hormonal and HER2 receptor is an independent risk factor for poorer Disease-Free Survival (DFS) and Overall Survival (OS).[10] TNBC is more common to be diagnosed in premenopausal, younger women with the cut-point to be set at 40 years old and African American women.[23] Approximately 20% of patients with TNBC are identified to have BRCA germline pathogenic variants, with mutations in BRCA1 gene to be more common.[24] BRCA related tumors are histologically classified as TNBC in 75% of the cases.[25]

In a single center retrospective analysis of clinical data from 1601 patients diagnosed BC it was found that TNBC presents different clinical features compared to other intrinsic subtypes of BC. TNBC is diagnosed in younger patients comparing to other intrinsic subtypes. (53 vs 57,5 years, p<0,0001). Grade 3 was found more often found in TNBC (66% vs 28%, p<0,0001). The mean tumor size at diagnosis was larger in TNBC than other subtypes (3 vs 2,1 cm, p<0,0001). Interestingly only one third of TNBC primary tumors were smaller than 2 cm at the time of diagnosis. Also, TNBC presents higher mortality rate 42,2% comparing to 28% of non-TNBC (p<0,0001). The distance recurrence rate was about 34% and local recurrence was not a common event in this study. The poor prognosis of TNBC may be a result of the tendency of TNBC for hematogenous metastasis than lymphatic mutations.[26] Importantly, patients diagnosed with TNBC experience high rates of recurrence during the first 4 years from diagnosis. During a 17-year follow-up period no recurrences occurred after the first 8 years.

Another clinical characteristic of TNBC is that it is commonly presented as interval tumor since it has rapid progression, and it is not found in mammography that is conducted in screening programs.[27]

In a data analysis of SEER registry and Emory database examined the clinicopathological features of BC diagnosed in 158358 women. The follow up time was 35 months in SEER database and 144.1 months in Emory database. Statistically significant difference was once again noticed in patients with TNBC in terms of high tumor grade (p<0,0001) and younger age of diagnosis (58,9 vs 61,8 years p<0,0001). Survival analysis showed that older age, higher grade, African American ethnicity and lack of surgical or radiotherapy treatment were independent negative prognostic factors for OS. Patients with TNBC had worse prognosis in all AJCC staging subgroups. Only in stage IA and IB the difference was not statistically significant even though there was a trend towards shorter OS. Study showed that even early TNBC has worse prognosis compared to non-TNBC subtypes and this should be taken account by physicians and patients considering adding or omitting systemic therapy in treatment plan.[28]

Locoregional treatment of TNBC does not differ from other subtypes. Data about locoregional recurrence are conflicting. According to Haffty and colleagues locoregional recurrence rate of TNBC in breast conserving surgery combined with adjuvant radiotherapy was similar to Luminal types even though overall survival was much worse in TNBC group.[29] However, there are researchers suggesting that there is increase up to 50% of local recurrence rate in patients with TNBC.[30] There is a need for large sample and prospective analysis answering the question about optimal radiotherapy protocol and in general locoregional treatment according to different molecular subtypes of TNBC.[26]

According to the available literature and contemporary knowledge there is no receptor specific treatment in adjuvant, neo-adjuvant and first line treatment for TNBC. Thus, the backbone of systemic treatment remains non-target specific chemotherapy. In

terms of stage II and stage III St Gallen consensus suggest the use of neoadjuvant treatment with or without immunotherapy.[31] Patients achieving Pathologic Complete Response (pCR) have 6 times less chance of developing recurrence in contrast with patients who do not achieve pCR. Therefore, pCR is used as a surrogate endpoint for examining the efficacy of a neoadjuvant regiment. Neoadjuvant therapy helps patients to achieve breast conserving surgery in patients with large tumors.[32] Administration of Pembrolizumab in combination with chemotherapy presented statistically significant benefit in terms of pCR as neodjuvant treatment in patents with early TNBC (HR 0.63; 95% CI, 0.43 to 0.93), according to Keynote 522, a phase 3 multicentered, double-blind randomized trial.[33]

Adjuvant chemotherapy is strongly recommended to all patients diagnosed with TNBC. Systemic adjuvant treatment may be considered to omit only in patients with pT1a tumors with good prognostic features such as secretory or adenoid cystic BC. The most common combined regiments are combination of Anthracyclines with Taxanes. The standard anthracycline regiments are Doxorubicin (AC) or Epirubicin (EC) plus cyclophosphamide. In the case of contraindication or strong clinical indications of high toxicity due to anthracyclines 4 cycle of AC can be replaced from 6 cycles of Cyclophosphamide, Methotrexate and 5-FU (CMF). Taxanes have been proven to have clinical efficacy regardless of tumor size, grade, nodal and receptor status. Sequential administration of Anthracyclines and Taxanes has proven to be more efficient and with a better toxicity profile.[34,35,36,37,38] Adjuvant anthracycline chemotherapy regiments reduces the reduces the annual death rate for women under 40 years old approximately 38%, whereas the benefit for women in the age group 50-69 is reduced by 20%, regardless of hormonal and HER2 receptor status.[2] Even though chemotherapy can be omitted in a great proportion of patients with Luminal BC there is no similar indication for TNBC. There is no evidence of superiority of any chemotherapeutic agent in a particular breast cancer phenotype in neoadjuvant or

adjuvant setting. Currently, immunotherapy is not a treatment option for adjuvant treatment.[39]

Chemotherapy remains for many years the main available treatment option for metastatic setting, too. However, the administration of nab paclitaxel with Atezolizumab for de novo metastatic or recurrent disease after 6 months the completion of neoadjuvant chemo/immunotherapy showed clinical and statistically relevant benefit in terms of PFS in patients with PDL1 positive TNBC according to Impassion130, a phase 3, multicentered, double blind, randomized clinical trial.[40] In Keynote 355 a phase 3 randomized double blind clinical trial the administration of Pembrolizumab in combination with investigators choice chemotherapy presented statistically significant benefit in terms of OS (HR, 0.73; 95% confidence interval [CI], 0.55 to 0.95; P = 0.0185) in CPS>10 arm.[41] Tumor's stroma concentration to infiltrating lymphocytes is known as TILs is a promising biomarker in terms of assessing the sensitivity of TNBC to immunotherapeutic agents. However, currently the use of PDL1 is used in clinical practice.[42] Carboplatin is the preferred treatment for patients with germline BRCA mutation. Monotherapy regiments are preferred for metastatic or recurrent TNBC when there is no imminent organ failure. In case of visceral crisis or imminent organ failure combination regiment is preferred with anthracycline/taxane regiment to be the optimal if it was not priorly used. For second line therapies, capecitabine, eribulin, gemcitabine, vinorelbine, carboplatin, anthracycline and taxanes if not priorly used can be administrated. Sacituzumab govitecan-hziy is a drug antibody conjugate that target TROP2 receptors and presented statistically significant benefit compared to physician's choice in terms of PFS or death (5.6 months in SG arm vs and 1.7 months in control arm, HR 0.41; 95% CI,0.32 to 0.52; P<0.001) in beyond second line treatment in patients with TNBC.[43]

Chapter 2

Study Design and Results

2.1 Methods

This study is a retrospective collection and analysis of data from patients with TNBC. The data were extracted from medical documents and electronic registry of a single center (Bank of Cyprus Oncology Center). Patients that were histologically diagnosed with TNBC in the years 2008 to 2017 were included in the study. Patients that were included did not have history of prior malignancy and did not receive any type of treatment for Breast Cancer to any other medical center before being evaluated and treated to Bank of Cyprus Oncology Center. Patients that were simultaneously being diagnosed with a second non-Triple Negative Breast Cancer were excluded. No underaged nor male patients were included to this study.

The hormonal and HER2 status of the tumor was determined by immunochemistry strain. In case of inconclusive HER2 results by immunochemistry, FISH test was conducted to evaluate HER2 amplification. In the case that immunochemistry was run by two different laboratories pathology lab of Nicosia General Hospital results were preferred. Ki67 status was valid if it was measured before any systemic treatment.

Two cohorts were created according to the hormonal status of the tumor. In the first cohorts were included patients that had no detected Estrogen, Progesterone receptors and HER2 amplification was not identified. In the second cohort patients with “weak positive” Hormone Receptor profile were examined. Weak positive was determined as positive hormonal receptors to be less than 10% and no HER2 amplification. The data analysis presented in the study was based on the first cohort while the data of second cohort were used only for comparison of survival analysis. Staging was graded according to AJCC 8th edition guidelines.

This study was conducted after gaining approval from Research Ethics Committee of Cyprus. No patient was exposed to unnecessary risk for his mental and physical health. Written informed consent for the use of their medical data without exposing them was

obtained from all patients during their registration to the Oncology Center. All patients were treated according to the standards and received the best available treatment of the period. Treatment and clinical workup of the patients has been evaluated at Breast Cancer Multidiscipline Meetings. The personal data of all the patients remained protected during the time of the study and they will remain according to the study protocol. The study was executed in accordance with the provisions of the Declaration of Helsinki and local laws. Study protocol and Research Ethics Committee approval are attached in appendix (A and B).

Data analysis was conducted after the completion of data collection. Main time endpoints of the study were Overall Survival (OS), Progression Free Survival (PFS) and time from first PFS to OS. Other features that were examined were the stage at diagnosis, the rate of patients with bilateral metastasis, genetic BRCA mutations, age of diagnosis, site of mutations. Overall survival was determined as the time between diagnosis and last follow up or death. PFS was determined as the time from diagnosis until the first recurrence or in case of de novo metastatic until the time of first progression which was proven radiologically according to RECIST criteria, local or systemic from BC. The observation period determined as the day of the diagnosis of each participant until the end of January 2023. Last follow up was determined as the last time an individual had a clinical or radiological examination for follow up or the day of death according to death certificate in case of a mortal event. Statistical analysis was done via Excel and STATA MP software.

2.2 Results

330 patients were enrolled in TNBC cohort and fulfill inclusion and exclusion criteria. 25 patients were identified to suffer from week positive BC and registered in second cohort. The maximum follow-up time was 172 months and minimum intended follow up time was 60 months. Table A illustrates information about the year of diagnosis of patients and it is divided into two cohorts described above. Mean age of diagnosis was

57,56 years and median age was 58 years (IQS 49-66). According to our record 12 patients presented metachronous BC on the other test during follow up time (3,67%).

The stage of diagnosis is presented in Table B and Chart A. Interestingly, only 3 patients were diagnosed with de novo metastatic disease which is calculated as 0,92%. The most common stage of diagnosis was stage IIA (38,77%). AJJC 8th edition was established in 2017, so there was a change from the initial staging which was based in AJCC 7th and 6th edition. Most of the times downstaging was described.[44] 5 patients did not have adequate staging at the diagnosis.

Ki67 is an established proliferation index which is recommended by guidelines. In our study Ki67 was defined in 3 groups with the first cut-off set at 20% and the second at 50%. Median Ki67 score was 60% (IQS 30-80). Laboratory dependent bias was minimized by using mostly the same lab. Mean tumor size at diagnosis, based on histopathology report of the surgery was 1,20 cm. Median tumor size was 2,4 cm (IQS 1,7-3,48). Cancer was found in left breast in 177 cases (53,64%), right breast 151 (45,76%) and in two cases (0,06%) patients were diagnosed with bilateral cancers at diagnosis. Data are shown in Table D.

As was mentioned before germline mutations are connected with basal type of BC which overlaps at approximately 80% with TNBC. 137 patients were examined for BRCA germline mutations (41,5%). 8/137 patients (5,84%) presented to have pathogenic variant (PV) in BRCA1 gene and 7/137 patients (5,11%) presented PV in BRCA2 gene. Variants of Unknown clinical significance and benign variants were not included. In Table E and Chart B there is a detailed representation of germline BRCA mutational status of our registry.

