POSSIBLE TREATMENT OPTIONS FOR HER2 POSITIVE AND HER2 NEGATIVE BREAST CANCER: A REVIEW

A Research Report Submitted to the Department of Pharmacy, East West University in Partial Fulfillment of the Requirement for the Degree of Bachelor of Pharmacy.

Submitted by:

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Possible Treatment Options for HER2 Positive & HER2 Negative Breast Cancer: A Review"** is an authentic and genuine research work carried out by me under the guidance of **Ms. Farah Shahjin,** Senior Lecturer, Department of Pharmacy, East West University, Aftabnagar, Dhaka, Bangladesh.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled **"Possible Treatment Options for HER2 Positive & HER2 Negative Breast Cancer: A Review"** is a bona-fide research work done by **Rupali Ghosh (ID No: 2013-3-70-032)** in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

……..

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ENDORSEMENT BY THE CHAIRPERSON

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DEDICATION

This research paper is dedicated to my family, my dearest daughter who are my biggest inspiration and to my research supervisor

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LIST OF FIGURES

LIST OF ABBREVIATION

17AAG	17-*N*-Allylamino-17-demethoxygeldanamycin
ABC	Advanced breast cancer
ADCC	Antibody dependent cellular cytotoxicity
ADP	Adenosine di phosphate
Akt	Serine-threonine kinase
ASCO	American Society of Clinical Oncology
ATP	Adenosine tri phosphate
BBB	Blood brain barrier
BER	Base excision repair
BJC	British journal council
CAP	College of American Pathologists
CD3	Cluster of differentiation 3
CDK	Cycline dependent kinase
CDKI	Cycline dependent kinase inhibitor
CEP17	Copy number of the chromosome 17 centromere
CISH	Chromogenic in situ hybridization
CNS	Central nervous system
DFS	Disease free survival
DNA	Deoxyribosze nucleic acid
ECD	Extracellular domain
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeability and retention
ER	Estrogen receptor

Fc	Fragment crystallizable
FDA	Food & drug administration
FISH	Fluorescence in-situ-hybridization
GH	Growth hormone
HCL	Hydrochloric acid
HDAC	Histone deacetylase
HDI	Histone deacetylase inhibitor
HER	Human epidermal growth factor receptor
HER1	Human epidermal growth factor receptor 1
HER2	Human epidermal growth factor receptor 2
HER3	Human epidermal growth factor receptor 3
HER4	Human epidermal growth factor receptor 4
HIF-1	Hypoxia inducible factor 1
HR	Hormone receptor
HSP 90	Heat shock protein 90
HSP70	Heat shock protein 70
ICBSG	International Breast Cancer Study Group
IFN-γ	Interferon gamma
IGF	Insulin like growth factor
IGF-1	Insulin like growth factor 1
IGF-1R	Insulin like growth factor 1 receptor
IGF-2	Insulin like growth factor 2
IGF-2R	Insulin like growth factor 2 receptor
IgG	Immunoglobulin G
IHC	Immunohistochemistry

LDL	Low density lipoprotein
LVEF	Left ventricular ejection fraction
MAPK	Mitogen activated protein kinase
MBC	Metastatic breast cancer
MCC	N-maleimidomethyl cyclohexane-1-carboxylate
MEK	Mitogen activated protein kinase-kinase enzyme
mRNA	Messenger ribose nucleic acid
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
NER	Nucleotide excision repair
NK	Natural killer
ORR	Overall response rate
OS	Overall survival
PARP	Poly (ADP-ribose) polymerase
PARPi	Poly (ADP-ribose) polymerase inhibitors
PCR	Pathological complete response
PD-1	Programmed death-1
PD-L1	Programmed death- ligand 1
PFS	Progression free survival
PI3K	Phosphatidylinositol-3-kinases
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
PR	Progesteron receptor
PTEN	Phosphatase and tensin homolog
RR	Response rate

SISH	Silver enhanced in situ hybridization
T-DM1	Trastuzumab-emtansine conjugate
TIL	Tumor infiltating lymphocytes
TKI	Tyrosine kinase inhibitors
TNBC	Triple negative breast cancer
TTP	Time to Progression
USA	United states of America
VEG-A	Vascular endothelial growth factor ligand-A
VEGF	Vascular endothelial growth factor
VEGF-A.	Vascular endothelial growth factor-A
VEGFR-2	Vascular endothelial growth factor receptor 2
XP	Capecitabine and cisplatin combination

Abstract

This review will summarize currently available all possible treatment options for HER2 positive & HER2 negative breast cancer. Significant research have been conducted to find out and understand the biology and molecular features of HER2 positive & HER2 negative breast cancer and possible treatment options including conventional chemotherapy and targeted therapy and result of several clinical trials have also been published. In this research work, I have done computer based screening of these articles and compiled up currently available all treatment options for HER2 positive & HER2 negative breast cancer and pros and cons of these treatments also stated. The purpose of this study is to give an overview on already available treatment options for HER2 positive & HER2 negative breast cancer and anyone can choose their suitable options among these. In terms of result it is found that targeted therapy for HER2 positive breast cancer and combination chemotherapy for HER2 negative breast cancer is more effective.

Chapter 1

Introduction

1.1 Overview:

Breast cancer is among the most commonly diagnosed cancers worldwide and is the number one cancer found in women, with an estimated 14.1 million cases reported in 2012. (Schroeder, Stevens and Sridhar, 2014) Breast cancer is the most common malignancy and the second cause of cancer death in women in the USA. (Gajria and Chandarlapaty, 2011) In Bangladesh, the incidence rate of breast cancer was about 22.5 per 10000 in females. Breast cancer has been reported as the highest prevalence rate among Bangladeshi women between 15 and 44 years of age when compared to other types of cancer. (News.isncc.org, 2017) Although there are many risk factors known to increase the occurrence of breast cancer, how these risk factors contribute to the transformation of normal cells into cancer cells has remained incompletely understood. Accumulating evidence suggests that genetic alteration, including both inherited and acquired mutations of certain tumor suppressors and oncogenes are an important cause of breast cancer. (Shim et al., 2012) Breast cancer is a heterogeneous disease that can be classified by microscopic appearance and molecular profiles that includes the expression of estrogen receptor and amplification of HER2. (Gajria and Chandarlapaty, 2011) The ligand orphan receptor, human epidermal growth factor receptor 2 (HER2), represents a prominent target in breast cancer with approximately 20-30% of patients with primary invasive breast cancer overexpressing HER2 receptor. Indeed, high levels of HER2 overexpression or gene amplification have been definitively associated with a more aggressive disease phenotype with a shorter time to relapse after initial treatment and as a significant predictor of survival. Understanding the biology of HER2 is fundamental to maximizing its clinical therapeutic efficacy and ultimately deciphering mechanisms of resistance to anti-HER2 therapies. The HER2 gene, a proto-

oncogene that maps to chromosome 17q21, encodes the HER2 receptor, a 1255 amino acid, 185kD transmembrane glycoprotein with an intracellular domain with tyrosine kinase catalytic activity. (Subbiah and Gonzalez-Angulo, 2013)

Treatment decisions are guided by stage, tumor grade and hormone and HER2 status. (LoRusso, 2013) Chemotherapy has significantly improved DFS and OS for women with nonmetastatic breast cancer. Traditionally, systemic therapy has been given in the postoperative and adjuvant setting. In the last 2 decades, however, its use has been evaluated in the preoperative or neoadjuvant setting as well. (Zhang and Hurvitz, 2016) Before the advent of HER2 targeted therapeutics, patients with HER2 positive disease had an associated increase in mortality and recurrence. At present, there are several EGFR family inhibitors, but only 2 are approved for treatment of breast cancer. Trastuzumab is the only approved adjuvant treatment specific for early stage HER2 positive breast cancer. (Fink and Chipuk, 2013)

If breast cancer cells don't contain HER2 (HER2 gene isn't overexpressed), the condition is called HER2-negative breast cancer. HER2 negative breast cancer is considered breast cancer groups 1 and 4 when determining a treatment plan. Group 1 breast cancers are likely to benefit from hormone therapy and chemotherapy. Group 4 or basal like are likely to benefit from chemotherapy. (Perez, Patel and Moreno-Aspitia, 2010) Disease that is HER2-negative represents the largest and most heterogeneous advanced breast cancer (ABC) group because it includes both HR-positive tumors (which can be sensitive or resistant to endocrine therapy) and HR-negative tumors (that is basal like or triple negative breast cancers). (Joy et al., 2015)

Possible therapeutic targets for basal like HER2 negative breast cancer will be-targeting at gene level, receptors, immunomodulatory, signaling pathway and others and lots of researchers are working on this from different aspects. Patients with HER2 negative breast cancer are not candidates for HER2 targeted agents. Ongoing research is aimed at identifying and understanding the benefit of established and emerging therapies in this disease setting. (Perez, Patel and Moreno-Aspitia, 2010)

1.2 Biology of HER2 Breast Cancer:

HER2 belongs to the epidermal growth factor receptor (EFGR) family of receptor tyrosine kinase. This family consists of four receptors – HER1 HER2 HER3 HER4 – which are involved in regulating cell growth, survival, invasion, angiogenesis and differentiation.

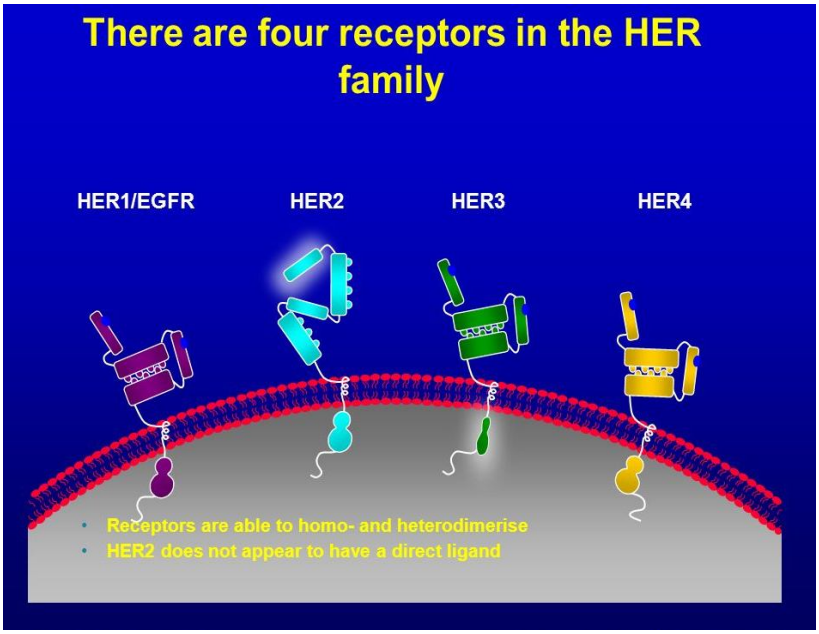

Figure 1.1: All receptors in HER family (Yarden & Sliwkowski, 2001)

HER receptors are inactive monomers, and to activate signaling pathways they have to undergo dimerization. HER dimerization leads to activation of two important signaling pathways– PI3K/Akt and Ras/Raf/MEK/MAPK. HER2 is always in active conformation and it is preferred partner for other HER receptors and the HER2-HER3 dimer (heterodimerization) is an important oncogenic unit that signals constitutively to PI3K and Akt. All breast cancer should be evaluated for HER2 overexpression. Amplification or overexpression of HER2 is present in around 22% of early breast cancers, 35% of locally advanced and metastatic tumors, and 40% of inflammatory breast cancers and is associated with aggressive disease and poor prognosis. (Callahan and Hurvitz, 2011) (Sevcikova, Vertakova-Krakovska and Spanik, 2013)

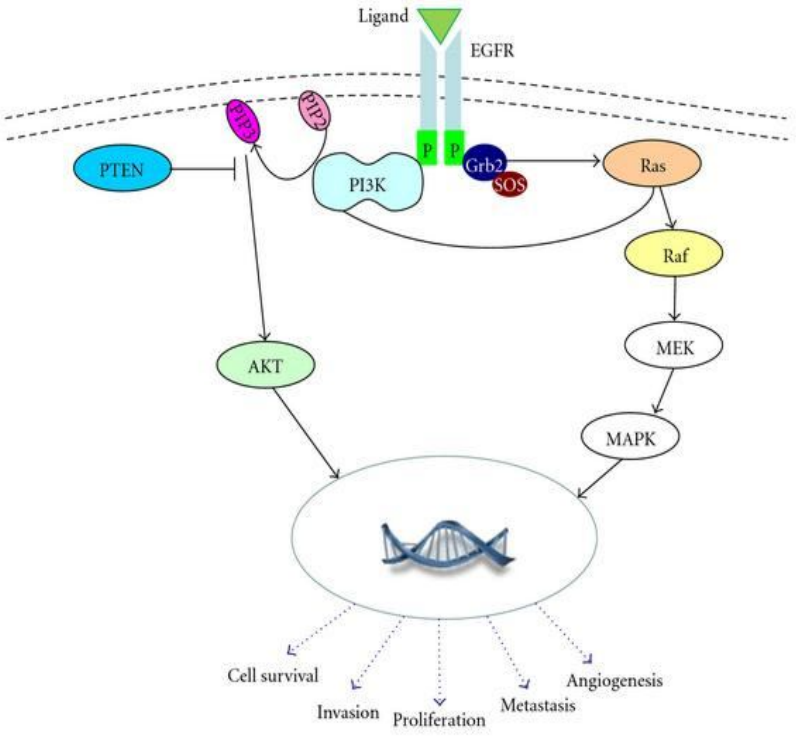

Figure 1.2: Ras/Raf/MEK/MAPK pathway (Callahan and Hurvitz, 2011)

1.3 Identifying HER2 and HER2 overexpression:

HER2 testing can be done by targeting protein and gene. The most widely used methods to detect HER2 amplification are immunohistochemistry (IHC) and fluorescence in-situ-hybridization (FISH).FISH detects the gene amplification by measuring the number of copies of HER2 genes in the tumor cells. (Viswanath and Pathak, 2016) Typically FISH is used to compare the HER2 gene copy number to the copy number of the chromosome 17 centromere (the HER2/CEP17 ratio) in a sample of patient's tumor tissue. Based on a joint update by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) in 2014, a ratio of 2.0 or greater is considered positive. If the score is below 2.0, the actual HER2 copy number should be assessed. A HER2 copy number of 6 or greater is considered negative. HER2 copy numbers between 4 and 6 are considered equivocal, in which case further testing should be performed. (Nye and Mutonga, 2016) IHC measures the number of receptors on the cell surface and therefore, detects overexpression (Viswanath and Pathak, 2016). While a score of 0 or 1 is HER2 negative and a score of 3 is HeR2 positive, a score of 2 is equivocal and indicates the need for further testing. (Nye and Mutonga, 2016)

Chromogenic in situ hybridization (CISH) uses chromogens for signal identification with several advantages over FISH: permanent staining, use of bright field microscopy, easy identification of the target cells. Silver enhanced in situ hybridization (SISH) is a highly sensitive technique with permanent staining thus allowing specimen archiving. HERmark® assay is a new assay that allows us measuring of total HER2 protein and the amount of HER2 homodimers in breast cancer tissue. (Dadic Plavetic, Kulic and Vebanec, 2013)

Chapter 2

Methods

2.1 Materials and Methods

A computer based literature search was carried out using PubMed, ResearchGate for original article that were written in English and published before April 01, 2017. Review of various literatures including published literature review on BJC and ASCO and data reported at clinicaltrials.gov related to this topic was carried out. Both prospective and retrospective studies were included here. The initial search used the term “Biology of HER2”, “treatment options for HER2 positive” and “treatment options for HER2 negative”.

Chapter 3

Treatment Options for HER2 Positive Breast Cancer

3.1 The monoclonal Antibody, Trastuzumab :

The use of trastuzumab with or without chemotherapy is the backbone of systemic treatment of HER2-positive breast cancer. It is currently the standard first line agent in the treatment of HER2 positive metastatic breast cancer (Tinoco et al., 2013) Trastuzumab, the first available HER2-targeted therapy, is a humanized murine IgG monoclonal antibody that binds to the HER2 extracellular domain. Its mechanism of action has not been fully ascertained, however, it has been shown to reduce signaling through the PI3K/Akt and Ras/Raf/MEK/MAPK pathways, leading to cell cycle arrest, inhibition of DNA repair after chemotherapy, inhibition of cleavage of the extracellular domain of the HER2, decreased intracellular signal transduction, inhibition of ligand- independent HER2 receptor dimerization, antiangiogenic effects and induction of apoptosis. Two small clinical studies have suggested that trastuzumab may promote antibody dependent cellular cytotoxicity (ADCC), in which the antibody Fc portion is bound to Fc receptors expressed by immune effector cells, such as NK cells. A recent analysis of genomic DNA samples from trastuzumab treated breast cancer patient did not support ADCC as a major mechanism of action of trastuzumab. (Callahan and Hurvitz, 2011) (Li, 2013) (Sevcikova, Vertakova-Krakovska and Spanik, 2013). Patients having HER2 amplified breast cancer, 70% of them show primary resiatance to trastuzumab. In addition, an important number of patients who achieve initial response to the treatment tend to develop secondary trastuzumab resistance. Telomerase inhibitors have shown synergy with trastuzumab in inhibiting HER2 positive cancer cell growth and restoring trastuzumab sensitivity in trastuzumab-resistant cell lines. (Tinoco et al., 2013)

Cardiac toxicity is an important side effect of trastuzumab treatment and has been observed in patients who received trastuzumab as a single agent or in combination

with chemotherapy for metastatic disease and in primary breast cancer. The data concerning cardiac events differ from one clinical study to the next. (Sevcikova, Vertakova-Krakovska and Spanik, 2013)

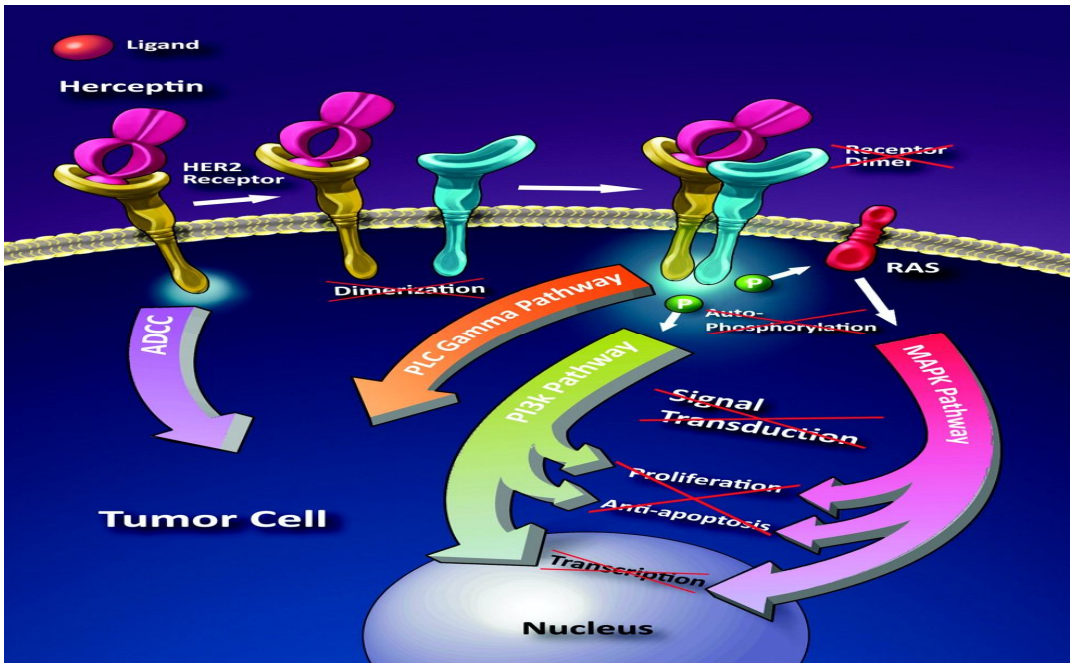

Figure 3.1: Mechanism of action of Trastuzumab (Herceptin) (Anon, 2017)

3.2 Combining pertuzumab and trastuzumab:

Pertuzumab is a humanized monoclonal antibody which binds to the her2 receptor, binding to a different domain of the extracellular portion of the HER2 receptor than trastuzumab and blocks the dimerization process of HER2 receptor. Active investigation has done by giving pertuzumab in combination with trastuzumab. Purpose of this study was to explore the theoretical advantage of using two HER2 blockade of the HER2 signaling pathway. (Tinoco et al., 2013) In the tumor xenograft models, combination of pertuzumab and trastuzumab showed enhanced anti-tumor activity than compared to pertuzumab or trastuzumab alone. (Viswanath and Pathak,

2016) The phase III trial showed that the addition of pertuzumab to trastuzumab plus docetaxel, when used as first-line treatment for HER2 positive metastatic breast cancer significantly prolonged median progression free survival (PFS) by 6.1 months, with no increase in cardiac toxicity. Based on the above data Perjeta® as the first line treatment for HER2 positive metastatic breast cancer (comination of pertuzumab, trastuzumab and docetaxel) got approval from FDA on June 8, 2012. Another trial named NeoSphere was also assessed the effect of pertuzumab and trastuzumab combination. In this trial, the group that received pertuzumab, trastuzumab and docetaxel in combination showed significantly improved pathological complete response (PCR) rate than the group that received trastuzumab and docetaxel. (Tinoco et al., 2013)

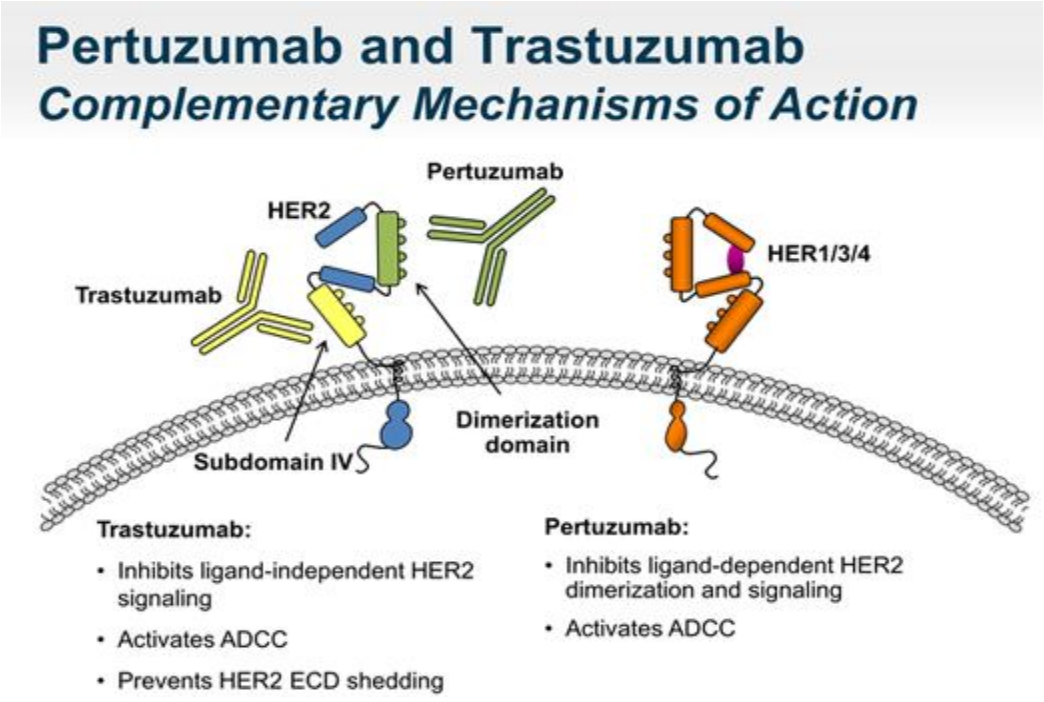

Figure 3.2: Combined mechanism of action of trastuzumab and partuzumab (Baselga J. et al; 2005)

3.3 Trastuzumab with chemotherapy:

The addition of trastuzumab to chemotherapy in patients with previously untreated metastatic breast cancer led to a significantly higher objective response rate, prolonged time to progression and improved overall survival compared with chemotherapy alone. Furthermore, in patients with early-stage breast cancer, the addition of trastuzumab to chemotherapy significantly improved disease-free survival and overall survival in multiple clinical trials in the early and locally advanced settings. (Drakaki and Hurvitz, 2017) Sequential anthracycline-taxane based chemotherapy in combination with trastuzumab gives a pCR (pathological complete response) of 40% compared with a pCR of 17% with the chemotherapy alone. (Sevcikova, Vertakova-Krakovska and Spanik, 2013)

Evidence support that continued use of 2nd and 3rd line chemotherapeutic agent with trastuzumab has significantly better median TTP (Time to Progression) than trastuzumab alone. (Callahan and Hurvitz, 2011) Most chemotherapy agents and HER2 targeted therapies do not cross the intact BBB or pumped out of the CNS by P-glycoprotein present in the BBB, so they cannot reach the sufficient therapeutic level to eradicate metastatic cells. A concept that has been proven by a number of labeled trastuzumab imaging studies is cranial surgery and brain radiotherapy disrupt the BBB and allow the access of systemic drugs to the tumor. Also several clinical studies have shown that the combination of chemotherapy with trastuzumab improved survival, even after the development of brain metastases. (Leone and Leone, 2015)

Patients with HER2 positive disease demonstrate adverse disease characteristics at presentation (large tumor size), have shorter survival time, and have a higher risk for

disease recurrence. So, the investigation of trastuzumab in combination with anthracycline based chemotherapy in several large, randomized, controlled trials have done. By combining their results in a meta analysis, 38% lower DFS event rate and a similar 34% difference in overall survival favoring the administration of trastuzumab. There have been a number of concerns regarding the use of trastuzumab and the incidence of cardiotoxicity. This analysis confirmed that benefits in disease control and survival came at the cost of serious cardiovascular adverse events, particularly heart failure accompanied by LVEF (left ventricular ejection fraction) decline.the incidence of cardiotoxicity to be significantly higher among trastuzumab treated patients than among control groups. (Dahabreh et al., 2008)

The National Surgical Adjuvant Breast and Bowel Projection Trial B-31 (March 15, 2005) compared adjuvant chemotherapy with or without concurrent trastuzumab in women with surgically removed HER2 positive breast cancer. Of these, 133 were in the trastuzumab group and 261 in the control group. It was found that, trastuzumab combined with paclitaxel after doxorubicin and cyclophosphamide improves outcomes among women with surgically removed HER2 positive breast cancer. (Romond and Perez, 2005) It was also reported that, Trastuzumab improves survival in the adjuvant treatment, although combined therapy with anthracyline based regimens has been associated with cardiac toxicity. (Slamon et al., 2011) In four large trials adjuvant trastuzumab was evaluated and on the basis of data from these trials, adjuvant trastuzumab has become the foundation of care for HER2 positive early breast cancer. (Perez et al., 2011) Cardiotoxicity is unacceptably high with the concurrent use of anthracyclines and trastuzumab and is not recommended. (Callahan and Hurvitz, 2011) The mechanism of cardiac dysfunction associated with trastuzumab is not clearly understood, although several hypotheses have been

proposed. These include the modification of anthracycline-induced cardiotoxicity, immunemediated destruction of cardiomocytes, the effects on HER2 signaling pathways that are required for the maintenance of normal cardiac contractility and the dependence on HER2 for myocyte survival, which is then impaired during trastuzumab treatment. Thus in the adjuvant setting, continued treatment with trastuzumab is contraindicated if there is any evidence of cardiac dysfunction and monitoring for early evidence of left ventricular dysfunction is important. (Viani et al., 2007)