Surgery remains the primary modality for curative intent for patients with non-metastatic BC. Surgical treatment can be applied primarily or after neoadjuvant treatment and is dependent on tumor stage and available treatment options. 119 patients (36,06%) had Mastectomy, 209 had Wide Local Excision (63,33%) and 2 (0,6%) patients were not submitted to any curative intend surgical treatment due to de

novo metastatic disease. Data about the type of surgery are represented in Table F. Anthracycline based regiments on adjuvant or neoadjuvant setting was administrated to 228 patients (69,09%) with TNBC of our registry. Non anthracycline based regiments were given to 80 patients (24,24%) and 22 patients (6,67%) did not receive any systemic adjuvant or neoadjuvant treatment. The most common reasons of administrating non anthracycline based chemotherapy were the high expected toxicity or contraindications such as heart failure or prior administration of anthracyclines. Patients who did not receive any systemic therapy based on the multimodality treatment for curative intent was due to poor performance status, the reluctance of patients to receive chemotherapy or de novo metastatic disease. Table G gives additional information about adjuvant systemic treatment.

69 patients experienced recurrence during the follow-up period which implies 21,10% recurrence rate. 19 cases (27,54%) of recurrence presented firstly as local relapse and 50 patients (72,46%) relapsed as metastatic disease. First site of metastatic disease was identified in the 50 women who had systemic recurrence by radiographic imaging. There were cases where at the time of the diagnosis of relapse there were multiple metastatic sites. Most common site of first metastasis was lower respiratory tract (50%). Other sites of metastasis were bones (26%), liver (18%), distant lymph nodes (16%) and CNS (16%). Data about initial metastatic site and recurrence trends are given in Table H and I. Trends of metastasis at any time during follow up period of patient with recurrent or de novo metastatic disease was examined. Lung remained the most common site of metastasis since it presented in 41 women (59,42%). Osseous lesions were identified in 29 cases (42,2%), liver metastasis in 27 cases (39,13%), distant lymph node spread in 25 cases (36,23%), 3 women had subcutaneous lesions (4,34) and 1 patient presented metastatic deposit on adrenal gland. Interestingly, 25 patients with recurrent disease were diagnosed with CNS involvement (brain metastasis or leptomeningeal disease) which is the 36,23% of patients with metastatic disease and 7,58% of all patients of our study. More detailed data is given in Table J.

5-year OS rate was examined to TNBC group. 249 patients, which is 75,41% of the cohort reached 5-year OS, 48 patients (14,63%) deceased before 60 months of follow-up and 31 (9,45%) patients were censored prior completing 5 years of follow-up. According to each stage 5-year OS was 94,81%, 91,30%, 73,17%, 70%, 50%, 63,16% and 0% for stage IA, IIA, IIB, IIIA, IIIB, IIIC and IV respectively. There were not any patients in the registry with stage IB disease. Data about 5-year OS are represented in Table K and Chart C. Mean 5-year OS according Ki67 score was 95,35% for 1-20% group, 92,90% for 21-50 group and 87,18% for Ki67>50% group. One way analysis of variance presented a trend towards difference between groups, but it was not statistically significant (p=0,072).

Overall survival analysis is given to Chart D where a Kaplan Meier curve shows that median OS was not reached, and 10-year OS rate is above 75%. Chart E is a Kaplan-Meier graph of OS according to AJCC staging. Log rank test proved a statistically significant difference in terms of OS between stages (HR 1,5; 95% CI 1,33-1,74; p<0,0001). In Chart F there is an illustration of survival analysis between 3 predefined groups according to Ki67% score, again there was a statistically significant difference between groups. Survival analysis according to the administration of Anthracycline based regiment or not is presented in Chart G. There was a statistically significant difference between groups according to cox test (HR 0,51; p<0,0001) between Anthracycline and non-Anthracycline regiments, in favor of the first. Even though, we should bear in mind that this type of analysis probably is biased from confounders such as patient's performance status and age. Age was an independent poor prognosis factor, according to cox regression analysis a HR of 1.015 was observed (1.004-1.027, p=0.006). Difference in OS between patients diagnosed in the years 2008-2012 and 2013-2017 was examined however, there were no statistically significant differences between two groups.

Graph H illustrates the time to event analysis of PFS for TNBC cohort. Median PFS was not reached. 75th percentile of PFS was at 101 months. In Graph I Kaplan-Meier curves of PFS according to AJCC staging are presented.

Separate analysis of cases that recurrence occurred during follow-up was conducted. The OS after the time of recurrence was examined by PFS to OS variable. PFS to OS time was defined as time between first recurrence and end of follow-up, lethal event or censoring of a patient. 69 individuals who were not present with de novo metastatic disease were examined. 6 patients were in stage 1, 35 patients were in stage 2, and 28 patients were in stage 3 BC. Median OS was 58 months (IQS 29-137 months)2. According to Log rank test there is a statistically relevant difference in OS between stages (p<0,0001). Table L, Graph J and K present data about OS for recurrent disease cohort. Median time of death after the time of recurrence (PFS to OS) was 23 months (IQS 9-53 months). Statistically significant difference between AJCC stages was observed (p<0,0001). PFS to OS time to event curve is presented in Chart L. Difference prognosis in terms of PFS to OS between patients who experienced local or systemic relapse were examined with Wilcoxon test. Worse prognosis was observed in women who experienced recurrence as systematic disease than as local relapse (HR 3,52, p=0,004). Patients who experienced involvement in CNS anytime during follow up did not statistically significant had worse prognosis (p=0,65). Kaplan Meier analysis is illustrated in Graph M. Median OS of recurrent systemic (from the time of recurrence) or metastatic disease was 17 months (IQS 9-46). Survival Curve is illustrated in Graph M.

Weak positive BC is characterized by low expression of hormone receptors and it does not present the same level of sensitivity to hormone treatment as tumors with strong expression of hormone receptors. In our study a comparison of OS between 25 cases that were indicated as weak positive BC and TNBC group. Neither of the two groups did not reach median OS and there was no difference in survival between to test according to Wilcoxon test (p=0,4825).

Chapter 3

Discussion

3.1 Discussion

This is the first large scale statistical analysis of clinical data of patients with TNBC in Cyprus. By setting the latest year of diagnosis in 2017 allowed to have minimum follow-up of 5 years. This suggests an adequate follow-up time for this registry. With this study trends and features of TNBC can be appreciated, however direct statistical comparison with results published in available literature cannot be assessed.

Interestingly the median age of diagnosis (58 years old) was similar to the data extracted from SEER registry and Croatian retrospective single Center analysis, in a Canadian study the median age was 53. The rate of patients diagnosed with de novo metastatic disease was remarkably low in comparison with data from available literature since it is described that de novo metastatic disease rates reach 3-6% in developed countries and 10-20% in low-income countries. This implies that there is high BC awareness in Cyprus, leading patients to earlier stage of diagnosis. As it was mentioned in epidemiology women in Cyprus does not prefer to participate to organized screening programs often in Cyprus, although they have high rate of participation opportunistic screening and mammography checkup. Mean tumor size of TNBC primary lesion was measured 2,4 which was smaller than other registry results.[4,27,45,46]

In this study the incidence rate of germline pathogenic mutations in BRCA genes reached 10,95%. But only 41,5% of the patients were examined for germline mutations. Patients were examined for BRCA mutations according to their age of diagnosis, family history and current guidelines. Patients who did not want to have genetic counseling did not proceed with germline mutations screening. Unfortunately, available data could allow us to assess this parameter. According to a multigene hereditary cancer analysis of 8753 patients with TNBC by a clinical testing laboratory, a rate of 12% of pathogenic

variants was detected from which 3,7 were non-BRCA mutations. Thus, the results in Cypriot population does not present an important difference from data available in literature.[47]

Prognosis of TNBC was proven to be dependent of Age of diagnosis, administration of Anthracyclines and primary staging. Age was found to be a statistically important independent poor prognostic factor in a similar Croatian and Slovenian study. Probably, age of diagnosis includes confound parameters such as comorbidities, performance status and non-cancer related decease. In case of neoadjuvant treatment, the use of anthracycline is mandatory and in case of adjuvant treatment the efficacy of anthracycline is well established.[2] However, cardiovascular adverse effects are the main cause of morbidity and mortality among BC survivors. Although guidelines have been established for early detection of heart toxicity, the management remains a clinical challenge.[48] For that reason there is a constant academic need for the omitting of anthracyclines from systemic therapy. But the administration of anthracyclines in patients diagnosed with TNBC remains a mandatory practice if there is no contraindication. This is something that is supporter from the results of current analysis since the use of Anthracycline improves OS independently of the cause of death.[45,49]

Ki67 expression level increases from G1 phase to mitosis, and then rapidly decreases immediately after mitosis. The median score of Ki67 measured in this study was comparable with other similar studies. Classification of Ki67 in three groups is based on the threshold placed in intrinsic subtypes (20%). A trend of difference in prognosis was observed between groups but it was not proven to be statistically significant. Ki67 is a known proliferation marker but there is remarkable interlaboratory difference in its measurement. Ki67% was proposed as a therapeutic target since it is expressed in malignant cells and not in normal cells. Since Ki67 is associated to stage and metastatic of tumor, there are confounders to the use of this marker as independent prognostic factor.[45,27,50]

The comparison between the years of diagnosis was held to examine if there was difference in the management during the years examined. There was no difference between 2008-2012 and 2013-2017. Most of the patients were diagnosed with early-stage disease and received curative indent treatment. There were few changes in the multimodality treatment during these years for patients with TNBC. Surgical management remains the main modality for the treatment and the optimization surgical technics has been well established for years. Also, there were few changes in the adjuvant radiotherapy and chemotherapy treatment in the last decade, before the addition of immunotherapy to neoadjuvant systemic treatment.[39,51]

The median OS from the time of recurrence was 23 months and it is remarkably shorter than OS of recurrent disease that is observed to other BC types, where novel medications were brought to clinical practice.[52] Until recently the management of recurrent and metastatic disease was mainly non-specific chemotherapeutic agents. The addition of immunotherapy to the therapeutic options and ADC had added clinical benefit for patients with mTNBC. The de novo metastatic or metastatic disease in literature is determined to 10-13 months according to our record the median OS is longer since it was measured to be 17 months.[41,43,53,54,55,56]

Still, the management of mTNBC continues to be challenging and novel therapeutic strategies are needed. CDK inhibitors are promising drug class that presented clinical efficacy in Hormone Receptors positive BC and their efficacy for TNBC has not yet been established. Palbociclib in combination with an mTOR inhibitor, Abemaciclib, Prexacertib and Trilaciclib are currently being investigated in clinical trials. The G1-S transition is significantly promoted in the tumor cell cycle, as noted in TNBC. Multi VEGF/VEGFR inhibitors efficacy is currently examined in ongoing clinical trials for TNBC. Another targetable mutation that is investigated is EGFR which presented outstanding results in other cancer types such NSCLC. The identification of Androgen receptors in TNBC cells relates to better prognosis and presented sensitivity to androgen receptor blockage in preclinical studies and phase 1/2 trials. 27,96% of

patients with TNBC express Androgen Receptors, according to meta-analysis. The results of phase 3 trials are anticipated.[21,22,57]. In addition to expression of the AR, the LAR subtype cell lines have a high rate of PIK3CA activating mutations and exhibit high sensitivity to PI3K inhibitors. Preclinical data suggest synergic effect of antiandrogen drugs with PI3K inhibitors.[58] PI3K/AKT/mTOR signaling pathway takes part in oncogenesis by enhancing proliferation and survival to affected cells. Alterations in PI3K/AKT/mTOR signaling pathway are rarer in TNBC, however they are found to approximately 10% of TNBC cancer cells. Patients with mutations affecting this pathway have longer OS and currently there are trials examining medications targeting this pathway.[15] Targeting genomic instability which is a result of homologous genomic instability remains an important aspect of research. As mentioned above BRCA mutations are found in approximately 10% of patients with TNBC and pathogenic variants of these genes have as a result defects in homologous recombination. Cancer cells with homologous recombination deficiency present sensitivity to platinum-based chemotherapy in early stage and metastatic TNBC.[59,60] Poly ADP-Ribose (PARP) inhibitors are effective against BRCA mutations, and they are biomarker dependent. Presently, two drugs gained approval for the treatment of germline mutant BRCA TNBC, Olaparib and Talazobarib.[61] Both medications have proven superior to single agent physician's choice treatment in the presence of germline BRCA mutations.[62,63]

Better understanding of TNBC pathophysiology and molecular biology will allow better treatment solutions. According to the classification of Lehman et al. there is a meaningful genomic differentiation between TNBC which can justify the variability of sensitivity in treatments and prognosis between patients diagnosed with TNBC. Phase 3 clinical trials based on gene expression of TNBC will result in more precise selection of available treatments.