3.4 Tyrosine Kinase inhibitor:

To continue improving upon oral based therapies for HER2 positive breast cancer, a new generation of irreversible HER2 Tyrosine Kinase Inhibitors (TKI) is being developed. Most of the TKIs form covalent, irreversible bonds with the kinase domain of HER2 and are being developed with a specific focus on patients with acquired resistance to current therapies. (Nye and Mutonga, 2016)

Lapatinib is a dual tyrosine kinase inhibitor of HER2 and epidermal growth factor receptor (EFGR). (Drakaki and Hurvitz, 2017) It is orally administered small molecule, which binds to the intracellular ATP binding pocket of the two receptors and inhibits receptor autophosphorylation, preventing the activation of downstream cellular signals that promote tumor cell survival and proliferation. The systemic analysis of lapatinib demonstrated that it is well tolerated with manageable toxic effects and the incidence of cardiac toxicity was lower with lapatinib compared with trastuzumab. (Li, 2013) Since the action of lapatinib is intracellular, it may avoid resistance mechanisms involving the HER2 extracellular domain. Lapatinib is primarily used to treat trastuzumab-resistant tumors based on a phase III study in

which patients pretreated with an antracycline, taxane and trastuzumab were randomized to receive capecitabine and laptinib versus caqpecitabine alone. A significant improvement in TTP was seen with the combination regimen. (Callahan and Hurvitz, 2011) Potential ability of lapatinib to cross the BBB has been extensively tested in the treatment of HER2 positive brain metastases. (Leone and Leone, 2015) The comparison of lapatinib versus trastuzumab was the aim of the *GeparQuinto* trial. This randomized phase III study included 620 patients with operable or locally advanced HER2 positive breast cancer. The results have confirmed a higher pCR rate for the trastuzumab arm compared with that of lapatinib. The most common adverse effects associated with lapatinib were diarrhoea and skin toxicity; trastuzumab caused more often oedema and dyspnoea. (Sevcikova, Vertakova-Krakovska and Spanik, 2013)

Neratinib is an irreversible, orally administered small molecule tyrosine kinase inhibitor of HER2 that covalently binds to the cysteine residues of the ATP binding portion of the HER tyrosine kinases. It has demonstrated promising preclinical and clinical antitumor activity in HER2 positive breast cancer. Phase II data in patients who had been previously treated with trastuzumab demonstrated that single-agent neratinib could produce an overall response rate (ORR) of 24% and in patients with no prior trastuzumab the ORR increased to 56% but 93% of patients experienced diarrhea. (Nye and Mutonga, 2016)

Afatinib is an irreversible small molecule inhibitor of ErbB-receptor family, targeting the intracellular tyrosine kinase of the HER2 molecule. (Tinoco et al., 2013) It binds covalently and irreversibly blocks all kinase-competent HER family members. (Li, 2013) In initial phase I studies, diarrhea, vomiting, nausea, fatigue and rash were the

most common adverse events. Results of an open-label phase II study in advanced HER2 positive breast cancer patients after failure of trastuzumab were recently published, showing clinical benefit in 53% of the patients enrolled. (Tinoco et al., 2013) Afatinib is also under investigation in various combinations and in the neoadjuvant settins. (Li, 2013)

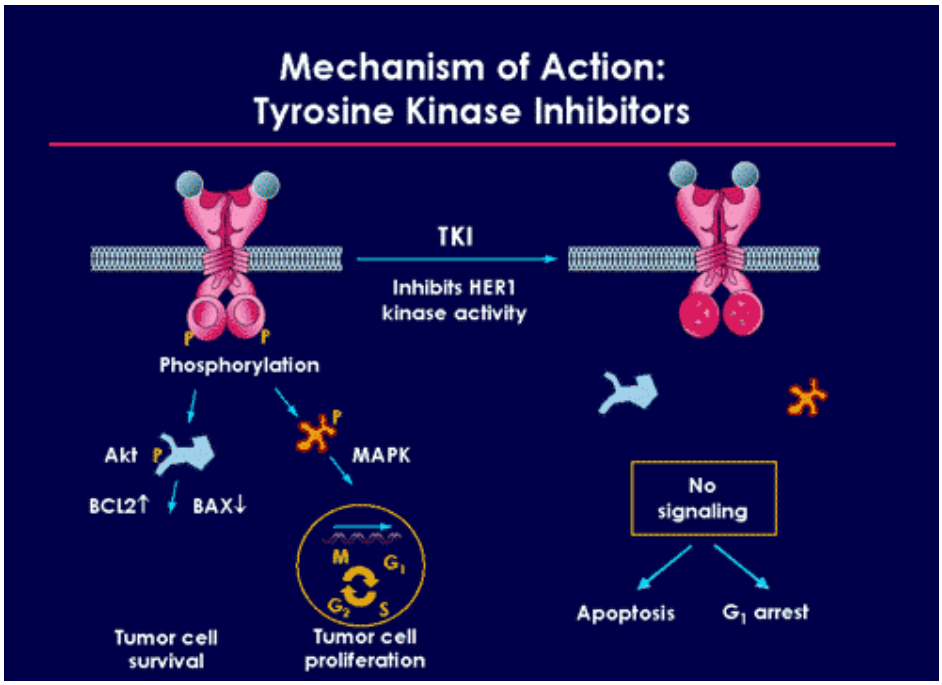

Figure 3.3: Mechanism of action of tyrosine kinase inhibitor (Moyer JD et al, 1997)

ONT-380 is another TKI that has a milder adverse effect profile. Unlike afatinib and neratinib, ONT-380 is a reversible TKI that selectively inhibits HER2 without significant inhibition of EGFR. ONT-380 is under investigation in three phases I studies in combination with other anti-HER2 therapy and found that with trastuzumab has activity in the CNS. (Nye and Mutonga, 2016)

TAK-285 is a novel oral small-molecule dual HER2/EGFR TKI. The initial phase I study demonstrated good tolerance of TAK-285, with elevation in aminotransferases and hyporexia being the most prominent dose limiting toxicities. **ARRY-380** is an orally active, reversible selective inhibitor of HER2 Tyrosine Kinase receptor with antitumor activity. Phase I trial found that ARRY-380 to be well tolerated, with rash and diarrhea as the most frequent adverse effects, along with promising signs of clinical activity, especially in pretreated patients with HER2 positive metastatic breast cancer. (Tinoco et al., 2013)

3.5 Neoadjuvant Therapy (trastuzumab and chemotherapy):

In locally advanced breast cancer, neoadjuvant (preoperative) chemotherapy has increased rates of breast conserving surgery. (Callahan and Hurvitz, 2011) The addition of trastuzumab to neoadjuvant treatment in the locally advanced setting is attractive. Initial data on trastuzumab in the neoadjuvant setting revealed that the pathologic complete response rate which is connected with a significantly improved outcome, increase from 26.0 to 65.2% with the addition of trastuzumab to sequential anthracycline and taxane based chemotherapy and long term follow up of the patients revealed a significantly lower relapse rate in patients receiving chemotherapy plus trastuzumab. Trastuzumab related cardiac dysfunction, which involves asymptomatic decrease in the left ventricular ejection fraction (LVEF), may be influenced by concurrent or sequential chemotherapy as well as type of chemotherapy. (Li, 2013) Concurrent administration of trastuzumab and paclitaxel followed by anthracycline based chemotherapy produced a higher pCR rate than the same chemotherapy alone. Updated safety and efficacy analyses conducted with an additional 22 patients who all received trastuzumab and chemotherapy,

demonstrated an improvement in 3-year disease free survival (DFS) with the addition of trastuzumab. (Callahan and Hurvitz, 2011) A retrospectic review of records of patients with metastatic breast cancer (MBC) showed that cardiac dysfunction is lower with trastuzumab and paclitaxel than trastuzumab & anthracycline. (Li, 2013) In GeparQuattro trial found that combining trastuzumab with anthracycline-taxane-based neoadjuvant chemotherapy results in high pCR rate without clinically relevant early toxicity. (Untch et al., 2010)

3.6 Combination of Lapatinib and trastuzumab:

Dual combination targeting both intracellular and extracellular domain of HER2 has also been tested in HER2 positive MBC, as demonstrated by preclinical studies in HER2 positive cell lines, that shown a synergistic interaction between lapatinib nad trastuzumab. (Fabi et al., 2016) The combination of lapatinib and trastuzumab has demonstrated synergy in preclinical models and has recently been shown to improve PFS in patients with MBC that had progressed on trastuzumab. (Callahan and Hurvitz, 2011) Based on these data, clinical evaluation of dual HER2 targeting was undertaken, and ultimately showed that in heavily pretreated, trastuzumab-resistant HER2 positive MBC, PFS were improved with trastuzumab plus lapatinib compared with lapatinib alone. (Drakaki and Hurvitz, 2017) In a phase III trial PFS was 12.0 weeks in the combination arm but only 8.1 weeks with lapatinib arm. This combination was well tolerated, with fewer serious adverse effects than would be expected with a chemotherapy combining regimen. (Callahan and Hurvitz, 2011)

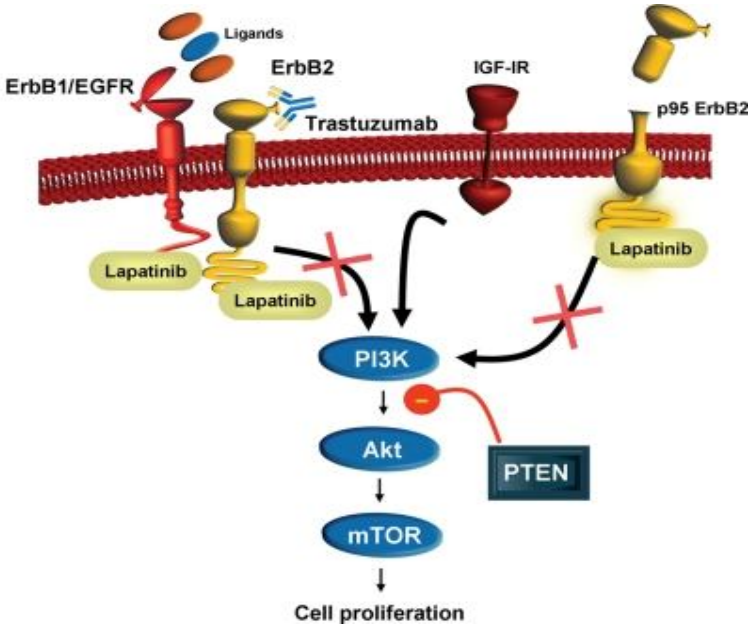

Figure 3.4: Combine action of lapatinib and trastuzumab (Callahan and Hurvitz, 2011)

3.7 Novel approach mTOR inhibitor:

mTOR is a serine/threonine protein kinase, which is found downstream of PI3K and Akt. It comprises two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which are structurally similar but functionally different. mTORC1 is the target of rapamycin and rapamycin analogues, such as everolimus, and leads to cell anabolic growth by promoting mRNA translocation and protein synthesis and also has roles in lipid synthesis and glucose metabolism via its action on 40S ribosomal protein S6 kinase 1 and eukaryotic initiation factor 4E binding protein. (Paplomata and O'Regan, 2014) (Lee, Loh and Yap, 2015) **Rapamycin** was the first available mTOR inhibitor. It was initially developed and used as an immunosuppressant in transplant recipients. (Lee, Loh and Yap, 2015) Preclinical models have shown that the combination of trastuzumab with rapamycin reduces the

activity of the PI3K, MAPK signaling pathway and show synergism in clinical activity. (Callahan and Hurvitz, 2011) (Tinoco et al., 2013)

Everolimus is an oral mTOR inhibitor, termed as rapalogues and work as allosteric inhibitors of mTORC1. The combination of everolimus and trastuzumab, with or without chemotherapy, has been explored in several phase I and II clinical trials. The overall response rate (ORR) of this combination therapy was approximately 20% in all 4 trials and toxicities were tolerable. (Lee, Loh and Yap, 2015) (Tinoco et al., 2013) The antidiabetic drug, **metformin** has been shown to inhibit mTOR and is being evaluated in breast cancer for this reason. (Callahan and Hurvitz, 2011) mTOR inhibitors may cause hyperglycemia, with elevations in both low density lipoprotein (LDL) cholesterol and triglycerides. Everolimus is contraindicated in patients with uncontrolled diabetes and requires optimization of glycemic control prior to initiation. (Lee, Loh and Yap, 2015) Other mTOR inhibitors under development in HER2 resistant breast cancer include temsirolimus and INK128, both undergoing phase 1/2 clinical trials. (Wilks, 2015)