3.2 Conclusion

Patients with TNBC present worse prognosis than non-TNBC. Clinical characteristics of TNBC are larger tumor size, higher Ki67% score and higher incidence of treatment

failure during the first years of diagnosis. Tumor stage according to AJCC guidelines, high Ki67 score, and age of diagnosis are poor prognostic factors whereas the administration of Anthracyclines is connected to better clinical outcome. Recurrent disease remains a clinical challenge suggesting the need for novel therapeutic agents based on gene expression assays, targetable mutations and more effective biomarkers.

Tables and Charts

Table A - Patients Diagnosed per Year		
Year	TNBC	Weak Positive
2008	38	4
2009	32	2
2010	42	1
2011	26	1
2012	25	4
2013	28	3
2014	35	5
2015	32	1
2016	37	4
2017	35	0
Total	330	25

Table B – Staging at Diagnosis		
Stage	n	Percentage
IA	86	26,46%
IB	0	0
IIA	126	38,75%
IIB	48	14,77%
IIIA	32	9,85%
IIIB	10	3,08%
IIIC	20	6,15%
IV	3	0,92%

Chart A - Staging

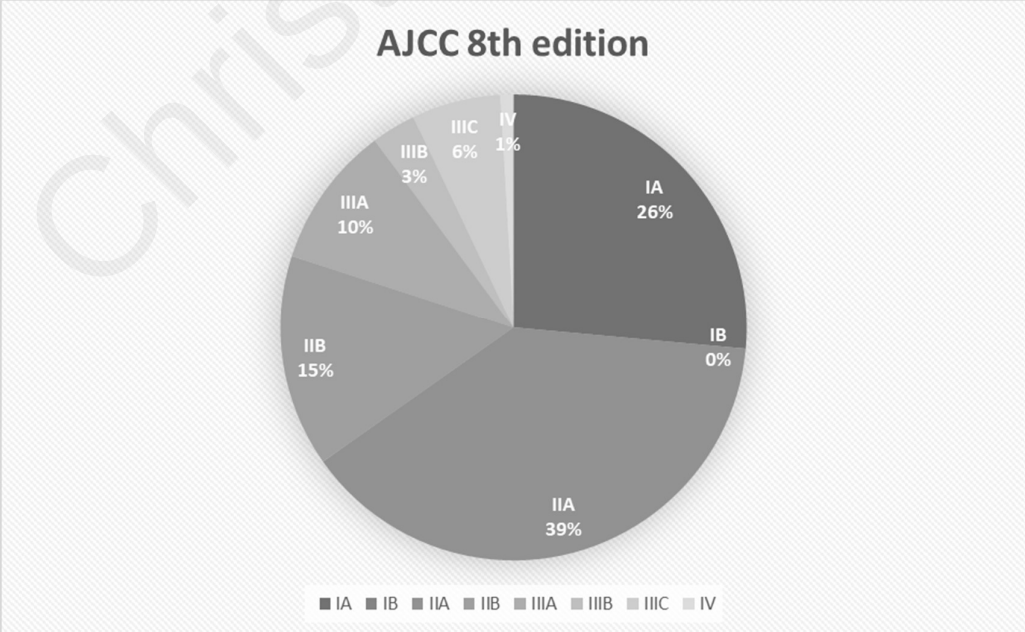


Table C Ki67 score		
Ki67	n	Percentage
1-20%	46	14,84%
21-50%	83	26,77%
>50%	181	58,39%

Table D - Breast Site			
Year of Diagnosis	Left	Right	Bilateral
2008	20	18	0
2009	20	12	0
2010	21	20	1
2011	14	12	0
2012	16	9	0
2013	16	12	0
2014	13	21	1
2015	19	13	0
2016	20	17	0
2017	18	17	0
Total	177	151	2
Percentage	53,64%	45,76%	0,06%

Table E – Germline Pathogenic BRCA Mutations				
Year	BRCA1	BRCA2	Checked	Percentage Checked
2008	1	2	14/38	36,8%
2009	0	0	14/32	43,7%
2010	1	1	17/42	40,5%
2011	0	0	7/26	40,5%
2012	0	0	9/25	36%
2013	1	1	8/28	28,6%
2014	1	2	11/35	31,4%
2015	2	0	18/32	56,2%
2016	2	0	18/37	48,6%
2017	0	1	21/35	60%
Total	8	7	137/330	41,5%
	5,84%	5,11%		

Chart B - Germline BRCA mutational Status and Percentage Checked

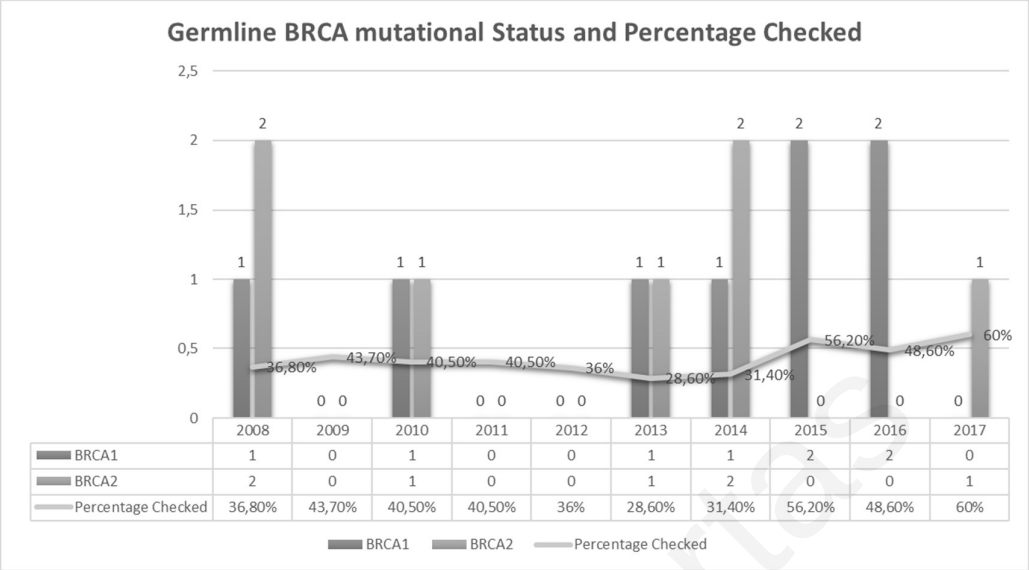


Table F – Type of Surgery		
Type of Surgery	n	Percentage
Mastectomy	119	36,06%
Wide Local Excision	209	65,34%
No Surgery	2	0,6%

Table G – Adjuvant or Neoadjuvant Systemic Treatment		
Type of Adjuvant Chemotherapy	n	Percentage
Anthracycline	228	69,09%
Non-Anthracycline	80	24,24%
No Chemotherapy	22	6,67%

Table H – Systemic or Local Recurrence			
Year	Local	Systemic	Total
2008	2	7	9
2009	3	7	10
2010	0	7	7
2011	1	3	4
2012	0	3	3
2013	1	4	5
2014	4	4	9
2015	3	3	6
2016	1	6	7
2017	4	6	10
Total	19	50	69
Percentage	27,54%	72,46%	

Table I – Site of Initial Metastatic Site		
Site	n	Percentage
Lung	25	50%
Bones	13	26%
Liver	8	16%
Distant Lymph Nodes	9	18%
CNS	8	16%

Table J – Site of Metastasis Anytime		
Site	n	Percentage
Lung	41	59,42%
Bones	29	42,02%
Liver	27	39,13%
CNS	25	36,23%
Distant Lymph Nodes	15	21,73%
Subcutaneous	3	4,34%
Adrenal	1	1,45%

Table K – 5 Year OS					
AJCC		5 Year OS	No 5 Year OS	Censored	Total
IA	n	73	4	9	86
	Percentage	84,88%	4,65%	10,47%	100%
IB					
IIA	n	105	10	10	125
	Percentage	84%	8%	8%	100%
IIB	n	30	11	7	48
	Percentage	62,50%	22,92%	14,58%	100%
IIIA	n	21	9	2	32
	Percentage	65,63%	28,13%	6,25%	100%
IIIB	n	5	5	0	10
	Percentage	50%	50%	0	100%
IIIC	n	12	7	1	20
	Percentage	60%	35%	5%	100%
IV	n	0	2	1	3
	Percentage	0	66,67%	33,33%	100%
		75,41%	14,63%	9,45%	

Chart C – 5 Year Overall Survival according to Stage

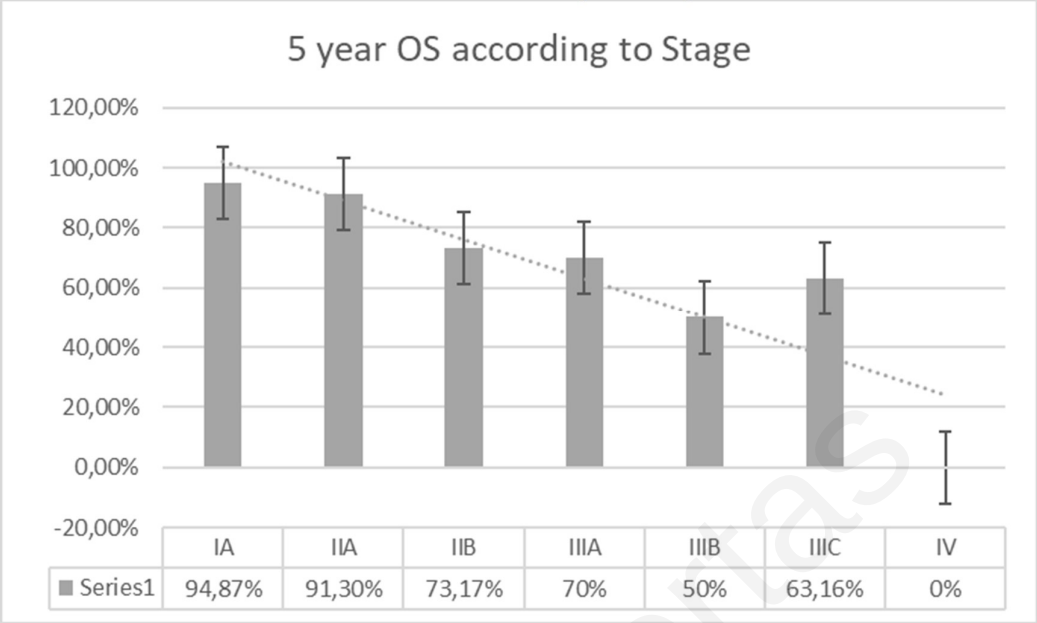


Chart D – Overall Survival

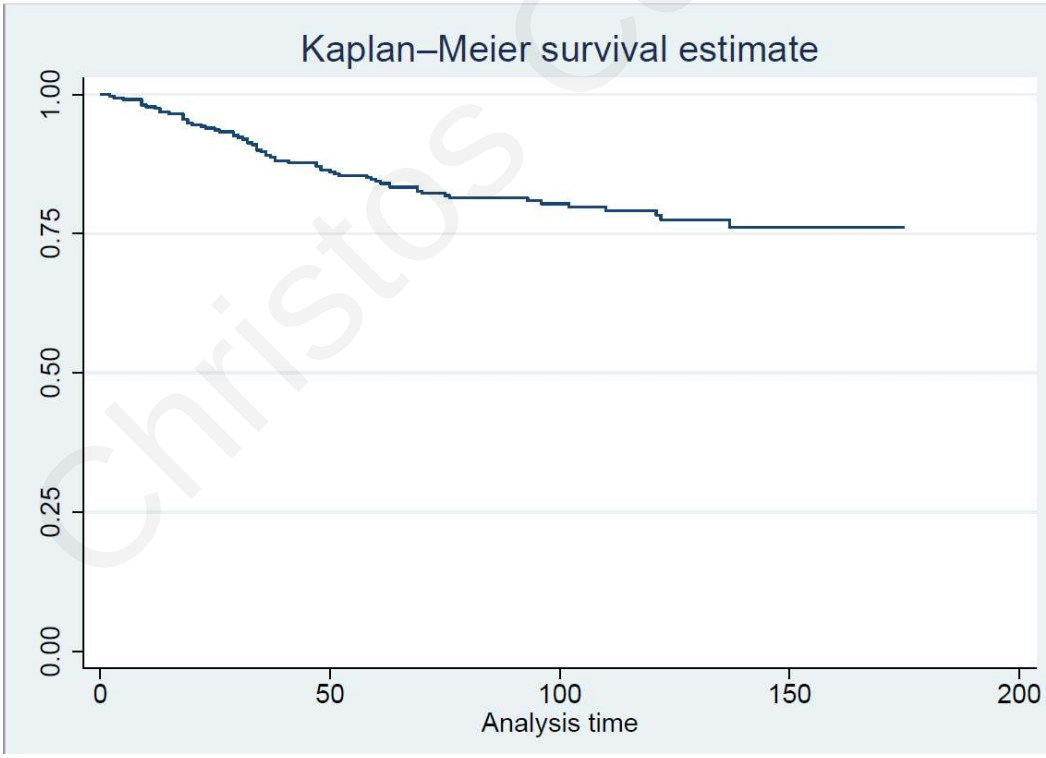


Chart E – Overall Survival According to Stage

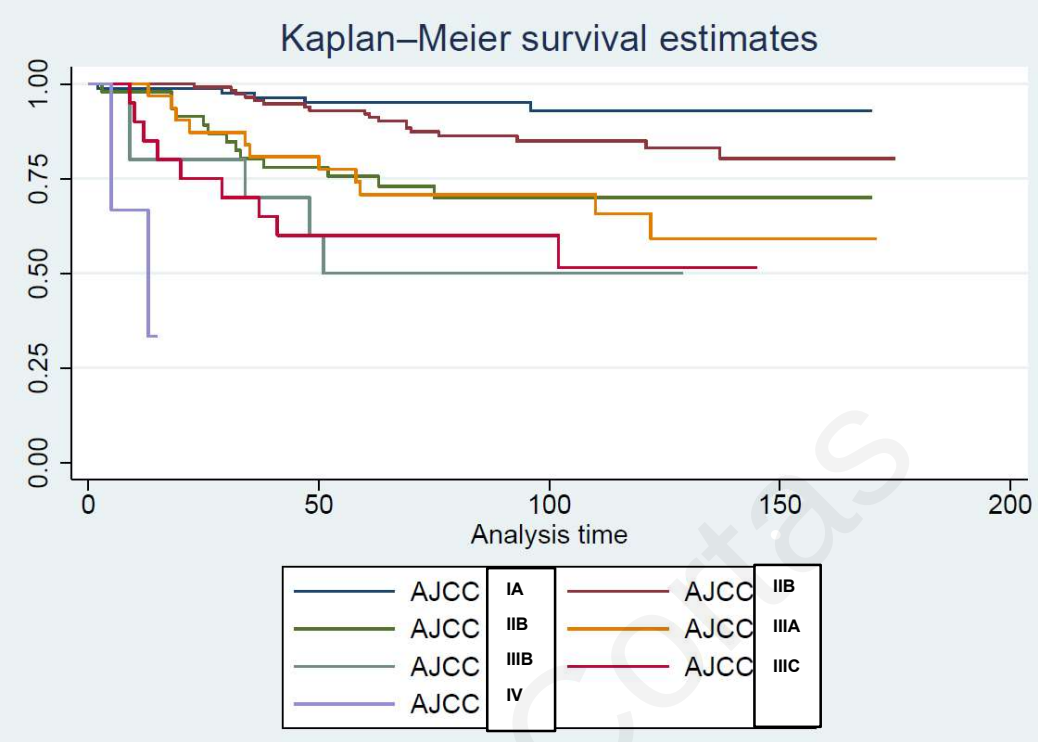


Chart F – Overall Survival According to Ki67 Score Group

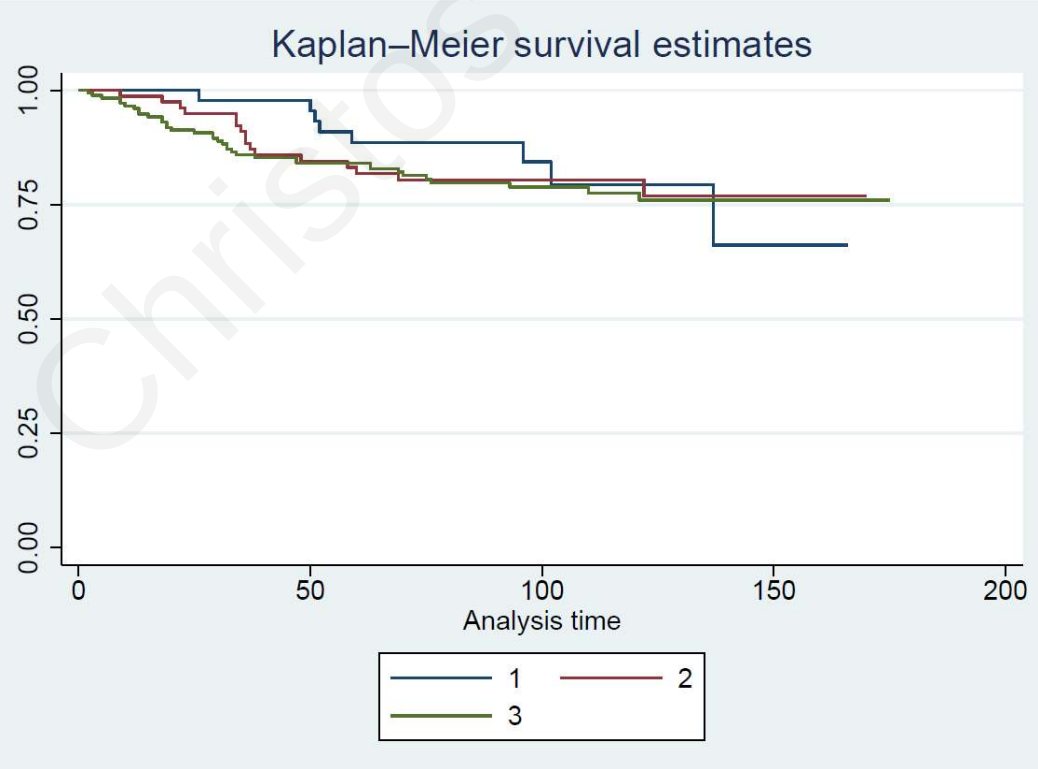


Chart G – Overall Survival According to Neoadjuvant or Adjuvant Treatment

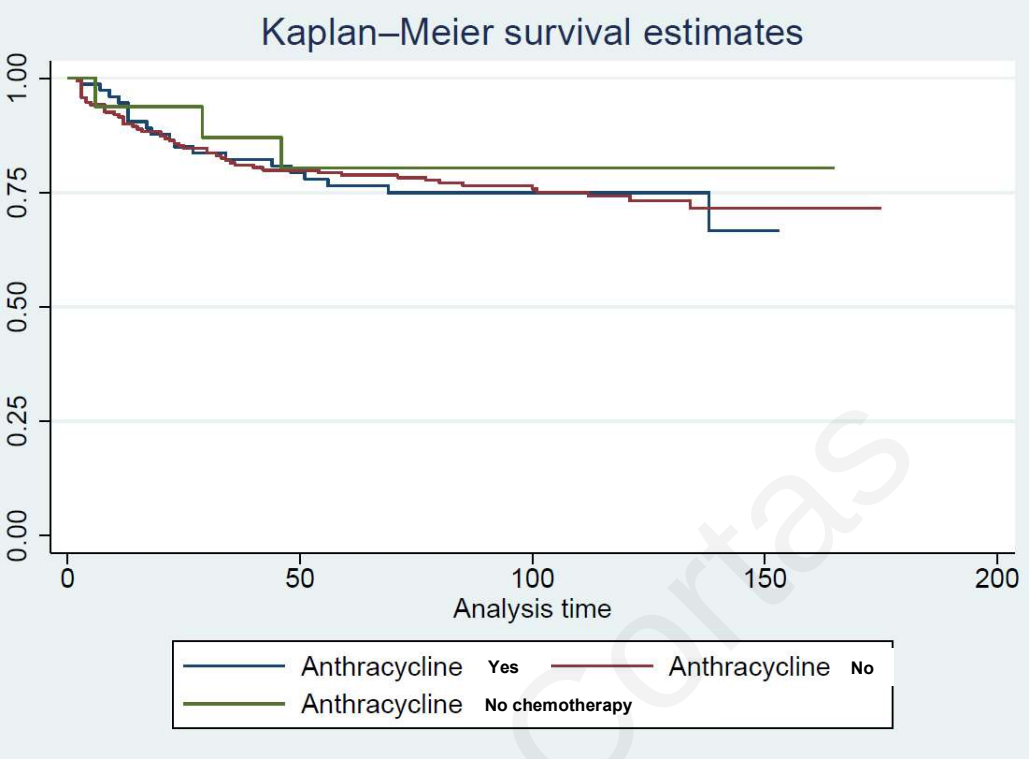
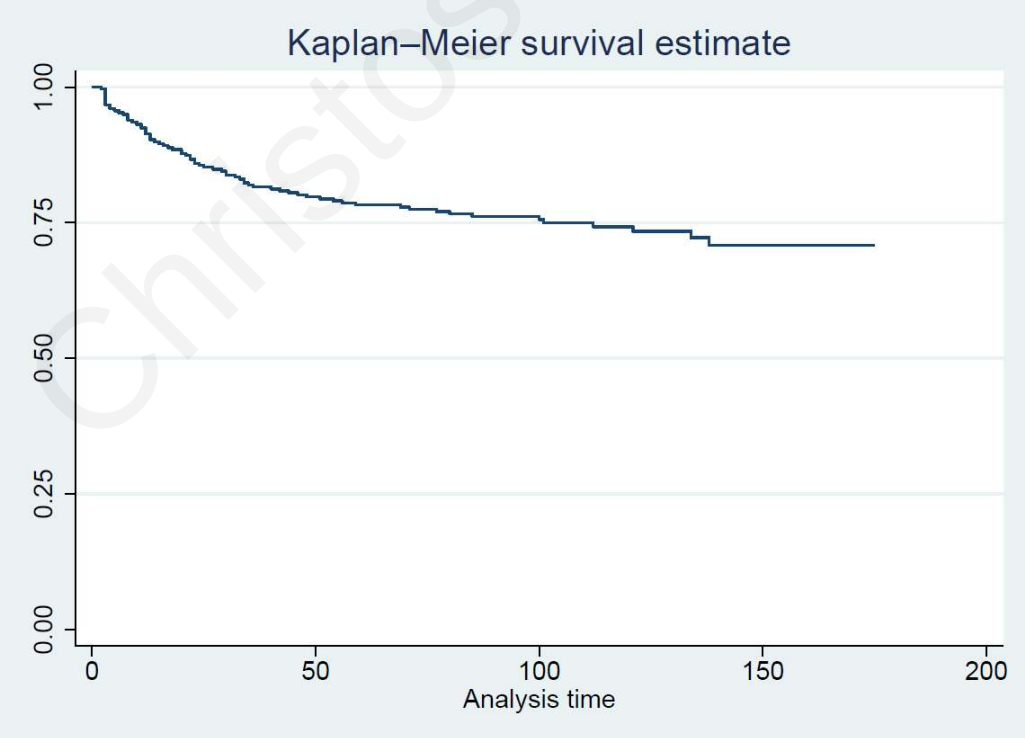