3.8 Angiogenesis inhibitor:

Angiogenesis is a pivotal component of cancer growth, including invasion and metastasis. Tumors induce blood vessel growth (angiogenesis) by secreting various growth factors (for example vascular endothelial growth factor, VEGF) which allows tumor expansion. It has been shows decades ago that without expanding vasculature, the tumor growth cannot exceed 2-3 mm. (Fakhrejahani and Toi, 2014) Thus, angiogenesis is a required step for the spread of tumor, invasion and/or metastasis. The inhibition of angiogenesis is emerging as a new, attractive therapeutic approach to control tumor progression. (Nielsen et al., 2010) Preclinical

studies shown that VEGF induced proliferation in breast cancer cell lines by interfering with the action of oestrogens and VEGF is overexpressed by hypoxic tumor cells and host macrophages. (Kümler et al., 2016) (Gordon, Mendelson and Kato, 2010) Thus current antiangiogenic strategies have therefore mainly aimed at blocking the action of VEGF. VEGFR-2 (receptor of VEGF) has direct involvement in pathological angiogenesis and has therefore become an important target for drug development. (Gordon, Mendelson and Kato, 2010) (Nielsen et al., 2010) Lymphatic dissemination is an important mechanism for tumor angiogenesis as it allows the tumor to disseminate into the systemic circulation. A protein named hypoxia inducible factor 1 (HIF-1) is upregulated in cells growing at low oxygen concentrations, promotes lymphatic metastasis in breast cancer. This study suggests that targeting HIF-1 may be a useful strategy to limit lymphoangiogenesis (blocked through administration of HIF-1 inhibitor digoxin and imatibin) and thus metastatic dissemination in breast cancer. (Hielscher and Patel, 2015)

Bevacizumab is a recombinant humanized monoclonal antibody that binds VEGF and prevents it from binding to its receptor. Thus, inhibits a single isoform of the VEGF ligand, VEGF-A. Clinical studies indicate that the anti-neoplastic activity of bevacizumab as monotherapy is modest. (Gordon, Mendelson and Kato, 2010) (Nielsen et al., 2010) Preclinical data demonstrated an interaction between the HER2 and VEGF pathway and providing a rationale for combining therapies targeting each. (Callahan and Hurvitz, 2011) A first and second line phase II trial of bevacizumab in combination with docetaxel demonstrated a RR (response rate) of 52% and PFS of 7.5 months. The tolerability of bevacizumab is generally acceptable and can readily be administered in combination with chemotherapeutic agents. (Nielsen et al., 2010)

Pazopanib is an investigational VEGFR inhibitor and recent phase II data have demonstrated potential synergy between pazopanib and lapatinib in the first line HER2+ breast cancer patients . (Gordon, Mendelson and Kato, 2010) Novel agents targeting VEGF include aflibercept which binds to all isoforms of VGRF and have 100 to 1000 fold higher affinity for VGRF than other VGRF antagonists. Another novel agent is TB-403 which is a anti-placental growth factor antibody, selectively binds VEGFR1 and inhibit the growth and metastasis of various tumors including those resistant to VEGF inhibitors. This drug is currently being investigated in a phase I trial and it did not affect healthy vessels. (Nielsen et al., 2010) Toxicity of antiangiogenic agents varies considerably depending on the mechanism of action and target specificity. Essentially all antiangiogenic agents cause hypertension, bleeding and thromboembolic events, impaired wound healing, gastrointestinal perforation. Interestingly, bevacizumab seems to be the least toxic of the approved antiangiogenic drugs. (Kerbel, 2011) (Nielsen et al., 2010) Another threat is change in biologic aggressiveness induced by antiangiogenic therapy. Antiangiogenic therapy can increase tumor hypoxia which results in the upregulation of a number of genes that involved in promoting growth, invasion and metastasis. (Kerbel, 2011)

3.9 Antibody conjugates T-DM1:

Chemotherapy has long been considered our most efficient weapon in the fight against breast cancer, but for patients who are unable to tolerate chemotherapy for those T-DM1 in the first line setting. (Nye and Mutonga, 2016) (Piccart-Gebhart, 2005) Trastuzumab-DM1 (T-DM1) is a novel anti-HER2 drug conjugate in development for treatment of patients with HER2 positive breast cancer. T-DM1 combines the HER2 targeting properties of trastuzumab with intracellular delivery of

DM1 (emtansine), a highly potent derivative of the antimicrotubule agent maytansine. DM1 binds to tubulin and inhibits microtubule assembly with greater potency than vincristin or vinblastin (polymerization inhibitor). In T-DM1, trastuzumab and DM1 are covalently linked via the thioether linker (N-maleimidomethyl) cyclohexane-1-carboxylate (MCC). The stability of MCC strongly contributes to the favorable activity and toxicity profiles of T- DM1.

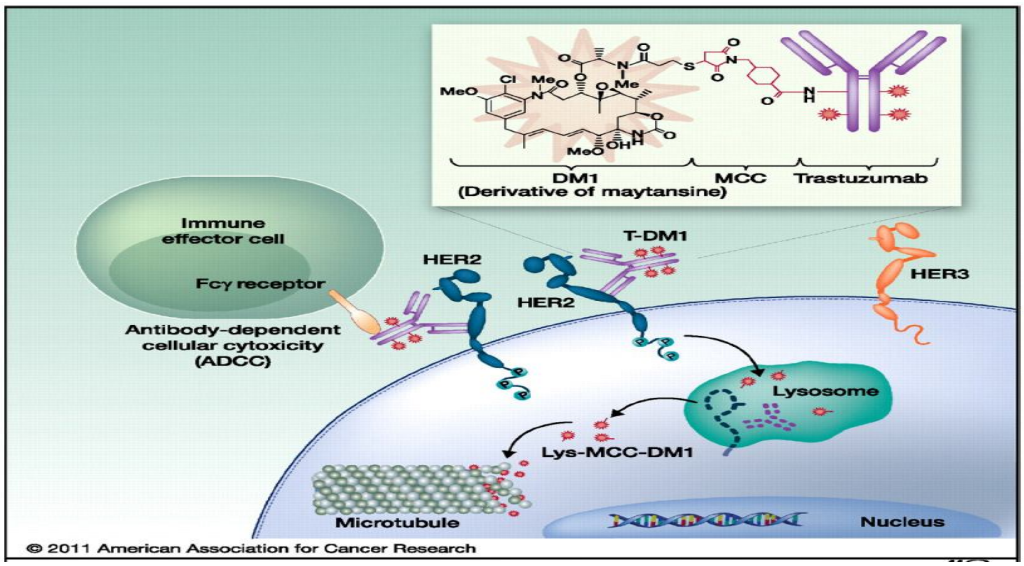

Figure 3.5: Mechanism of action of T-DM1 (American Association for Cancer Reasearch, 2011)

Additionally T-DM1 seems to retain the antitumor properties of trastuzumab including flagging HER2 positive tumor cells for destruction by ADCC and inhibiting HER2 signaling. (Burris et al., 2011) (Eroglu, Tagawa and Somlo, 2014) In preclinical models, T-DM1 has demonstrated inhibition of cellular proliferation and promotion of cell death in trastuzumab-resistant breast cancer cells. (Eroglu, Tagawa and Somlo, 2014) EMILIA group (a phase III trial) had shown that T-DM1 significantly prolonged

PFS and OS with less toxicity than lapatinib+capecitabine in patients with HER2-positive advanced breast cancer previously treated with trastuzumab and a taxane. This study led to the approval of T-DM1 in 2013. (Viswanath and Pathak, 2016) T-DM1 was better tolerated than lapatinib, with the most commonly reported adverse events including thrombocytopenia and elevated serum concentrations of both aspartate aminotransferase and alanine aminotransferase. While the rate of serious toxicity is low, it should be noted that lower grade toxicities, including fatigue, nausea, diarrhea and neuropathy, may occur and can potentially affect the quality of life. (Nye and Mutonga, 2016) No dose limiting cardiotoxicity was observed. (Callahan and Hurvitz, 2011) In the pharmacokinetic profile T-DM1 shows relatively slow clearance, a small volume of distribution mainly (distributed in plasma), and a half life of approximately 4days. On the basis of these results, a phase II study was initiated to evaluate T-DM1 treatment in patients with HER2+ MBC who experienced progression on HER2 directed therapy. (Burris et al., 2011) Trials comparing first-line treatment with T-DM1 to combination chemotherapy and anti-HER2 agents are ongoing. T-DM1 is also currently being evaluated in combination with pertuzumab. (Callahan and Hurvitz, 2011)

3.10 Ongoing clinical trials with other HER2 targated agents:

Clinical trials are ongoing for following compounds:

Ertumaxomab is a hybrid trifunctional monoclonal antibody that targets the CD3 antigen on T-cells and the HER2 expressed on the tumor cell. This compound forms a HER2-ertumaxomab-CD3 complex, leading to the aggregation and activation of T-cells, macrohages, dendritic cells and natural killer cells, which result in the phagocytosis and death of the tumor cells. Further clinical studied are required to

determine the clinical relevance of this medication. (Tinoco et al., 2013) Another one is **MM-111** which is a novel antibody fusion protein that targets the HER2/HER3 heterodimer, and blocks the ligand binding to HER2-3 heterodimers, thereby inhibiting downstream signaling. The fusion protein is conformed by two human single-chain variable fragment antibodies linked to a modified human serum albumin. An ongoing phase I/ II trial is evaluating the safety and tolerability of MM-111 in patients with HER2 MBC. (Tinoco et al., 2013) One example of drug resistance to HER2 targeted therapy is the lack of aberrant overexpression of the drug target p185HER2. In addition to this simplistic mechanism other mechanisms of resistance have been elucidated such as- activation of parallel signaling receptors- insuline like growth factor receptor, activation of downstream signaling events caused by PTEN loss and mutation of PI3K pathway. These findings lead to discover and investigation of new drug targets for HER2 positive breast cancer. (Pegram, 2011)

3.11 PI3K inhibitors:

It is well established that HER2 amplified tumors show significant dependence on the PI3K pathway, and the antitumor effect of HER2 targeted agents is at least in part mediated by inhibition of PI3K pathway activity. (Ma, 2017) Phosphatidylinositol-3-kinases (PI3K) are a family of enzymes involved in multiple important cellular functions including proliferation, cell growth, differentiation, motility and survival. (Tinoco et al., 2013) The central role in PI3K/Akt/mTOR pathway is played by the PI3K heterodimer, which belongs to the class IA of PI3Ks. The heterodimer consists of two subunits, the regulatory subunit p85 and the catalytic subunit p110. p110 subunits are encoded by PIK3CA gene and mutation of PIK3CA can lead to tumorigenesis. (Paplomata and O'Regan, 2014) The PI3K pathway involves a

complex network of interactions with many parallel cascades, so its inhibition releases negative feedback resulting in activation of compensatory signaling pathways, including PTEN loss. (Lee, Loh and Yap, 2015) PTEN, the phosphate and tensin homolog, is an important tumor suppressor and its loss involve in maligancies. (Paplomata and O'Regan, 2014) Mutations in PIK3CA and/or loss of expression of PTEN gene protein product have been identified in 20%-50% of HER2 positive MBC. (Nye and Mutonga, 2016)

In HER2 amplified cancers, the heterodimer of HER2 with kinase deficient HER3 is a major activator of PI3K-Akt signaling and when HER3 is phosphorylated, can directly couple to the p85 subunit of PI3K. Inhibition of PI3K-Akt signaling is believed to be an essential component of the antitumor effect of HER2 directed therapies. (Rexer et al., 2014)

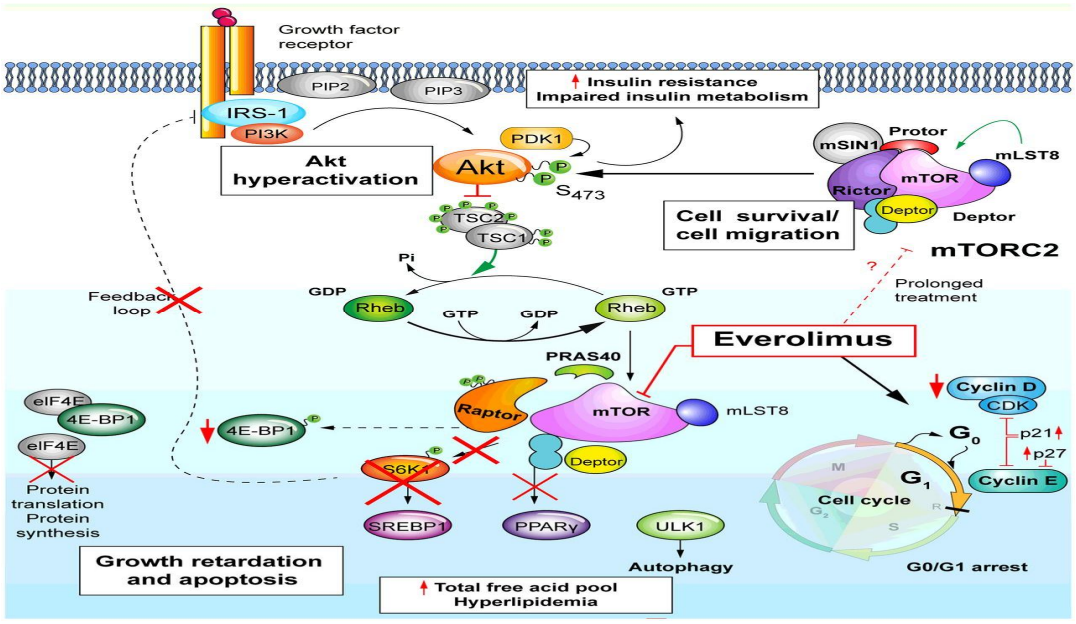

Figure 3.6: PI3K/Akt/mTOR pathway (Rexer et al., 2014)

Multiple PI3K/Akt inhibitors are currently in clinical or preclinical studies but no agent is currently FDA approved. **Wortmannin** is a fungal metabolite and potent pan-specific irreversible PI3K inhibitors, which targets the p110 subunit. Wortmannin was found to inhibit cell growth and it has also been found to potentiate chemotherapy effects. (Paplomata and O'Regan, 2014) **Buperlisib** is another PI3K inhibitor which is more advanced in clinical development. Buperlisib is an oral selective inhibitor of pan class I PI3K, which equally inhibits class IA PI3Ks, but has no activity against class III PI3Ks. (Lee, Loh and Yap, 2015) This compound was used in a phase I dose escalation study of 35 patients with advanced solid tumors and it was found to be safe and well tolerated. **BKM120** is undergoing multiple clinical trials in breast cancer and is currently being studied in combination with chemotherapy. (Paplomata and O'Regan, 2014)

Recent clinical studies have suggested that targeting HER2-PI3K signaling with combination of agents that inhibit HER2 by different mechanisms is more effective than a single HER2 inhibitor. PIK3CA mutation is a mechanism of acquired resistance to lapatinib and this mutation partially uncouple PI3K from HER2 to allow for the development and maintenance of resistance. Targeting of PI3K itself, in combination with maximal HER2 blockade with both an antibody and a TKI, is more effective than HER2 targeting alone for HER2 tumors without PIK3CA mutations and is required for HER2 tumors with PIK3CA mutations. (Rexer et al., 2014) There is also evidence that tumors with PIK3CA mutations are good targets for inhibitors of the PI3K pathway but this has yet to be validated in the clinical setting. PI3K inhibitors have been studied less extensively but most common side effects encountered in phase I studies were gastrointestinal toxicity, such as diarrhea,

nausea and vomiting. Rash, hyperglycemia and mood changes can also occur. These drugs appear to cross the BBB. (Paplomata and O'Regan, 2014)

3.12 Insulin like growth factor inhibitors:

Insulin like growth factor (IGF) system is comprised of two ligands, IGF-1 and IGF-2, which exhibit their effects through binding to IGF-1R (primarily), IGF-2R, and insulin receptor (IR), all belonging to the tyrosine kinase receptor family. Drugs that work by targeting HER2 in HER2 positive breast cancer have met with significant tumor resistance. Trastuzumab, which is commonly used in therapy, but limited drug efficacy, is appeared due to IGF signaling. In models of breast cancer cells that overexpressed HER2, trastuzumab activity is disrupted by increased expression of IGF-1R. (Denduluri et al., 2015) One possible strategy to block IGH-1R signaling pathway would be to lower IGF-1 concentrations. During puberty, growth hormone (GH) release results in the production of IGF-1 by the liver. A pegylated mutant GH (pegvisomant) has been developed to disrupt GH signaling in patients with excess GH and this compound also have antitumor activity. As both GHRH (GH releasing hormone) and GH antagonists may have direct antitumor activity, they both result in suppression of serum IGF-1 levels. (Yee, 2006)

IGF-1R antisense oligonucleotides have been used to down-regulate expression. The transfection of IGF-1R antisense oligonucleotides effectively reduces IGF-1R mRNA, protein, and IGF induced gene transcription and cell growth. Recent reports on the use of IGF-1R antisense in the clinical setting to treat patients with cancer have been positive. (Ibrahim and Yee, 2005)

The bulk of drug development has been directed towards targeting IGF-1R. Several monoclonal antibodies directed against IGH-1R have been created. All antibodies seem to have a similar mechanism of action and result in IGF-1R downregulation. (Yee, 2006)

Five agents that target the IGF-R pathway have been studied in the treatment of breast cancer. Ganitumab, frigitumumab, dalotuzumab and cixutumumab are monoclonal antibodies that target IGF-1R while BMS-754807 is an IGF-1R/IR kinase inhibitor. (Tinoco et al., 2013) **Gefitinib** has been shown to reduce cell proliferation and tumor growth in breastcancer cell lines with different level of HER2 expression. Different combinatorial regimes have been approached and the combination of gefitinib with calcitriol or their synthetic analogs resulted in a greater antiproliferative effect than with either of the agents alone in HER2 positive breast cancer cells. (Voudouri et al., 2015) IGF-1R is ubiquitously expressed in most normal tissue. Thus, disruption of IGF-1R could result in many potential toxicities. The ongoing clinical trials will determine whether long or short term inhibition will IGF-1R results in unacceptable toxicity. (Yee, 2006)

3.13 Heat shock protein 90 (HSP 90):

Proteosomal degradation of oncoproteins such as HER2 is caused by compounds called HSP90 inhibitors. (Singh, Jhaveri and Esteva, 2014) HSP 90 is a molecular chaperone protein which assists in the folding and stabilization of proteins vital to cell survival. (Tinoco et al., 2013) HSP 90 required for the stabilization of essential components of HER2 signaling pathway. HER2 is among the most sensitive client proteins of HSP 90 and its inhibition mediates degradation of HER2, as well as PI3K and Akt in HER2 overexpressing cancer cells. Resistance of breast cancer cells to

chemotherapy is known to involve PI3K pathway which is modulated by HSP90 by virtue of one of its key signaling protein (Akt) being a client protein of HSP 90. Inhibition of HSP 90 has also been known to modulate angiogenesis of breast cancer xenografts. Finally, expression of HSP90 has been shown to correlate with adverse clinical outcomes, further validating HSP90 as a target in breast cancer. (Zagouri et al., 2012) Expression of HSP 90 from invasive breast cancer was associated with an increased risk of early recurrence. Coexpression of HSP90 and PI3K or expression of HSP90 in combination with the loss of PTEN was associated with significantly worse RFS in her2 positive breast cancer. (Song et al., 2010)

Galdanamycin bind directly to the ATP binding pocket in the *N*-terminal domain of Hsp90, blocks the binding of nucleotide to Hsp90. 17-*N*-Allylamino-17-demethoxygeldanamycin **(17AAG)** is the semisynthetic derivative of Geldanamycin, exhibiting a less toxic profile, but with the same therapeutic potential as Geldanamycin. It is the first HSP90 inhibitor that has been tested in clinical trials. HSP90 inhibitors plus trastuzumab have significant anticancer activity in patients with HER2+ MBC previously progressing on trastuzumab. (Zagouri et al., 2012)

17-AAG was combined with trastuzumab in 31 patients with advanced HER2+ breast cancer who had progressed on trastuzumab. The ORR was 22%, median PFS was 6 months and the adverse events include fatigue, headache and transaminitis. **Retaspimycin** was also combined with trastuzumab in 26 patients with advanced, HER2+ breast cancer. More specifically retaspimycin HCL has shown promising result with a safe toxicity profile and antitumor activity in a phase I trial. (Tinoco et al., 2013) (Zagouri et al., 2012) **Ganetespib** is an Hsp90 inhibitor with broader activity then 17-AAG was studied as single agent therapy. (Tinoco et al., 2013) **Metformin**

can restrain the proliferation and promote apoptosis of cancer cells, and it is possible that metformin restrain the expression of HSP90 protein, which down-regulates the key downstream molecule activation of Akt and MAPK pathways (activation of AKT and MAPK and high expression of HSP90 are common in HER2 positive breast cancer). (Tian-wen and Ya-nan, 2013) There are many other Hsp90 inhibitors with better pharmacological and toxicological properties that are currently under investigation. (Zagouri et al., 2012)

3.14 Histone deacetylase inhibitors:

In eukaryotes, DNA bound to histones forms chromatin. Histone acetylation occurs on lysines in the N-terminal tails of histones, causing histone methylation, leading to chromatin relaxation and elevated gene expression. Histone deacetylases (HDACs) enzymes catalyze the deacetylation of histones and counter the activity of histone acetyltransferase by inducing hydrolysis of the ε-amino acetyl moiety on specific acetylated lysine residues within core histones, are associated with tumerogenesis through the repression of tumor suppressor gene expression. HDAC inhibitors promote histone acetylation and consequently allow re-expression of tumor suppressor genes, which can repress malignancies. (Perera and Hergovich, 2017) (Nolan et al., 2008)

HDAC inhibitors have been shown to indirectly and directly negatively regulate HIF-1 α (hypoxia inducible factor-1 alpha, promotes angiogenesis) offering promising options for the treatment of tumors, which proliferate via stimulation from VEGF. (Tinoco et al., 2013) HDAC inhibitors are epigenetic drugs targeting specific parts of cancer cell signaling. In contrast, standard chemotherapy indiscriminately kills rapidly

dividing cancerous and non-cancerous cells, which can cause undesired side effects and anticancer therapy resistance.

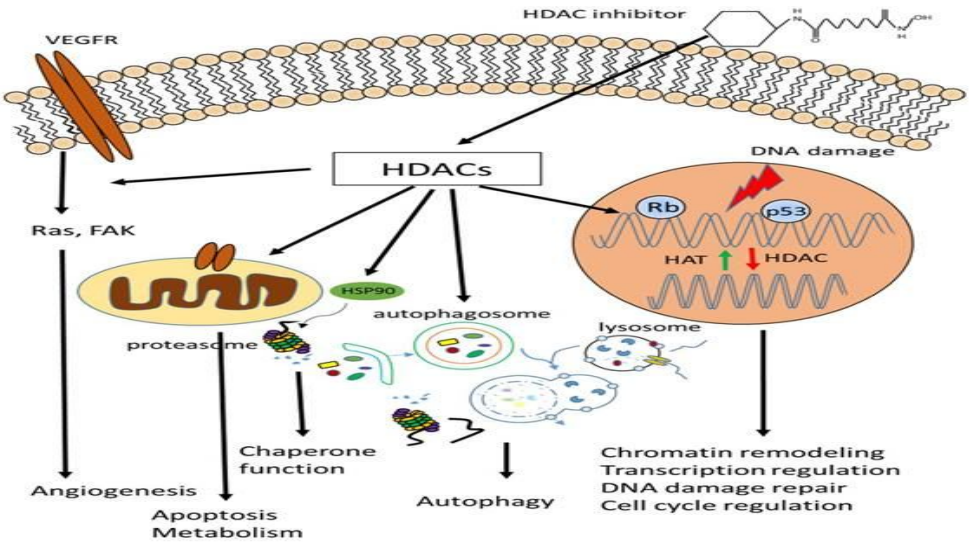

Figure 3.7: Mechanism of action of HDAC inhibitors. (Perera & Hergovich, 2017)

HDAC in the treatment of breast cancer cells, with some promising results showing positive effects associated with low side effects. (Perera and Hergovich, 2017) In vitro studies indicate that histone deacetylase inhibitors (HDIs) have single agent activity in HER2 overexpressing breast cancer cell lines including attenuation of HER2 expression, its tyrosine kinase activity, its cell membrane localization and dimerization with HER3. Combination with trastuzumab produced synergistic induction of apoptosis. Synergy may result from counteracting HER2 overexpression as HDAC inhibition reduced HER2 mRNA transcript expression and induced HER2 protein degradation. On the basis of clinical trials of trastuzumab-HDI combination

are in progress for locally advanced and metastatic breast cancer. (Nolan et al., 2008)

HDI are differentiated by their structure and further characterized into different sub groups. There are short chain fatty acids (valproic acid), benzamide (entinostat), cyclic tetrapeptides, and hydroxamic type (vorinostat). (Truong and Munster, 2013) Valproic **acid** as an HDI was tested in phase I/II clinical trial. (Tinoco et al., 2013) It causes hyperacetylation of the *N*- terminal tails of H3 and H4 and inhibits HDAC activity, probably by binding to the catalytic center and blocking the access of the substrate. HER2 overexpressing breast cancer cells were more sensitive to valproic acid treatment than HER2 negative ones. This antiproliferative activity of valproic acid seems to be a result of HSP90 dysfunction, which hyperacetylates HSP70. The hyperacetylation of HSP70 affects HER2 protein, which is a client protein of HSP90. (DAMASKOS et al., 2017)

Three other HDAC inhibitors have been clinically evaluated to date in breast cancer patients: **vorinostat, entinostat, panobinostat**.initial studies used an HDI as an adjunct to angiogenesis inhibitor therapy in patients who had progressed on prior angiogenesis inhibitor therapy. (Tinoco et al., 2013) In a subset of patients, an association of histone deacetylase inhibition with protein lysine acetylation and improved clinical outcome was demonstrated. (Kümler et al., 2016)

3.15 MM302 Nanoparticle:

Studies of T-DM1 demonstrated that using targeted vehicles to deliver highly toxic chemotherapy can yield significant clinical benefit. In a similar approach, new therapeutic strategies are exploring the potential of packaging common toxic

chemotherapies in nanoparticles conjugated with anti-HER2 antibodies. **MM-302,** a HER2 targeted nanoparticle containing doxorubicin, was designed to deliver doxorubicin to HER2 positive breast cancer cells. (Nye and Mutonga, 2016) It is a novel antibody-drug conjugate that offers targeted delivery of pegylated liposomal doxorubicin to cancer cells overexpressing the HER2 protein. Based on a phase I monotherapy study, MM-302 is well tolerated with promising activity, especially in patients with anthracycline-naïve breast cancer. (Drakaki and Hurvitz, 2017) Another phase I study demonstrated that patients treated with MM-302 alone, in combination with trastuzumab, or in comination with cyclophosphamide had an ORR of 11% and median PFS of 7.6 months. Cardiac toxicity was considered acceptable, with only 9% of patients experiencing reduction in their LVEF of below 50%. (Miller et al., 2016) Another clinical trial was done to assess EPR (enhanced permeability and retention) effect in patients using 64Cu-labelled nanoparticle, 64Cu-MM-302. This trial provide important evidence and quantification of the EPR effect in human metastatic breast cancer and support imaging nanoparticle deposition in tumors as a potential means to identify patientswell-suited for treatment with therapeutic nanoaparticle. (Lee et al., 2017) These data have led to a larger phase II study, now underway, which is designed to support an application for accelerated approval of MM-302. (Nye and Mutonga, 2016)