Chart H – Progression Free Survival



Graph I – Progression Free Survival According to Stage

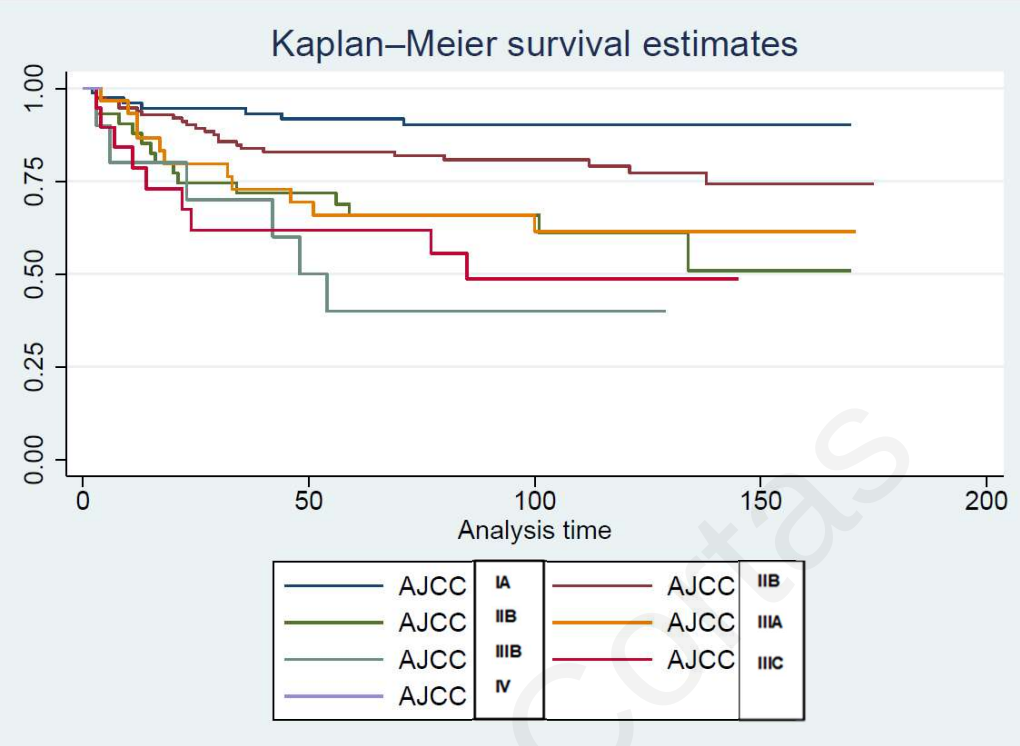
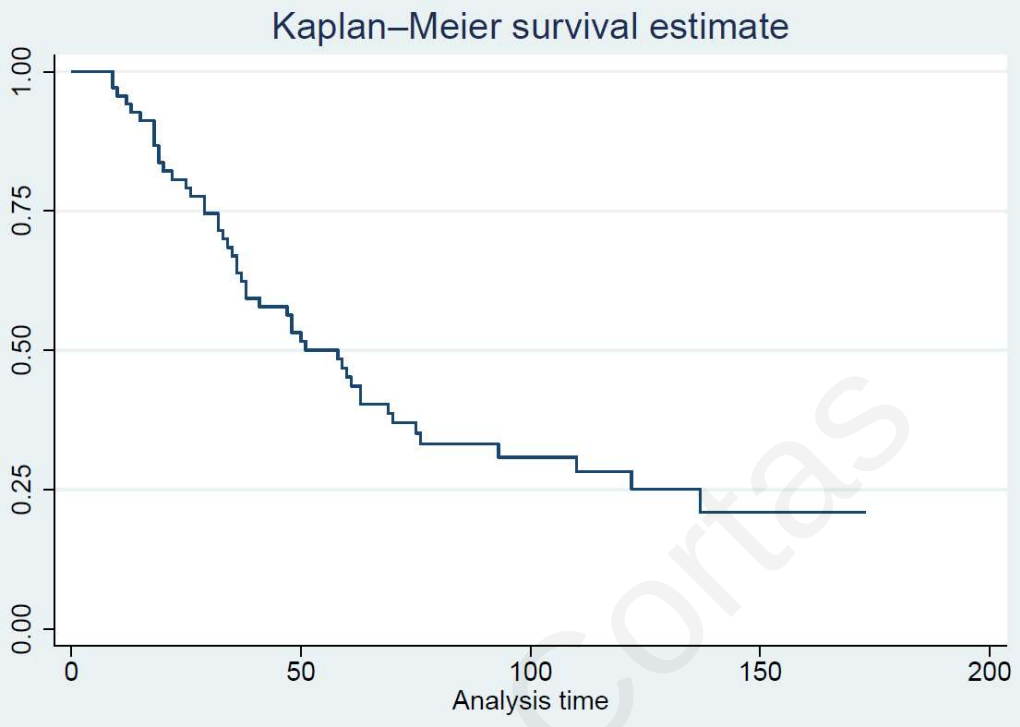


Table L – Overall Survival for Recurrent Disease			
AJCC	n	Percentage	Median (Months)
IA	6	8,70%	n/a
IIA	23	33,33%	93
IIB	12	17,39%	36
IIIA	13	18,84%	47
IIIB	5	7,25%	18
IIIC	10	14,49%	29
Total	69	100%	58

Graph J – Overall Survival for Recurrent Disease



Graph K – Overall Survival for patients with Recurrent Disease According to Stage

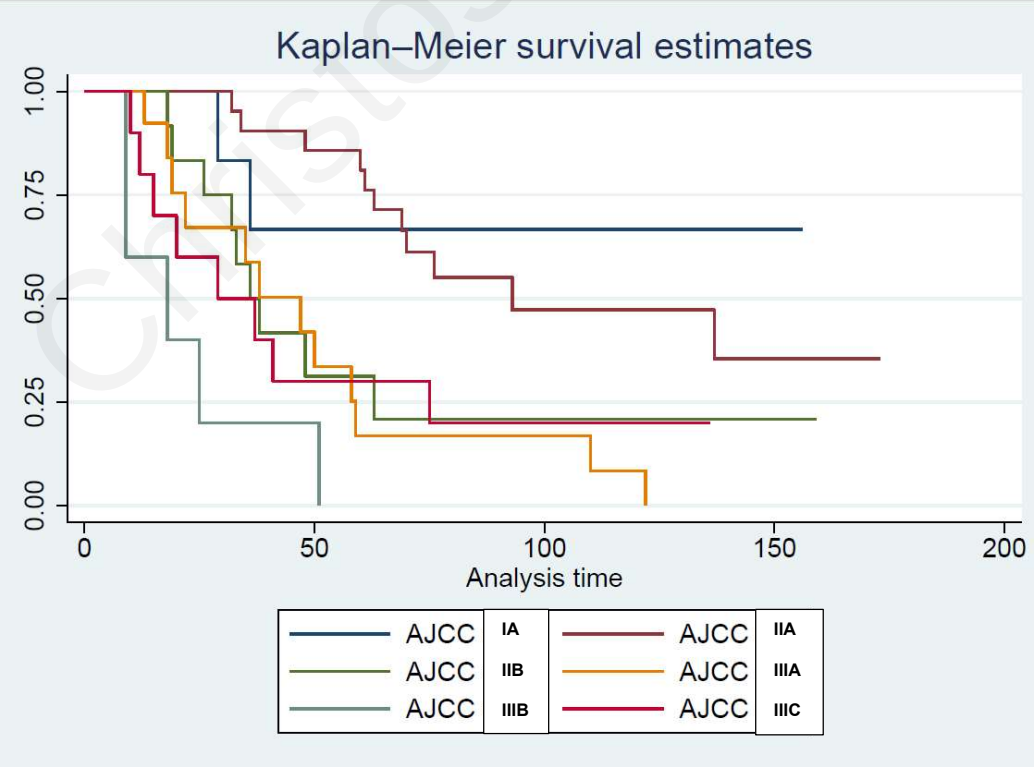
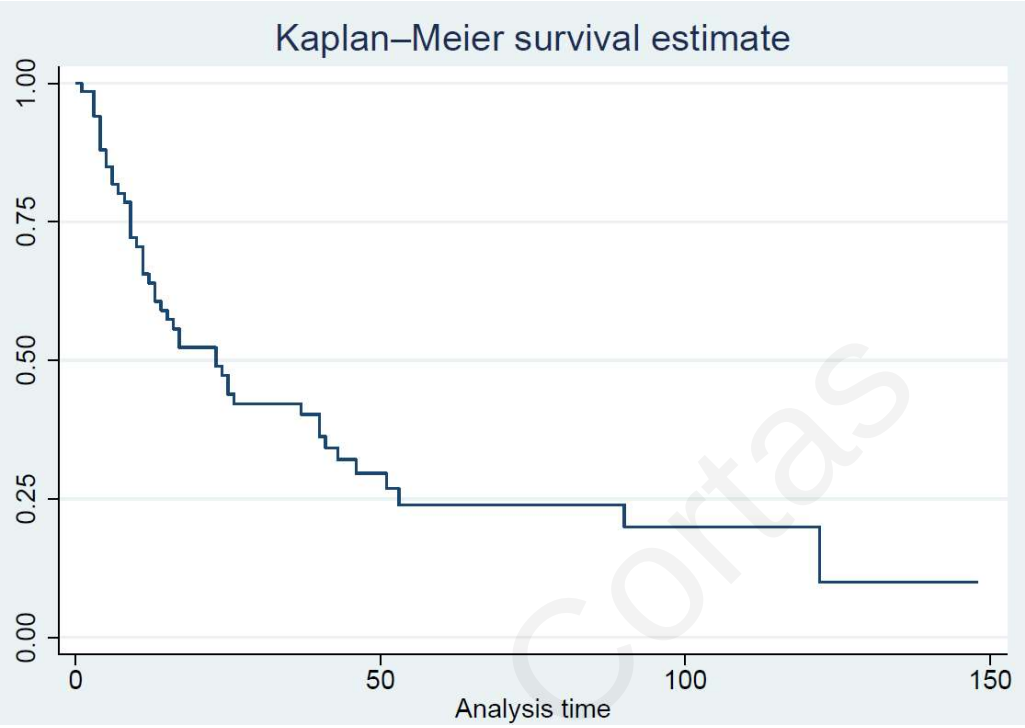
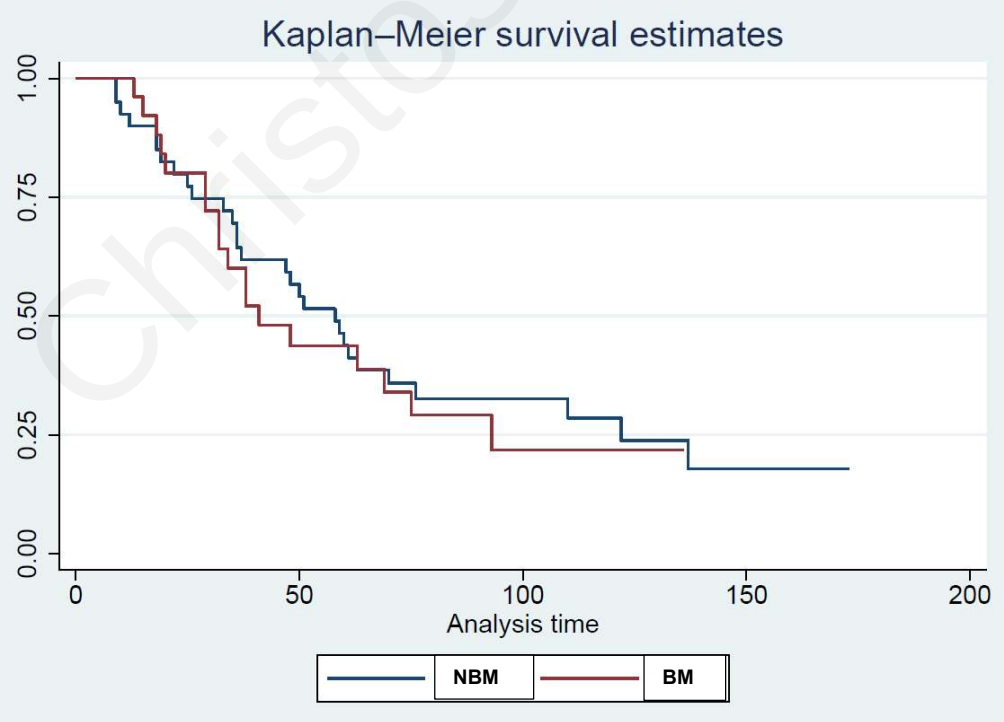