3.16 Trastuzumab plus PD 1 inhibitor (Immune Blockade Inhibition):

In HER2 positive breast cancer, immune checkpoint inhibition may be an important therapeutic strategy. (Nye and Mutonga, 2016) FDA approved checkpoint antibodies targets inhibitory receptor, PD-1 (programmed death-1), or its ligand, PD-L1 (programmed death ligand-1). PD-1 is a co-inhibitory receptor and act as a negative

regulator of the immune system. When T-cells are activated and infiltrate tumor, they release interferon gamma (IFN-γ), which in turn upregulate PD-L1 expression by tumor cells. PD-L1 binds to PD-1, which is expressed by activated T-cells and generated a signal that leads to T-cell exhaustion. Thus, PD-1/PD-L1 blocking antibodies may impede the exhaustion signal, and thus reinvigorate tumor specific T-cells to destroy the cancer. (Lavaud and Andre, 2014) (McArthur and Page, 2016)

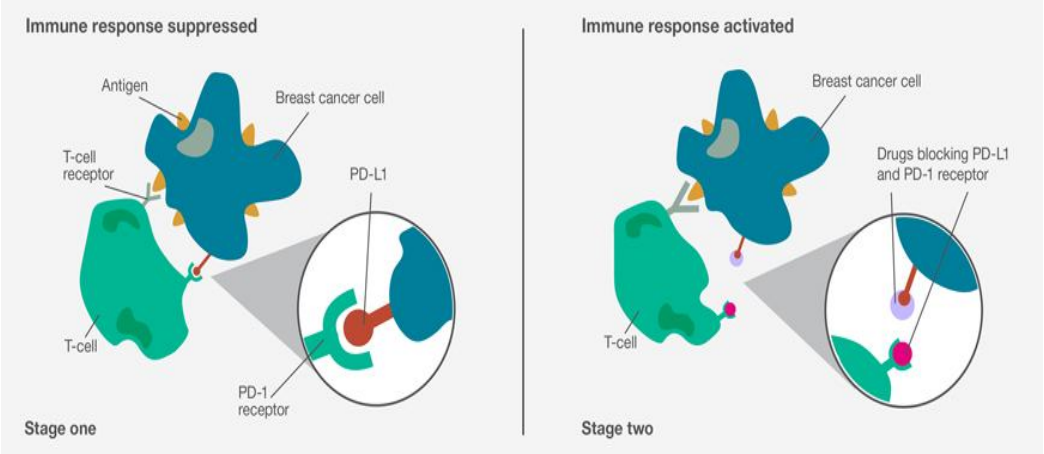

Figure 3.8: Mechanism of action of PD1 inhibitors (Lavaud and Andre, 2014)

Biomarker studies have shown that PD-1+TIL (tumor infiltating lymphocytes) are associated with poor prognosis in HER2 positive breast cancer and preliminary data also suggest a relationship between PD-L1 expression on tumor cells and anti PD-1 therapy. The effects of anti PD-1 and anti PD-L1 antibodies have been investigated in phase I trials and have shown encouraging responses rate 6 to 28% and stabilized disease rate 12% to 41%. (Lavaud and Andre, 2014)

The International Breast Cancer Study Group (ICBSG) is conducting a phase II investigation of trastuzumab combined with anti PD-1 inhibitor **perbrolizumab** found that trastuzumab and anti PD-1 antibodies show synergism. (Nye and Mutonga,

2016) (Perey et al., 2006) A study examining the PD-L1 monoclonal antibody **avelumab** had included 168 patients pretreated with locally advanced breast cancer and 15.5% were with HER2 positive breast cancer. Median duration of response in responding patients exceeded 6 months and tolerance was acceptable and ORR was only 4.8%. (VOUTSADAKIS, 2016)

3.17 CDK4/6 inhibitor:

The recurrent tumor cells that are resistant to HER2-targeted therapies are sensitive to cyclin D1 downregulation by RNA interference or the CDK (cycline dependent kinase) 4/6 inhibitor **abemaciclib**. These findings not only re-enforce the interest of CDK4/6 inhibitors in treating primary HER2 positive tumors but also predict their efficacy in relapses generated after HER2 targeted therapies and it is a findings of great clinical importance. The role of cycline D1-CDK4 complexes as downstream effectors for cell-cycle entry in HER2 positive breast tumors is well established. Whereas recurrent use tumors are insensitive to lapatinib or trastuzumab, the combination of abemaciclib with these compounds have synergistic effects –i.e., inhibition of CDK4/6 restores the sensitivity of relapses to HER2 directed therapies. (Malumbres, 2016)

Chapter 4

Treatment Options for HER2 Negative Breast Cancer

4.1 Monotherapy for only HER2 negative:

Timing the initiation of chemotherapy is a complex decision, with multiple variables at play. In the case of HR-positive, HER2-negative metastatic disease, guideline suggests that endocrine therapy, rather than chemotherapy, should be offered as first line treatment. But an exception would be the circumstances in which the disease is rapidly growing and there is chance of endocrine resistance. A Meta analysis of 11 randomized clinical trials evaluating the duration of first line chemotherapy for ABC demonstrated that longer treatment is associated with a significant benefit in PFS and improved OS. As a result it was recommended that each therapy be maintained until maximum disease control or limiting toxicity was observed. (Joy et al., 2015)

4.1.1 Capecitabine:

Capecitabine is an oral pro-drug that is preferentially converted to the antimetabolite 5-fluorouracil (inhibit DNA synthesis by inhibiting conversion of deoxyuracil monophosphate to deoxythymidine monophosphate) by thymidine phosphorylase, an enzyme found at higher levels in tumor cells versus normal cells. (Liu, 2012) It should be avoided in patients who have previously exhibited hypersensitivity to 5-fluorouracil and who have a known dihydropyrimidine dehydrogenase (needed for excretion of capecitabine) deficiency. One of the main advantages of capecitabine is that, being an oral drug, it allows for an additional degree of patient convenience with respect to medicine administration. (Joy et al., 2015)

The efficacy of capecitabine was first established in a phase II study in patients who had previously received either anthracycline, taxane or combined therapy. In women with such prior exposures the RR was 20%, with median response duration of 8.3 months. (Joy et al., 2015)

And found that it is a potent and safe agent that can be used after anthracycline and taxane treatment in patients with MBC. Another study was done to investigate the efficacy and safety of capecitabine monotherapy as a first line treatment in HER2 negative patients. In this single centre trial, a total of 109 HER2 negative patients with MBC who received capecitabine monotherapy as first line treatment between 2003 and 2014 were retrospectively analyzed and found that capecitabine monotherapy is an effective and safe (grade 3-4 toxicities were lower and low toxicity profile compared with two other intravenous cytotoxic agents) regimen for HER2 negative patients. (Babacan et al., 2015) In this same condition another 75 medical records of HER2 negative patients were evaluated and ORR was 29.3%. The median OS is approximately 18 months but HER2 negative patients have a low probability of response or disease control to capecitabine and require innovative therapies. Adjuvant capecitabine improves outcome in women with HER2 negative breast cancer who still have invasive disease after neoadjuvant chemotherapy, according to the findings of the CREATE-X trial reported at the San Antonio Breast Cancer Symposium. (Gilabert et al., 2010)

Complete hair loss and myelosuppression were rare adverse event of capecitabine. The most common adverse effect was hand-foot syndrome, a cutaneous side effect that may be debilitating but is always reversible. Emollients, with or without vitamin B6 preparations are common preventive/supportive measures. Gastrointestinal adverse events were the next most common side effect seen but were largely moderate to mild in intensity and could be effectively managed with medical intervention. (Gelmon, 2006) Grade 3/4 adverse events associated with capecitabine include palmar-plantar erythrodysesthesia and diarrhea. Nausea, emesis, and stomatitis were also reported but6 the majority of toxicities were mild to moderate in

severity, with few grade 4 adverse events and there were no documented treatment related deaths. (Liu, 2012)

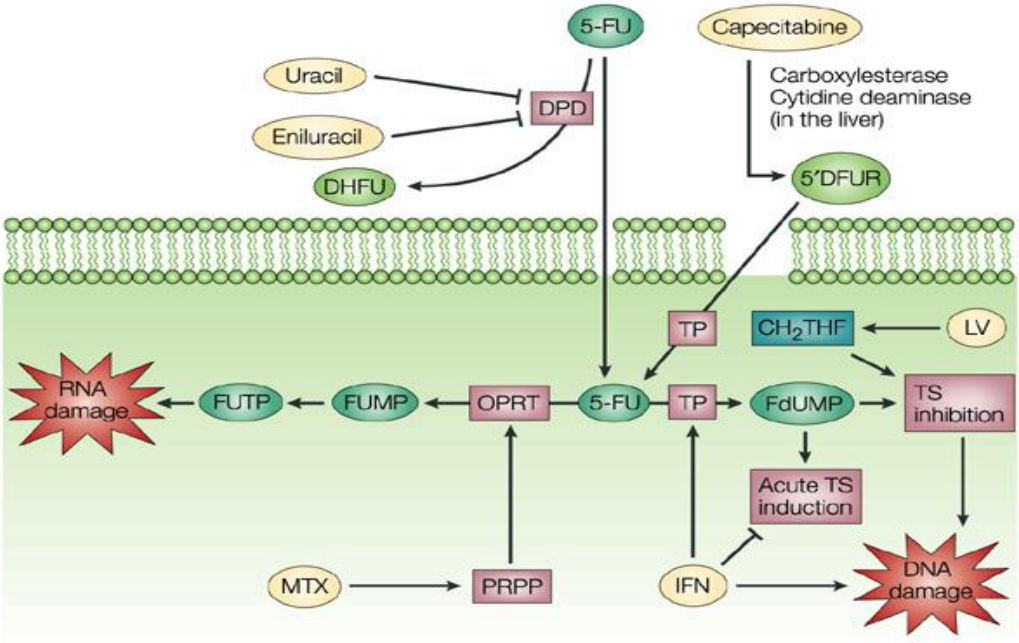

Figure 4.1: Mechanism of action of Capecitabine (Liu, 2012)

4.1.2 Ixabepilone:

Ixabepilone is the first in class member of epothilone class of antineoplastic agent a relatively new class of antimicrotubule agents. Epothilones are cytotoxic macrolides with a similar mechanism of action to paclitaxel but with the potential advantage of activity in taxane resistant settings in preclinical models. (Tkaczuk and Tkaczuk, 2011) The epothilones are a new class of microtubule-stabilizing drugs produced by the myxobacterium *Sorangium cellulosum*. Epothilones bind to ß-tubulin to stabilize microtubule polymers, thereby inducing cell-cycle arrest and triggering tumor cell

apoptosis. (Monero-Aspitia and Perez, 2009) In addition, Ixabepilone prolong activation of spindle assembly checkpoint which may promote cancer cell death in mitosis or following mitotic exit. (Tkaczuk and Tkaczuk, 2011) However, the epothilones bind to a different site on ß-tubulin than do taxanes and they induce apoptosis by distinct epothilone-selective mechanisms. These factors together may explain why these agents remain effective against tumor refractory or resistant to taxane. Notably, Ixabepilone can bind to a wider variety of ß-tubulin isoforms that can the taxanes. Ixabepilone has also been shown to induce apoptosis via a Bcl-2-suppressible pathway that controls a conformational change of the proapoptotic Bax protein. Ixabepilone is metabolized in the liver and caution should be used when considering patients with liver impairment for therapy with this agent. (Monero-Aspitia and Perez, 2009) In preclinical models, ixabepilone demonstrared low susceptibility to mechanisms that confer tumor resistance, such as overexpression of efflux transporters (eg: P-glycoprotein and multidrug resistance protein-1) and class III isoform of ß-tubulin. In phase II studies, single-agent ixabepilone showed clinical activity in metastatic breast cancer, with objective response rate from 12-42%, median TTP of 2.2 months and median OS of 7.9 months. (Thomas et al., 2007) Peripheral neuropathy was common in clinical trials with ixabepilone although many patients who entered these trials had preexisting peripheral neuropathy due to prior exposure to other neurotoxic chemotherapy agents (taxane). (Tkaczuk and Tkaczuk, 2011) A second, multicenter phase II study demonstrated grade 3/4 treatment-related side effects included peripheral sensory neuropathy, fatigue, myalgia, and stomatitis. Other adverse events such as nausea and emesis were manageable and classified as grade 1 or 2. In 2007, on the basis of these data, the FDA approved ixabepilone

as single agent therapy following failure of an anthracycline, a taxane or capecitabine in MBC. (Liu, 2012)

4.1.3 Eribulin:

Eribulin mesylate is a novel compound derived from halichondrin B, a large polyether macrolide found in marine sponges including *Halichondria okadai.* Structurally, eribulin is a simplified halichondrin analog with biochemical effects similar to those of the parent compound since the active macrocyclic lactone moiety is preserved. Eribulin has a unique mechanism of action, with a tubulin binding site that appears to be different from the taxane and vinca binding site on the positive end of the microtubule. (Doherty and Morris, 2015) In vitro studies revealed that the activity of eribulin is more specific compared to other tubulin-tareting egent. This degree of specificity is achieved by its ability to bind selectively and with high affinity to the growing positive ends of microtubule, suppressing dynamic instability. (Liu, 2012) Eribulin exerts its cytotoxic effect by inhibiting microtubule growth and sequestering tubulin, ultimately causing G2-M cell cycle arrest and cell death through apoptosis. Eribulin was more potent than vinblastin to paclitaxel. Eribulin was also found to have extensive distribution, with prolonged elimination primarily through feces and was predominately metabolized by cytochrome P450 3A4. (Doherty and Morris, 2015) A total of 325 patients with HER2 negative were assessed in a study and several retrospective and prospective studies have demonstrated the therapeutic benefits of eribulin. (Watanabe, 2015) Three phase II studies investigated the efficacy and tolerability of eribulin in heavily pre-treated MBC patients and found that ORR is 11.5%, median PFS of 2.6 months and median OS of 9.0 months. (Liu, 2012) The phase III EMBRACE (open label study in 762 patients) study demonstrated that

eribulin provided a survival benefit in women with MBC who had previously received at least two chemotherapeutic regimens including anthracycline and taxanes. In that study eribulin was well tolerated and mostly grade 1-2 toxicities were observed. Based on the EMBRACE study eribulin was approved by FDA for MBC. (Ates and Babacan, 2016) Based on these findings eribulin is currently used as one of the options in 2nd and 3rd line chemotherapy for HER2 negative breast cancer. The most common adverse effects associated with eribulin include fatigue, netropenia and peripheral neuropathy. (Joy et al., 2015) (Kotake, 2016)