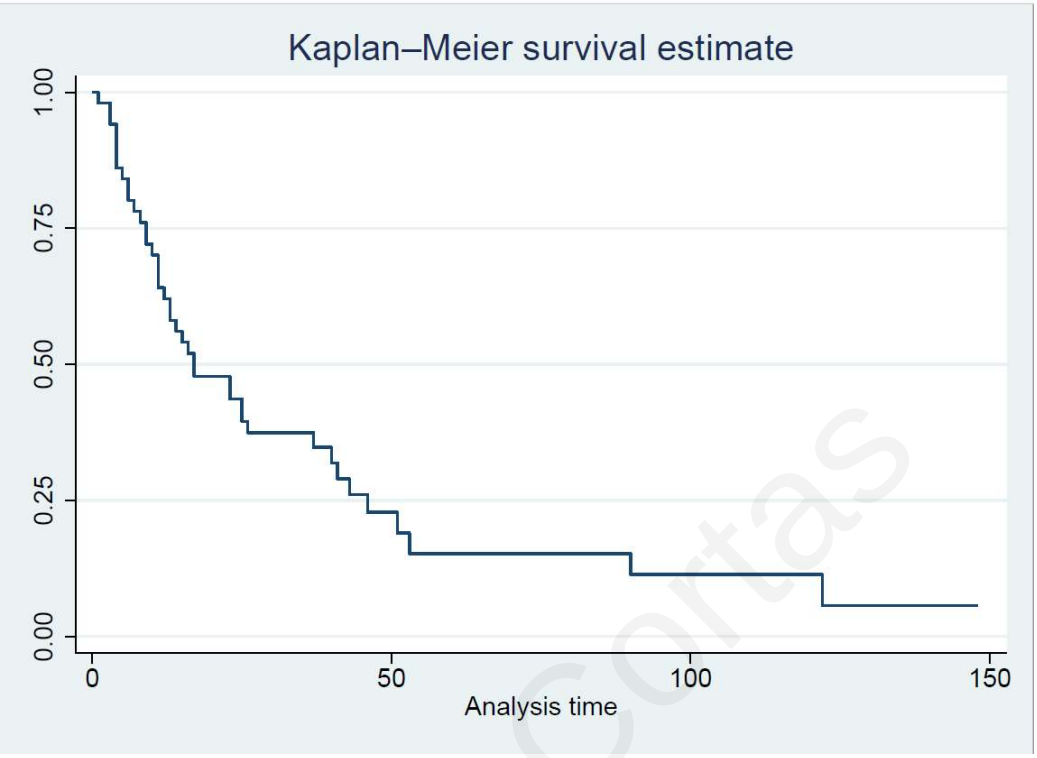
Chart L – Overall Survival from the Time of Recurrence



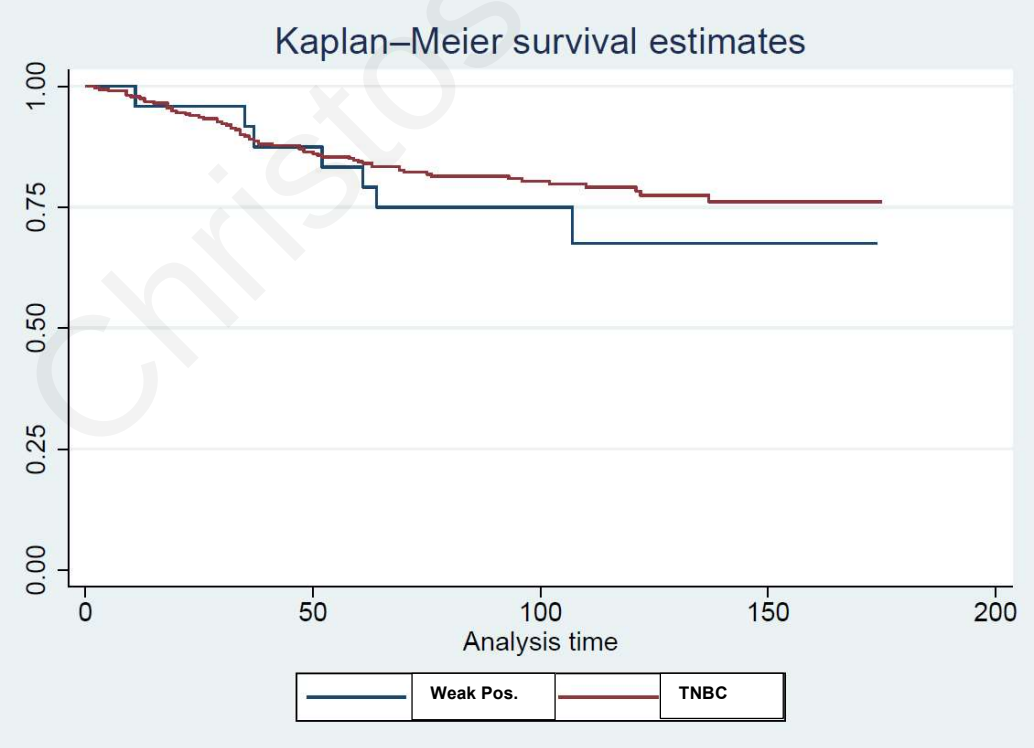
Graph M – Overall Survival According to the Presence of Brain Metastasis



Graph M – Overall Survival from the time of systemic Recurrence



Graph O – Overall Survival for TNBC versus Weak Positive TNBC



Appendix A

Study Protocol as it was submitted to Cyprus National Bioethics Committee (in Greek)

<u>Αναδρομική μελέτη διερεύνησης επιδημιολογικών δεδομένων που αφορά ασθενής με τριπλά αρνητικό καρκίνο μαστού.</u>

Ερευνητές:	Αναστασία Κωνσαντινίδου[1,2] *(Επιβλέπουσα καθηγήτρια για την εκπόνηση διπλωματικής εργασίας του μεταπτυχιακού φοιτητή Χρίστου Κόρτα)* Χρίστος Κόρτας[1,2]
Εμπλεκόμενα Ιδρύματα:	[1]Πανεπιστήμιο Κύπρου, [2]Ογκολογικό Κέντρο Τράπεζας Κύπρου
Υπεύθυνος Αλληλογραφίας:	Χρίστος Κόρτας *Ογκολογικό Κέντρο Τράπεζας Κύπρου* *Λεωφ. Ακροπόλεως 32, Στρόβολος, Λευκωσία Κύπρος* *0035799983099* christoscortas@hotmail.com

Εισαγωγή

Επιστημονικό υπόβαθρο

Ο καρκίνος του μαστού αποτελεί την συχνότερη κακοήθεια στις γυναίκες στην Ευρώπη. Κάθε χρόνο διαγιγνώσκονται περίπου 350000 νέες περιπτώσεις και αποτελεί το 28,7% των νέων περιστατικών κακοήθειας στην Ευρωπαϊκή Ένωση. Παρόλη την μείωση της θνησιμότητας από τον καρκίνο του μαστού αποτελεί την πρώτη αιτία θανάτου από καρκίνο στις γυναίκες στις χώρες της Ευρωπαϊκής Ένωσης.[1]

Ο καρκίνος μαστού διαχωρίζεται σε τέσσερα ανοσοιστοχημικά προφίλ (Luminal A, Luminal B, HER2+, Triple Negative) ανάλογα με την έκφραση πρωτεϊνικών υποδοχέων στη μεμβράνη του καρκινικού κυττάρου. Ο διαχωρισμός καθορίζει την θεραπευτική προσέγγιση και πρόγνωση της νόσου.[2,3]

Ο τριπλά αρνητικός καρκίνος μαστού παρουσιάζει μέχρι σήμερα την χειρότερη πρόγνωση και δυστυχώς έχει παρουσιαστεί η λιγότερη πρόοδος στην εξέλιξη της θεραπευτικής αντιμετώπισης.

Η παρούσα μελέτη καταγράφει αναδρομικά κλινικά δεδομένα ασθενών με τριπλά αρνητικά καρκίνο μαστού από το μεγαλύτερο κέντρο αντιμετώπισης ογκολογικών περιστατικών στην Κύπρο. Όλες οι ασθενείς έλαβαν την ενδεδειγμένη θεραπεία σύμφωνα με τις σύγχρονες κατευθυντήριες οδηγίες. Δεν έχει διενεργηθεί αντίστοιχη μελέτη μέχρι σήμερα.

Είδος Μελέτης

Η εν λόγω μελέτη αφορά αναδρομική συλλογή δεδομένων από του φακέλους των ασθενών που διαγνώστηκαν με τριπλά αρνητικό καρκίνο μαστού και έλαβαν θεραπεία στο Ογκολογικό Κέντρο της Τράπεζας Κύπρου, από το 2008 μέχρι το 2017. Αυτή η έρευνα είναι μίας ευκαιρία να αναλυθούν τα δεδομένα από τον κυπριακό πληθυσμό

τον ασθενών που διαγνώστηκαν με τον εν λόγω υπότυπο καρκίνο μαστού και να συγκριθούν με τα δεδομένα από την διεθνή βιβλιογραφία.

Στόχος Μελέτης

Η συλλογή και ανάλυση δεδομένων ασθενών

Σχεδιασμός Μελέτης

- Η συλλογή δεδομένων θα αφορά γυναίκες άνω των 18 ετών με ιστοποθαλογικά επιβεβαιωμένο καρκίνο μαστού που έλαβε θεραπεία στο Ογκολογικό Κέντρο της Τράπεζας Κύπρου.
- Ο πληθυσμός θα αποτελείται μόνο από ασθενείς που διαγνώστηκαν με καρκίνο μαστού την περίοδο 2008-2017.
- Όλες οι ασθενείς έλαβαν την βέλτιστη θεραπεία σύμφωνα με την οδηγίες της εποχής που λάμβαναν αγωγή και τις διαθέσιμες θεραπευτικές επιλογές. Καμία ασθενής δεν μπήκε σε αχρείαστο κίνδυνο για την σωματική της και ψυχική της υγεία.
- Η εν λόγω έρευνα δεν αποτελεί πειραματική μελέτη αλλά αφορά συλλογή “real world” ψευδοανώνυμων δεδομένων που έχουν λάβει θεραπεία στο Ογκολογικό Κέντρο Τράπεζας Κύπρου την περίοδο 2008-2017.
- Θα διενεργηθεί αναδρομική συλλογή δεδομένων από τον ηλεκτρονικό φάκελο και έντυπο φάκελο των ασθενών.
- Τα δεδομένα που θα συλλεχθούν αφορούν κυρίως τα χαρακτηρίστηκα της νόσου (σταδιοποίηση, ιστολογικός υπότυπος, ανοσοιστοχημικό προφίλ), την θεραπεία που έλαβαν, την ανταπόκριση στη θεραπεία και την επιβίωση των ασθενών.
- Τα δεδομένα που θα ελεγχθούν θα αφορούν την περίοδο από τη διάγνωση τους μέχρι και τον Δεκέμβριο του 2022.
- Τα δεδομένα να βρίσκονται σε ψευδοανώνυμη μορφή και στο αρχείο θα έχουν πρόσβαση μόνο οι 2 ερευνητές.

- Η ανάλυση των δεδομένων θα γίνει από τους 2 καταγεγραμμένους ερευνητές και τα δεδομένα θα φυλάσσονται στον υπολογιστή του με κωδικό ασφαλείας που θα γνωρίζουν μόνο οι ίδιοι.
- Τα δεδομένα θα παραμείνουν φυλαγμένα για περίοδο 20 ετών και για τυχών χρήση τους θα ζητηθεί ανάλογη έγκριση από την επιτροπή βιοηθικής Κύπρου.
- Από την μελέτη θα αποκλειστούν οι ασθενείς που δεν έχουν υπογράψει ή δεν βρίσκεται στο φάκελο τους το ανάλογο έντυπο του Ογκολογικού Κέντρου Τράπεζας Κύπρου που επιτρέπει την χρήση των κλινικών τους δεδομένων.

Βιβλιογραφία.

1. http//ecis.jrc.ec.europa.eu
2. H. J. Burstein et al. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 2021 Oct;32(10):1216-1235.
3. A Gennari et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021 Dec;32(12):1475-1495.