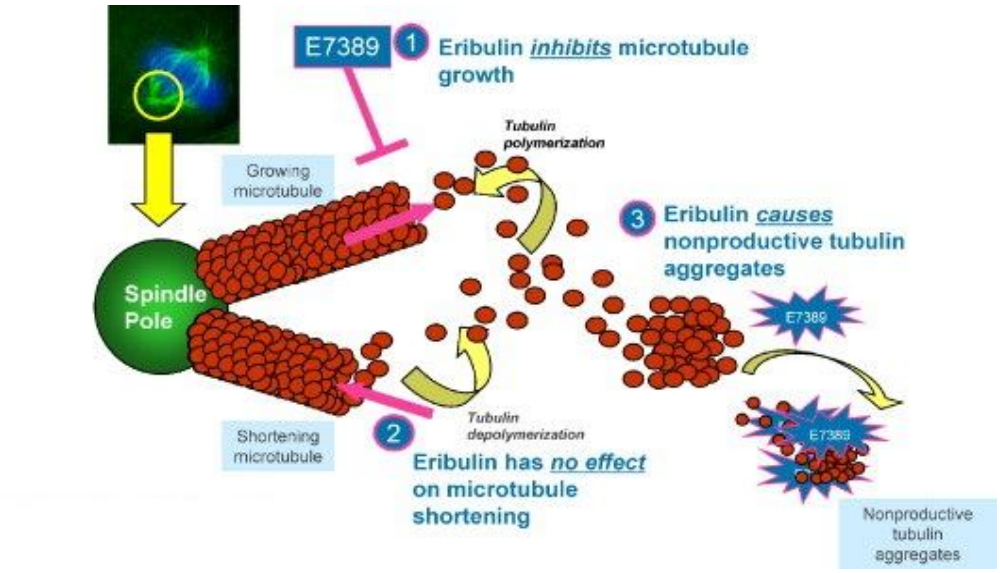

Figure 4.2: Mechanism of action of Eribulin (Joy et al., 2015)

4.1.4 Vinorelbine:

Vinorelbine is a semi-synthetic vinca alkaloid that suppress microtubule formation by binding to tubulin monomers (inhibit tubulin polymerization), resulting in cytotoxicity and cellular apoptosis. Several clinical studies have reported on its efficacy in the treatment of MBC and in a phase II trial of 40 patients previously treated with

anthracyclin and taxane, single agent vinorelbine resulted in an ORR of 25%, median OS is 6 months. Other phase II studies have further demonstrated the activity of vinorelbine monotherapy in pretreated MBC. (Liu, 2012)

It is metabolizes primarily in the liver, and transient elevations in liver enzymes can be observed. It is well tolerated intravenously (oral formulations are not currently available in North America)) with minimal nausea and alopecia. The main side effects of vinorelbine are cytopenias. Gastrointestinal toxicity is seen particularly if the drug is taken orally. Vinorelbine has also occasionally reported to cause pain, primarily abdominal. (Joy et al., 2015) Vinorelbine is not FDA-approved in the US for the treatment of resistant MBC. (Liu, 2012)

4.2 Combination chemotherapy for only HER2 negative:

Combination therapy is a mainstay of anticancer treatment, with optimal combinations producing synergistic antitumor responses. This is achieved by combining agents with established safety profiles and non-overlapping mechanisms of action. (Monero-Aspitia and Perez, 2009)

4.2.1 Gemcitabine based combination:

Gemcitabine is pyrimidine nucleoside analog (antimetabolite) that becomes phosphorylated in the cell and incorporated into DNA as a triphosphate, interferes with DNA synthesis and induce apoptosis. Antimetabile therapy should be considered in women with prior exposure to anthracycline and taxane therapy. (Gogineni and DeMichele, 2012) When used as a single agent, its RR ranges from 14% to 37% in the chemotherapy-naïve population and from 12% to 30% in patients with anthracycline and taxane exposure. The addition of gemcitabine or capecitabine

to an anthracyclin regimen is not recommended for adjuvant chemotherapy. (Denduluri et al., 2016) The main clinical benefit of gemcitabin was demonstrated in a phase III setting in which it was given in combination with paclitaxel and compared with single agent paclitaxel, was associated with a higher RR (41.4% VS. 26.2%), longer TTP and improvrd OS. Other drugs combinations have also evaluated such as vinorelbine-gemcitabine combination showed improved PFS compared with vinorelbine alone. Gemcitabine-docetaxel shows efficacy with less hematologic toxicity comared with capecitabine-docetaxel combination. (Joy et al., 2015)

Gemcitabine also evaluated in combination with carboplatin in a phase II study of patients with hormone receptor –negative, HER-2 negative MBC pre treated with anthracycline and taxane, the ORR was 32%, median TTP was 5.5 months and median OS was 11 months. (Liu, 2012) Toxicity was higher with combination treatment than with single agent chemotherapy. Gemcitabine as a single agent is relatively well tolerated overall, with asthenia and transient flu-like symptoms being the most notable adverse effects. Gemcitabine does not cause a great deal of alopecia or nausea. Gemcitabine has been associated with chemotherapy-induced thrombotic micropangiopathy along with chemotherapy induced cytopenias. The drug is excreted by kidneys mainly and it therefore has to be used with caution in patients with renal dysfunction. (Joy et al., 2015)

4.2.2 Ixabepilone plus capecitabine:

Ixabepilone has single agent activity and preclinical synergy with capecitabine has been demonstrated. Two large phase III studies were done to evaluate efficacy of Ixabepilone in combination with capecitabine in MBC patients. In an international phase III study studied the efficacy of the combination compared to capecitabine

alone in patients with anthracycline-pretreated or resistant and taxane-resistant. The primary end point was progression–free survival evaluated by blinded independent review combination treatment resulted in prolonged PFS relative to capecitabine (median, 5.2 v 4.2 months), with a 25% reduction in the estimated risk of disease progression. (Tkaczuk and Tkaczuk, 2011) These findings were supported by a second phase III trial of 1221 patients, which demonstrated a statistically significant improvement in PFS in combination treatment but no improvement in OS is observed with thw drug combination compared with capecitabine monotherapy. (Liu, 2012) Ibexapilone plus capecitabine significantly improved OS compared with capecitabine in patients with symptomatic disease, (12.3 v 9.3 months respectively) demonstrated by another study. (Fornier, 2010) Another phase III trial noted that, compared with capecitabine alone, combination of ixabepilone and caecitabine was associated with improved RR (43% v 29%). (Joy et al., 2015)

Treatment related adverse events were mostly grade ½ and generally reversible. The most frequently reported grade 3/4 adverse events in the combination group were peripheral sensory neuropathy, hand-foot syndrome, fatigue, myalgia and diarrhea. Myelosuppression was common in patients treated with ixabepilone plus capecitabine and consisted primarily of leucopenia and neutropenia, with a 5% incidence of febrile neutropenia. (Thomas et al., 2007)

4.2.3 Ixabepilone in combination with some other drugs:

Ixabepilone was combined with Sorafenib to evaluate the efficacy and toxicity in the treatment of patients with HER2 negative MBC. In a sudy, 67 patients entered the phase II portion. The median PFS was 4.8 months and this combination was poorly tolerated as first-line treatment. 20 patients discontinued because of oxicity and dose

reduction were frequent. The common toxicities included neutropenioa, fatigue, rash and neuropathy. (Yardley et al., 2016)

Ixabepilone can be combined with cetuximab, which might be more effective at targeting cancer stem cell than other antitubulins (target of ixabepilone alone) as a possible way to increase antitumor activity. Findings of study suggest that ixabepilone produces therapeutic synergism with cetuximab only in a small subset of TNBCs and provide additional evidence that clinical trials using cetuximab in combination with ixabepilone should be applied with caution. (Tanei et al., 2016)

4.2.4 Cetuximab in combination with Carboplatin:

EGFR/HER1 is perhaps the most well-known protein overexpressed among triple-negative breast cancer for which several MoAbs and small molecular inhibitors exist. A multicenter randomized phase II study of the anti-EGFR MoAb cetuximab alone and in combination with carboplatin was performed to determine benefit in the setting of triple-negative MBC. (Anders and Carey, 2009) Anti-EGFR antibody cetuximab with carboplatin was added after progression or as concomitant therapy from the beginning. In 102 patients with TNBC, RRs were 6% to cetuximab and 16% to cetuximab plus carboplatin after progression. The cetuximab plus carboplatin regimen was well tolerated, but both TTP and OS were short at 2.1 months and 10.4 monhs respectively. (Carey et al., 2012)

4.2.5 Capecitabine and cisplatin:

Capecitabine and cisplatin combination (XP) regimen is widely used in gastrointestinal cancer, but there are relatively scarce data in breast cancer patients with different administration schedule and different patient population. In a study, XP

combination regimen was administered to heavily pre-treated patients. About 40% of patients received 4 or more lines of systemic chemotherapy prior to XP administration. Median PFS in total population was 4.33 months. In this study, XP was well tolerated. Grade 3-4 neutropenia occurred in 37% of total delivered chemotherapy. Anemia and thrombocytopenia was detected during chemotherapy with grade 1-2 toxicity. This study showed there might be a potential clinical benefit of XP regimen in heavily pretreated breast cancer patients with favorable toxicity profile. (Lee et al., 2017)

4.3 Standard chemotherapeutic approaches:

4.3.1 Bevacizumab:

Bevacizumab is a humanized monoclonal antibody directed against VEG-A ligand, is the most target-based agent with antiangiogenetic activity. (Morabito et al., 2007) Preclinical data suggested the potential for synergy by combining targeted antiangiogenic agents with traditional cytotoxic chemotherapy. Anti-VEGF therapies may help to normalize the chaotic architecture of vessels within tumors, reducing vascular permeability and interstitial fluid pressure and potentially improving cytotoxic drug delivery. (Lorusso, 2008) The addition of bevacizumab to paclitaxel or capecitabine has demonstrated improved PFS and ORR as compared with chemotherapy alone in patients with HER2 negative MBC. Bevacizumab has a UK marketing authorization for the first line treatment, not for the second line treatment. (Lam et al., 2014) Eastern Cooperative Oncology Group (ECOG)2100 study found that adding bevacizumab to paclitaxel in unselected patients with metastaic breast cancer improved PFS (11.8 v 5.9 months). (Santa-Maria and Gradishar, 2015) The randomized, doubleblind, placebo-controlled, phase III AVADO study investigated the

combination of bevacizumab and docetaxel as first line therapy. Result indicated an improvement in both ORR and PFS. (Anders and Carey, 2009) However a phase III trial of bevacizumab and capecitabine versus capecitabine alone in A/T preated patients failed to show a difference in PFS and OS. (Morabito et al., 2007) Bevacizumab appeared safe and manageable in patients with MBC, with minimal additional toxicity seen when combined with other agents. The addiyion of bevacizumab increased the incidence of hypertension and proteinuria. Some authors have suggested a preference for calcium channel antagonists, while others favor angiotensin-convertibg-enzyme inhibitors in patients with coexisting proteinuria. It is hoped that an ongoing trial in the adjuvant setting will clarify the impact of bevacizumab on cardiac function. (Lorusso, 2008) In MBC, due to lack of a clear survival benefit associated with bevacizumab, the FDA recommended against bevacizumab in 2010. The FDA revocation resulted from concerns about safety, considering that bevacizumab did not improve OS in different randomized studies. Regarding cost, bevacizumab seems expansive and not cost effective. (Sini et al., 2016) The addition of bevacizumab to epirubicin and cyclophosphamide followed by docetaxel (neoadjuvant settings) was associated with an increased number of grade 3 and 4 toxic effects. (Minckwitz and Rezai, 2012)

4.3.2 PARP1 inhibitors:

DNA damage is caused by exposure of cells to a variety of agents, including environment carcinogens, relative oxygen species from cellular metabolism, UV, ionizing radiation, and chemotherapeutic drugs that target DNA. Lesser and subtle forms of DNA damage, such as oxidative lesions, alkylation of bases, DNA adducts and single strands breaks, are repaired by the base excision repair (BER) or

nucleotide excision repair (NER). (Ha et al., 2014) Poly (ADP-ribose) polymerase (PARP) is a family of enzymes involved in cellular processes, such as genomic stability, DNA repair, cell cycle progression, and apoptosis. PARP-1, a DNA binding protein, localizes to DNA strand breaks as part of the BER process. (Anders et al., 2010) TNBC comprise about 15% of breast cancer overall, about 70% of breast cancers in individuals harboring a germline BRCA1 mutation and 20% in BRCA2 mutation carriers. One characteristic of BRCA mutated cancers is defective function of one of the major DNA damage repair pathways, the homologous recombination (HR) pathway. Since cancer is a disease in which DNA replication is critical, replication errors are prominent, and deficiencies in DNA-repair pathways are common, the involvement of PARPs in DNA-repair pathways stimulated the development of agents capable of targeting PARP activity. (Livraghi and Garber, 2015)