Appendix B

Approval from Cyprus National Bioethics Committee “in Greek”

ΚΥΠΡΙΑΚΗ ΔΗΜΟΚΡΑΤΙΑ

ΕΘΝΙΚΗ ΕΠΙΤΡΟΠΗ ΒΙΟΗΘΙΚΗΣ ΚΥΠΡΟΥ

Αρ. Φακ.: ΕΕΒΚ ΕΠ 2023.01.66
Αρ. Τηλ.: 22809038/039, 22819101/122
Αρ. Φαξ: 22353878

22 Φεβρουαρίου, 2023

Δρ Αναστασία Κωνσταντινίδου
Επίκουρη Καθηγήτρια
Ιατρική Σχολή
Πανεπιστήμιο Κύπρου
Παλαιός Δρόμος Λευκωσίας Λεμεσού Αρ. 215/6
2029 Αγλαντζιά
Λευκωσία

Δρ Χρίστος Κόρτας
Λεωφ. Ακροπόλεως 32
2006 Στρόβολος
Λευκωσία

Αγαπητοί Δρ Κωνσταντινίδου και Δρ Κόρτα,

Αίτηση γνωμοδότησης για την πρόταση με τίτλο:
«Αναδρομική μελέτη διερεύνησης επιδημιολογικών δεδομένων που αφορά ασθενείς με τριπλά αρνητικό καρκίνο μαστού»

Αναφορικά με την αίτηση σας ημερομηνίας 17 Φεβρουαρίου 2023 για το πιο πάνω θέμα, επιθυμώ να σας πληροφορήσω ότι από τη μελέτη του περιεχομένου των εγγράφων που έχετε καταθέσει η Εθνική Επιτροπή Βιοηθικής Κύπρου (ΕΕΒΚ) **γνωμοδοτεί θετικά υπέρ της διεξαγωγής της εν λόγω έρευνας.**

2. Η Επιτροπή επιθυμεί να τονίσει ότι παραμένει ευθύνη δική σας η διεξαγωγή της έρευνας με τρόπο που να τηρούνται οι πρόνοιες του νέου Ευρωπαϊκού Γενικού Κανονισμού Προστασίας Προσωπικών Δεδομένων (2016/679) και του περί της Προστασίας των Φυσικών Προσώπων Έναντι της Επεξεργασίας των Δεδομένων Προσωπικού Χαρακτήρα και της Ελεύθερης Κυκλοφορίας των Δεδομένων αυτών Νόμος του 2018 (Ν. 125(Ι)/2018), ως αυτός εκάστοτε τροποποιείται.

3. Σας ενημερώνουμε ότι για σκοπούς καλύτερου συντονισμού και αποφυγής επανάληψης ερευνών με το ίδιο θέμα ή/και υπό εξέταση πληθυσμό μέσα σε σύντομο σχετικά χρονικό διάστημα, η ΕΕΒΚ δημοσιεύει στην ιστοσελίδα της το θέμα της έρευνας, τον φορέα και τον υπό εξέταση πληθυσμό.

4. Κατά τη διάρκεια εκπόνησης της έρευνας, ο συντονιστής / επιστημονικός υπεύθυνος θα ενημερώνει την ΕΕΒΚ για κάθε τροποποίηση των αρχικά κατατεθειμένων εγγράφων (πρωτόκολλο ή άλλα ερευνητικά έγγραφα) και θα υποβάλλει τις απαιτούμενες έντυπες τροποποιήσεις στην Επιτροπή.

…/2

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5. Σε περίπτωση διακοπής της έρευνας, ο συντονιστής / επιστημονικός υπεύθυνος θα ενημερώσει γραπτώς την Επιτροπή κάνοντας αναφορά και στους λόγους διακοπής της έρευνας.

6. Ο συντονιστής / επιστημονικός υπεύθυνος θα ενημερώσει την Επιτροπή σε περίπτωση αδυναμίας να συνεχίσει ως συντονιστής και θα υποβάλει τα στοιχεία επικοινωνίας του αντικαταστάτη του.

7. Με το πέρας της ερευνητικής πρότασης, ο συντονιστής / επιστημονικός υπεύθυνος θα ενημερώσει εγγράφως την Επιτροπή ότι το υπό αναφορά ερευνητικό πρωτόκολλο ολοκληρώθηκε.

8. Σας ευχόμαστε κάθε επιτυχία στη διεξαγωγή της έρευνάς σας.

Με εκτίμηση,

Καθ. Κωνσταντίνος Ν. Φελλάς
Πρόεδρος
Εθνικής Επιτροπής Βιοηθικής Κύπρου

Bibliography

1. Review of mortality results in randomised trials in early breast cancer. Lancet. 1984 Nov 24;2(8413):1205.
2. Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. N Engl J Med. 1988 Dec 29;319(26):1681-92. doi: 10.1056/NEJM198812293192601.
3. (https://gco.iarc.fr/) last accessed April 2023
4. Daily K, Douglas E, Romitti PA, Thomas A. Epidemiology of De Novo Metastatic Breast Cancer. Clin Breast Cancer. 2021 Aug;21(4):302-308. doi: 10.1016/j.clbc.2021.01.017. Epub 2021 Jan 31.
5. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S, Soerjomataram I. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022 Dec;66:15-23. doi: 10.1016/j.breast.2022.08.010. Epub 2022 Sep 2.
6. Zielonke N, Kregting LM, Heijnsdijk EAM, Veerus P, Heinävaara S, McKee M, de Kok IMCM, de Koning HJ, van Ravesteyn NT; EU-TOPIA collaborators. The potential of breast cancer screening in Europe. Int J Cancer. 2021 Jan 15;148(2):406-418. doi: 10.1002/ijc.33204. Epub 2020 Jul 30.
7. Derakhshan F, Reis-Filho JS. Pathogenesis of Triple-Negative Breast Cancer. Annu Rev Pathol. 2022 Jan 24;17:181-204. doi: 10.1146/annurev-pathol-042420-093238.
8. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, Evans DG, Izatt L, Eeles RA, Adlard J, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Tischkowitz M, Douglas F, Hodgson S, Walker L, Porteous ME, Morrison PJ, Side LE, Kennedy MJ, Houghton C, Donaldson A, Rogers MT, Dorkins H, Miedzybrodzka Z, Gregory H, Eason J, Barwell J, McCann E, Murray A, Antoniou AC, Easton DF; EMBRACE. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst. 2013 Jun 5;105(11):812-22. doi: 10.1093/jnci/djt095. Epub 2013 Apr 29.
9. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, van Leeuwen FE, Milne RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC; BRCA1 and BRCA2 Cohort Consortium; McGuffog L, Evans DG, Barrowdale D, Frost D, Adlard J, Ong KR, Izatt L, Tischkowitz M, Eeles R, Davidson R, Hodgson S, Ellis S, Nogues C, Lasset C, Stoppa-Lyonnet D, Fricker JP, Faivre L, Berthet P, Hooning MJ, van der Kolk LE, Kets CM, Adank MA, John EM, Chung WK, Andrulis IL, Southey M, Daly MB, Buys SS, Osorio A, Engel C, Kast K, Schmutzler RK, Caldes T, Jakubowska A, Simard J, Friedlander ML, McLachlan SA, Machackova E, Foretova L, Tan YY, Singer CF, Olah E, Gerdes AM, Arver B, Olsson H. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017 Jun 20;317(23):2402-2416. doi: 10.1001/jama.2017.7112. PMID: 28632866.
10. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature. 2000 Aug 17;406(6797):747-52. doi: 10.1038/35021093. PMID: 10963602.

11. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011 Aug;22(8):1736-47. doi: 10.1093/annonc/mdr304. Epub 2011 Jun 27.
12. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013 Sep;24(9):2206-23. doi: 10.1093/annonc/mdt303. Epub 2013 Aug 4.
13. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thürlimann B, Senn HJ; Panel Members. Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol. 2015 Aug;26(8):1533-46. doi: 10.1093/annonc/mdv221. Epub 2015 May 4.
14. Medina MA, Oza G, Sharma A, Arriaga LG, Hernández Hernández JM, Rotello VM, Ramirez JT. Triple-Negative Breast Cancer: A Review of Conventional and Advanced Therapeutic Strategies. Int J Environ Res Public Health. 2020 Mar 20;17(6):2078. doi: 10.3390/ijerph17062078.
15. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, Chen Y. Recent advances in therapeutic strategies for triple-negative breast cancer. J Hematol Oncol. 2022 Aug 29;15(1):121. doi: 10.1186/s13045-022-01341-0.
16. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010 Nov 11;363(20):1938-48. doi: 10.1056/NEJMra1001389.
17. Rakha EA, Aleskandarany M, El-Sayed ME, Blamey RW, Elston CW, Ellis IO, Lee AH. The prognostic significance of inflammation and medullary histological type in invasive carcinoma of the breast. Eur J Cancer. 2009 Jul;45(10):1780-7. doi: 10.1016/j.ejca.2009.02.014. Epub 2009 Mar 14.
18. Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, Macgrogan G, Bergh J, Cameron D, Goldstein D, Duss S, Nicoulaz AL, Brisken C, Fiche M, Delorenzi M, Iggo R. Identification of molecular apocrine breast tumours by microarray analysis. Oncogene. 2005 Jul 7;24(29):4660-71. doi: 10.1038/sj.onc.1208561.
19. Arpino G, Clark GM, Mohsin S, Bardou VJ, Elledge RM. Adenoid cystic carcinoma of the breast: molecular markers, treatment, and clinical outcome. Cancer. 2002 Apr 15;94(8):2119-27. doi: 10.1002/cncr.10455.
20. Bando Y, Kobayashi T, Miyakami Y, Sumida S, Kakimoto T, Saijo Y, Uehara H. Triple-negative breast cancer and basal-like subtype: Pathology and targeted therapy. J Med Invest. 2021;68(3.4):213-219. doi: 10.2152/jmi.68.213.
21. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011 Jul;121(7):2750-67. doi: 10.1172/JCI45014.
22. Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME, Pietenpol JA. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One. 2016 Jun 16;11(6):e0157368. doi: 10.1371/journal.pone.0157368.
23. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, Smith LV, Labbok MH, Geradts J, Bensen JT, Jackson S, Nyante S, Livasy C, Carey L, Earp HS, Perou CM. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat.

2008 May;109(1):123-39. doi: 10.1007/s10549-007-9632-6. Epub 2007 Jun 20. Erratum in: Breast Cancer Res Treat. 2008 May;109(1):141. Dressler, Lynn G.

24. Gonzalez-Angulo AM, Timms KM, Liu S, Chen H, Litton JK, Potter J, Lanchbury JS, Stemke-Hale K, Hennessy BT, Arun BK, Hortobagyi GN, Do KA, Mills GB, Meric-Bernstam F. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res. 2011 Mar 1;17(5):1082-9. doi: 10.1158/1078-0432.CCR-10-2560. Epub 2011 Jan 13. PMID: 21233401;

25. Rakha EA, Reis-Filho JS, Ellis IO. Basal-like breast cancer: a critical review. J Clin Oncol. 2008 May 20;26(15):2568-81. doi: 10.1200/JCO.2007.13.1748.

26. He MY, Rancoule C, Rehailia-Blanchard A, Espenel S, Trone JC, Bernichon E, Guillaume E, Vallard A, Magné N. Radiotherapy in triple-negative breast cancer: Current situation and upcoming strategies. Crit Rev Oncol Hematol. 2018 Nov;131:96-101. doi: 10.1016/j.critrevonc.2018.09.004. Epub 2018 Sep 12.

27. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007 Aug 1;13(15 Pt 1):4429-34. doi: 10.1158/1078-0432.CCR-06-3045.

28. Li X, Yang J, Peng L, Sahin AA, Huo L, Ward KC, O'Regan R, Torres MA, Meisel JL. Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer. Breast Cancer Res Treat. 2017 Jan;161(2):279-287. doi: 10.1007/s10549-016-4059-6. Epub 2016 Nov 25.

29. Haffty BG, Yang Q, Reiss M, Kearney T, Higgins SA, Weidhaas J, Harris L, Hait W, Toppmeyer D. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol. 2006 Dec 20;24(36):5652-7. doi: 10.1200/JCO.2006.06.5664. Epub 2006 Nov 20.

30. Kümmel A, Kümmel S, Barinoff J, Heitz F, Holtschmidt J, Weikel W, Lorenz-Salehi F, du Bois A, Harter P, Traut A, Blohmer JU, Ataseven B. Prognostic Factors for Local, Loco-regional and Systemic Recurrence in Early-stage Breast Cancer. Geburtshilfe Frauenheilkd. 2015 Jul;75(7):710-718. doi: 10.1055/s-0035-1546050.

31. Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, Senn HJ, Winer EP, Gnant M; Panelists of the St Gallen Consensus Conference. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol. 2021 Oct;32(10):1216-1235. doi: 10.1016/j.annonc.2021.06.023. Epub 2021 Jul 6.

32. Thomssen C, Balic M, Harbeck N, Gnant M. St. Gallen/Vienna 2021: A Brief Summary of the Consensus Discussion on Customizing Therapies for Women with Early Breast Cancer. Breast Care (Basel). 2021 Apr;16(2):135-143. doi: 10.1159/000516114. Epub 2021 Apr 7.

33. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, Takahashi M, Foukakis T, Fasching PA, Cardoso F, Untch M, Jia L, Karantza V, Zhao J, Aktan G, Dent R, O'Shaughnessy J; KEYNOTE-522 Investigators. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020 Feb 27;382(9):810-821. doi: 10.1056/NEJMoa1910549.

34. Berry DA, Cirrincione C, Henderson IC, Citron ML, Budman DR, Goldstein LJ, Martino S, Perez EA, Muss HB, Norton L, Hudis C, Winer EP. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA. 2006 Apr 12;295(14):1658-67. doi: 10.1001/jama.295.14.1658. Erratum in: JAMA. 2006 May 24;295(20):2356.

35. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Clarke M, Coates AS, Darby SC, Davies C, Gelber RD, Godwin J, Goldhirsch A, Gray R, Peto R,

Pritchard KI, Wood WC. Adjuvant chemotherapy in oestrogen-receptor-poor breast cancer: patient-level meta-analysis of randomised trials. Lancet. 2008 Jan 5;371(9606):29-40. doi: 10.1016/S0140-6736(08)60069-0.
36. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, Cutter D, Darby S, McGale P, Taylor C, Wang YC, Bergh J, Di Leo A, Albain K, Swain S, Piccart M, Pritchard K. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012 Feb 4;379(9814):432-44. doi: 10.1016/S0140-6736(11)61625-5. Epub 2011 Dec 5.
37. Nitz U, Gluz O, Huober J, Kreipe HH, Kates RE, Hartmann A, Erber R, Moustafa Z, Scholz M, Lisboa B, Mohrmann S, Möbus V, Augustin D, Hoffmann G, Weiss E, Böhmer S, Kreienberg R, Du Bois A, Sattler D, Thomssen C, Kiechle M, Jänicke F, Wallwiener D, Harbeck N, Kuhn W. Final analysis of the prospective WSG-AGO EC-Doc versus FEC phase III trial in intermediate-risk (pN1) early breast cancer: efficacy and predictive value of Ki67 expression. Ann Oncol. 2017 Nov 1;28(11):2899. do
38. Shao N, Wang S, Yao C, Xu X, Zhang Y, Zhang Y, Lin Y. Sequential versus concurrent anthracyclines and taxanes as adjuvant chemotherapy of early breast cancer: a meta-analysis of phase III randomized control trials. Breast. 2012 Jun;21(3):389-93. doi: 10.1016/j.breast.2012.03.011. Epub 2012 Apr 26.
39. Kumar P, Aggarwal R. An overview of triple-negative breast cancer. Arch Gynecol Obstet. 2016 Feb;293(2):247-69. doi: 10.1007/s00404-015-3859-y. Epub 2015 Sep 4.
40. Schmid P, Rugo HS, Adams S, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Henschel V, Molinero L, Chui SY, Maiya V, Husain A, Winer EP, Loi S, Emens LA; IMpassion130 Investigators. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2020 Jan;21(1):44-59. doi: 10.1016/S1470-2045(19)30689-8. Epub 2019 Nov 27.
41. Cortes J, Rugo HS, Cescon DW, Im SA, Yusof MM, Gallardo C, Lipatov O, Barrios CH, Perez-Garcia J, Iwata H, Masuda N, Torregroza Otero M, Gokmen E, Loi S, Guo Z, Zhou X, Karantza V, Pan W, Schmid P; KEYNOTE-355 Investigators. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2022 Jul 21;387(3):217-226. doi: 10.1056/NEJMoa2202809.
42. Loizides S, Constantinidou A. Triple negative breast cancer: Immunogenicity, tumor microenvironment, and immunotherapy. Front Genet. 2023 Jan 12;13:1095839. doi: 10.3389/fgene.2022.1095839.
43. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, Brufsky A, Sardesai SD, Kalinsky K, Zelnak AB, Weaver R, Traina T, Dalenc F, Aftimos P, Lynce F, Diab S, Cortés J, O'Shaughnessy J, Diéras V, Ferrario C, Schmid P, Carey LA, Gianni L, Piccart MJ, Loibl S, Goldenberg DM, Hong Q, Olivo MS, Itri LM, Rugo HS; ASCENT Clinical Trial Investigators. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. N Engl J Med. 2021 Apr 22;384(16):1529-1541. doi: 10.1056/NEJMoa2028485.
44. Jessica S. Young, Kazuaki Takabe, Mariko Asaoka, Stephen B. Edge Comparing the American Joint Committee on Cancer (AJCC) breast cancer staging eighth versus seventh edition and breast cancer biology in large databases.10.1200/JCO.2019.37.15_suppl.e12077 Journal of Clinical Oncology 37, no. 15_suppl
45. Tečić Vuger A, Šeparović R, Vazdar L, Pavlović M, Lepetić P, Šitić S, Bajić Ž, Šarčević B, Vrbanec D. CHARACTERISTICS AND PROGNOSIS OF TRIPLE-

NEGATIVE BREAST CANCER PATIENTS: A CROATIAN SINGLE INstitution RETROSPECTIVE COHORT STUDY. Acta Clin Croat. 2020 Mar;59(1):97-108. doi: 10.20471/acc.2020.59.01.12.

46. Sharma B; Satyanarayan; Kalwar A, Sharma N, Kapoor A, Kumar N. Five year retrospective survival analysis of triple negative breast cancer in North-West India. Indian J Cancer. 2013 Oct-Dec;50(4):330-2. doi: 10.4103/0019-509X.123616..

47. Shimelis H, LaDuca H, Hu C, Hart SN, Na J, Thomas A, Akinhanmi M, Moore RM, Brauch H, Cox A, Eccles DM, Ewart-Toland A, Fasching PA, Fostira F, Garber J, Godwin AK, Konstantopoulou I, Nevanlinna H, Sharma P, Yannoukakos D, Yao S, Feng BJ, Tippin Davis B, Lilyquist J, Pesaran T, Goldgar DE, Polley EC, Dolinsky JS, Couch FJ. Triple-Negative Breast Cancer Risk Genes Identified by Multigene Hereditary Cancer Panel Testing. J Natl Cancer Inst. 2018 Aug 1;110(8):855-862. doi: 10.1093/jnci/djy106.

48. Saleh Y, Abdelkarim O, Herzallah K, Abela GS. Anthracycline-induced cardiotoxicity: mechanisms of action, incidence, risk factors, prevention, and treatment. Heart Fail Rev. 2021 Sep;26(5):1159-1173. doi: 10.1007/s10741-020-09968-2.

49. Ovcaricek T, Frkovic SG, Matos E, Mozina B, Borstnar S. Triple negative breast cancer - prognostic factors and survival. Radiol Oncol. 2011 Mar;45(1):46-52. doi: 10.2478/v10019-010-0054-4. Epub 2010 Dec 31.

50. Yang C, Zhang J, Ding M, Xu K, Li L, Mao L, Zheng J. Ki67 targeted strategies for cancer therapy. Clin Transl Oncol. 2018 May;20(5):570-575. doi: 10.1007/s12094-017-1774-3. Epub 2017 Oct 20.

51. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, Zackrisson S, Senkus E; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019 Aug 1;30(8):1194-1220. doi: 10.1093/annonc/mdz173. Erratum in: Ann Oncol. 2019 Oct 1;30(10):1674. Erratum in: Ann Oncol. 2021 Feb;32(2):284

52. Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, Dent R, Fenlon D, Gligorov J, Hurvitz SA, Im SA, Krug D, Kunz WG, Loi S, Penault-Llorca F, Ricke J, Robson M, Rugo HS, Saura C, Schmid P, Singer CF, Spanic T, Tolaney SM, Turner NC, Curigliano G, Loibl S, Paluch-Shimon S, Harbeck N; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol. 2021 Dec;32(12):1475-1495. doi: 10.1016/j.annonc.2021.09.019. Epub 2021 Oct 19.

53. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010 Nov 11;363(20):1938-48. doi: 10.1056/NEJMra1001389.

54. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, Gallardo C, Lipatov O, Barrios CH, Holgado E, Iwata H, Masuda N, Otero MT, Gokmen E, Loi S, Guo Z, Zhao J, Aktan G, Karantza V, Schmid P; KEYNOTE-355 Investigators. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. Lancet. 2020 Dec 5;396(10265):1817-1828. doi: 10.1016/S0140-6736(20)32531-9.

55. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im SA, Shaw Wright G, Henschel V, Molinero L, Chui SY, Funke R, Husain A, Winer EP, Loi S, Emens LA; IMpassion130 Trial Investigators. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018 Nov 29;379(22):2108-2121. doi: 10.1056/NEJMoa1809615. Epub 2018 Oct 20.

56. Waks AG, Winer EP. Breast Cancer Treatment: A Review. JAMA. 2019 Jan 22;321(3):288-300. doi: 10.1001/jama.2018.19323.
57. Traina TA, Miller K, Yardley DA, Eakle J, Schwartzberg LS, O'Shaughnessy J, Gradishar W, Schmid P, Winer E, Kelly C, Nanda R, Gucalp A, Awada A, Garcia-Estevez L, Trudeau ME, Steinberg J, Uppal H, Tudor IC, Peterson A, Cortes J. Enzalutamide for the Treatment of Androgen Receptor-Expressing Triple-Negative Breast Cancer. J Clin Oncol. 2018 Mar 20;36(9):884-890. doi: 10.1200/JCO.2016.71.3495. Epub 2018 Jan 26.
58. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res. 2020 Jun 9;22(1):61. doi: 10.1186/s13058-020-01296-5.
59. Chai Y, Chen Y, Zhang D, Wei Y, Li Z, Li Q, Xu B. Homologous Recombination Deficiency (HRD) and BRCA 1/2 Gene Mutation for Predicting the Effect of Platinum-Based Neoadjuvant Chemotherapy of Early-Stage Triple-Negative Breast Cancer (TNBC): A Systematic Review and Meta-Analysis. J Pers Med. 2022 Feb 21;12(2):323. doi: 10.3390/jpm12020323.
60. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, Owen J, Abraham J, Barrett S, Barrett-Lee P, Brown R, Chan S, Dowsett M, Flanagan JM, Fox L, Grigoriadis A, Gutin A, Harper-Wynne C, Hatton MQ, Hoadley KA, Parikh J, Parker P, Perou CM, Roylance R, Shah V, Shaw A, Smith IE, Timms KM, Wardley AM, Wilson G, Gillett C, Lanchbury JS, Ashworth A, Rahman N, Harries M, Ellis P, Pinder SE, Bliss JM. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med. 2018 May;24(5):628-637. doi: 10.1038/s41591-018-0009-7. Epub 2018 Apr 30.
61. Cortesi L, Rugo HS, Jackisch C. An Overview of PARP Inhibitors for the Treatment of Breast Cancer. Target Oncol. 2021 May;16(3):255-282. doi: 10.1007/s11523-021-00796-4. Epub 2021 Mar 12.
62. Robson ME, Tung N, Conte P, Im SA, Senkus E, Xu B, Masuda N, Delaloge S, Li W, Armstrong A, Wu W, Goessl C, Runswick S, Domchek SM. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol. 2019 Apr 1;30(4):558-566. doi: 10.1093/annonc/mdz012.
63. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, Roché H, Im YH, Quek RGW, Markova D, Tudor IC, Hannah AL, Eiermann W, Blum JL. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. N Engl J Med. 2018 Aug 23;379(8):753-763. doi: 10.1056/NEJMoa1802905. Epub 2018 Aug 15.