Clinical development of PARP inhibitors (PARPi) started in 2003 and focused on 2 strategies: utilizing PARPi in combination with other drugs in a range of solid malignancies or using PARPi monotherapy in specific cancer types with features that would be predicted to be highly sensitive to PARP inhibition. Clinical testing of PARPi was initially slowed by negative results from a phase 3 trial of iniparib, a compound inaccurately classified as a PARPi. Currently, five compounds with the ability to inhibit the activity of various PARPs are being investigated in clinical trials. (Livraghi and Garber, 2015)

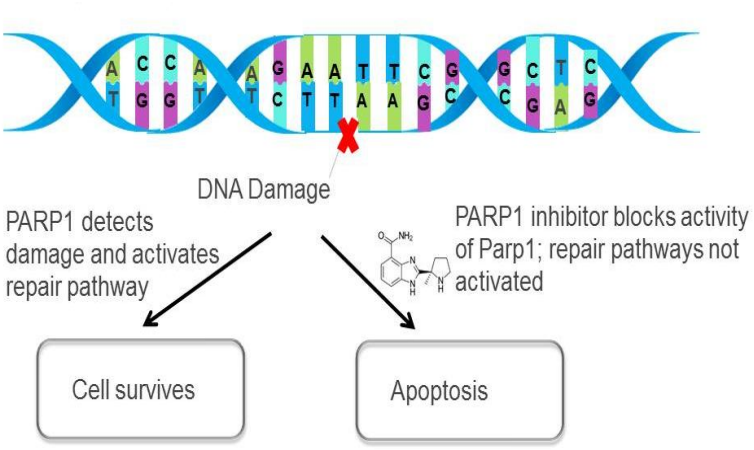

Figure 4.3: Mechanism of action of PARP1 inhibitors (Livraghi & Garber, 2015)

Veliparib, **Olaparib** and **BSI-201** are some PARPi. In july 2009, a randomized phase III registration study of gemcitabine and carboplatin with or without BSI-201 was launched to examine the safety and efficacy of this combination in a larger patient population. (Telli and Ford, 2010) A recent update at the 2009 ASCO annual meeting reported that the addition of BSI-201 significantly increased ORR, clinical benefit rate, median PFS in patients with metastatic triple-negative breast cancer. (Anders and Carey, 2009) Although olaparib and BSI-201 are the PARPi that are most advanced in clinical development, a number of agents with similar activity are in early clinical trials. Formulations and routes of delivery differ, but it remains to be seen whether different agents manifest significant differences in efficacy or toxicity. (Comen and Robson, 2010)

4.3.3 Dasatinib:

Gene expression profiling has suggested that basal-like breast cancers might be preferentially sensitive to inhibition of proto-oncogene, SRC. Dasatinib, a potent

orally available inhibitor of Src-family kinases with antiproliferative antiosteoclastic and antimetastatic activity, was recently studied in the setting of triple negative MBC. (Anders and Carey, 2009) Preclinical data suggest that dasatinib may be effective in treating TNBC by inhibiting proliferation, migration and invasion of metastatic breast cancer. Single agent dasatinib showed limited efficacy in patients with TNBC. (Finn et al., 2011) A phase II study reports a clinical benefit rate of 9.2% among 43 response-evaluable patients. As dramatic response to single-agent biologic agents is not expected, novel combinations of dasatinib and chemotherapy are warranted and are currently being explored. (Anders and Carey, 2009) Dose of dasatinib was interrupted, reduced or treatment suspended in the event of drug related grade 3 or 4 toxicity. (Finn et al., 2011)

4.3.4 mTOR inhibitors:

mTOR is a cell cycle regulator as well as an effector of the final common pathway of phosphatidylinisitol 3-phosphate phosphatase and PTEN/AKT. This metabolic pathway is damaged in breast cancer. Loss of the PTEN tumor suppressor gene is common in TNBC, which causes increased mTOR activation. This would be the rationale for the use of mTOR inhibitors for this condition. (Chacón and Costanzo, 2010) The mTOR inhibitor everolimus was evaluated in first-or-second line treatment of ABC in a phase II trial. When administered at a dose of 10mg/day, everolimus produced objective responses in 12% of patients, with HER2 negative status being predictive of clinical benefit. However the activity of everolimus in patients with TNBC in this trial was not specified. 16% of total patients receiving daily everolimus discontinued the therapy due to pneumonititis. (Dyar and Moreno-Aspitia, 2011) Although not yet given as a standard combination with chemotherapy in HER2

negative ABC patients. The BOLERO-2 study examined the addition of everolimus plus exemestane in the postmenoposal HR positive patients. An improvement in PFS was found. The most common grade 3 or 4 adverse events were stomatitis, anemia, dyspnea and hyperglycemia. (Joy et al., 2015)

4.3.5 Fulvestrant and CDK inhibitors (CDKI):

Fulvestrant, a serum ER downregulator, act as a pure estrogen antagonistenhancing the proteasomal degradation of ER. Fulvestrant's main benefits appear to be linked to combined use with aromatase inhibitors. When fulvestrant is used as a single agent, the higher loading dose regimen seems to be the most beneficial in terms of efficacy. The main toxicities of fulvestrant are not unexpected and include hot flashes, gastrointestinal discomfort. CDKI protein is an endogenous protein that interacts with cyclin-CDK complex to block kinase activity, usually during G_1 or in response to signals from the environment or damaged DNA. Cyclin D1 amplification is seen in nearly 60% of breast cancers. There may be also amplification of genes that encode CDK4 orCDK6, leading to their overexpression. Most commonly mutation, deletation or promoter methylation of the genes encoding endogeneous CDK4/6 inhibitors occurs; absence of these inhibitors allows CDK4 and 6 to continually drive the cell cycle progression that gives cancer cells a growth advantage. (Serkan et al., 2014) 12 postmenopausal women with OR positive, HER2 negative MBC were enrolled in a phase1 study to investigate the safety and tolerability of paibociclib (CDKI) plus letrozole for first line treatment. No drug-drug interactions were observed. Most important dose limiting toxicity was grade 4 neutropenia. Palbociclib granted by FDA in February 2015 for use in combination with PALAMO-1 trial and in February 2016 for in combination with fulvestrant based

on PALAMO-3 trial. (Polk et al., 2017) In the PALAMO-3 trial palbococlib and fulvestrant was associated with significant improvement in PFS compared with fulvestrant plus placebo in patients with MBC. The combination could be considered as a therapeutic option for patients with recurrent hormone receptor positive, HER2 negative metastatic breast cancer that has progressed on previous endocrine therapy. (Cristofanilli et al., 2016)

Chapter 5

Conclusion

Above stated data demonstrated that, the treatment of breast cancer is progressed now-a-days. Targeted therapy for HER2 positive breast cancer minimizes the toxicity which is much encountered by chemotherapy. In case of HER2 negative, if it is only HER2 negative and either HR or ER or both positive, then targeted therapy along with chemotherapy is effective. In case of TNBC (does not express ER, PR and lacks of HER2 amplification and account for approximately 15% of all diagnosed breast cancers) (Liu et al., 2011) there is no preferred standard chemotherapy. So, this will be an area of further research.

Chapter 6

References

American Association for Cancer Reasearch, (2011) [online] Available at: 2. https://www.dovepress.com/profiling-and-targeting-her2-positive-breast-cancer-using-trastuzumab--peer-reviewed-fulltext-article-PGPM [Accessed 17 Jan. 2017].

Anders, C. and Carey, L. (2009). Biology, Metastatic Patterns, and Treatment of Patients with Triple-Negative Breast Cancer. *Clinical Breast Cancer*, 9, pp.S73-S81.

Anders, C., Winer, E., Ford, J., Dent, R., Silver, D., Sledge, G. and Carey, L. (2010). Poly (ADP-Ribose) Polymerase Inhibition: "Targeted" Therapy for Triple-Negative Breast Cancer. *Clinical Cancer Research*, 16(19), pp.4702-4710.

Anon, (2017). [online] Available at: 3. http://www.ajnr.org/content/32/8/1373 [Accessed 17 Jan. 2017].

Ates, O. and Babacan, T. (2016). efficacy and safety of eribulin monotherapy in patients with heavily pretreated metastatic breast cancer.

Babacan, T., Efe, O., Hasirci, A., Demirci, F., Buyukhatipoglu, H., Balakan, O., Sarici, F., Kertmen, N., Esin, E., Akin, S., Ates, O., Aksoy, S., Sever, A. and Altundag, K. (2015). Efficacy of capecitabine monotherapy as the first-line treatment of metastatic HER2-negative breast cancer. *Tumori Journal*, 101(4), pp.418-423.

Burris, H., Rugo, H., Vukelja, S., Vogel, C., Borson, R., Limentani, S., Tan-Chiu, E., Krop, I., Michaelson, R., Girish, S., Amler, L., Zheng, M., Chu, Y., Klencke, B. and O'Shaughnessy, J. (2011). Phase II Study of the Antibody Drug Conjugate Trastuzumab-DM1 for the Treatment of Human Epidermal Growth Factor Receptor 2 (HER2) –Positive Breast Cancer After Prior HER2-Directed Therapy. *Journal of Clinical Oncology*, 29(4), pp.398-405.

Callahan, R. and Hurvitz, S. (2011). Human epidermal growth factor receptor-2-positive breast cancer: current management of early, advanced, and recurrent disease. *Current Opinion in Obstetrics and Gynecology*, 23(1), pp.37-43.v

Carey, L., Rugo, H., Marcom, P., Mayer, E., Esteva, F., Ma, C., Liu, M., Storniolo, A., Rimawi, M., Forero-Torres, A., Wolff, A., Hobday, T., Ivanova, A., Chiu, W., Ferraro, M., Burrows, E., Bernard, P., Hoadley, K., Perou, C. and Winer, E. (2012). TBCRC

001: Randomized Phase II Study of Cetuximab in Combination With Carboplatin in Stage IV Triple-Negative Breast Cancer. *Journal of Clinical Oncology*, 30(21), pp.2615-2623.

Chacón, R. and Costanzo, M. (2010). Triple-negative breast cancer. *Breast Cancer Research*, 12(S2).

Comen, E. and Robson, M. (2010). Poly(ADP-Ribose) Polymerase Inhibitors in Triple-Negative Breast Cancer. *The Cancer Journal*, 16(1), pp.48-52.

Cristofanilli, M., Turner, N., Bondarenko, I., Ro, J., Im, S., Masuda, N., Colleoni, M., DeMichele, A., Loi, S., Verma, S., Iwata, H., Harbeck, N., Zhang, K., Theall, K., Jiang, Y., Bartlett, C., Koehler, M. and Slamon, D. (2016). Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *The Lancet Oncology*, 17(4), pp.425-439.

Dadic Plavetic, N., Kulic, A. and Vebanec, D. (2013). Role of HER2 signaling pathway in breast cancer: biology, detection and therapeutical implication. *PERIODICUM BIOLOGORUM*, 114(4), pp.505-510.

Dahabreh, I., Linardou, H., Siannis, F., Fountzilas, G. and Murray, S. (2008). Trastuzumab in the Adjuvant Treatment of Early-Stage Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *The Oncologist*, 13(6), pp.620-630.

Damaskos, C., Valsami, S., Kontos, M., Spartalis, E., Kalampokas, T., Kalampokas, E., Athanasiou, A., Moris, D., Daskalopoulou, A., Davakis, S., Tsouroufl is, G., Kontzoglou, K., Perrea, D., Nikiteas, N. And Dimitroulis, D. (2017). Histone Deacetylase Inhibitors: An Attractive Therapeutic Strategy Against Breast Cancer. *Anticancer Research*, 37(1), pp.35-46

Denduluri, N., Somerfield, M., Eisen, A., Holloway, J., Hurria, A., King, T., Lyman, G., Partridge, A., Telli, M., Trudeau, M. and Wolff, A. (2016). Selection of Optimal Adjuvant Chemotherapy Regimens for Human Epidermal Growth Factor Receptor 2

(HER2) –Negative and Adjuvant Targeted Therapy for HER2-Positive Breast Cancers: An American Society of Clinical Oncology Guideline Adaptation of the Cancer Care Ontario Clinical Practice Guideline. *Journal of Clinical Oncology*, 34(20), pp.2416-2427.

Denduluri, S., Idowu, O., Wang, Z., Liao, Z., Yan, Z., Mohammed, M., Ye, J., Wei, Q., Wang, J., Zhao, L. and Luu, H. (2015). Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. *Genes & Diseases*, 2(1), pp.13-25.

Doherty, M. and Morris, P. (2015). Eribulin for the treatment of metastatic breast cancer: an update on its safety and efficacy. *International Journal of Women's Health*, p.47.

Drakaki, A. and Hurvitz, S. (2017). HER2-Positive Breast Cancer: Update on New and Emerging Agents. *BREAST CANCER*, 11(4).

Dyar, S. and Moreno-Aspitia, A. (2011). Triple-negative breast cancer and new treatment developments. *Clinical Investigation*, 1(8), pp.1107-1118.

Eroglu, Z., Tagawa, T. and Somlo, G. (2014). Human Epidermal Growth Factor Receptor Family-Targeted Therapies in the Treatment of HER2-Overexpressing Breast Cancer. *The Oncologist*, 19(2), pp.135-150.

Fabi, A., Malaguti, P., Vari, S. and Cognetti, F. (2016). First-line therapy in HER2 positive metastatic breast cancer: is the mosaic fully completed or are we missing additional pieces?. *Journal of Experimental & Clinical Cancer Research*, 35(1).

Fakhrejahani, E. and Toi, M. (2014). Antiangiogenesis Therapy for Breast Cancer: An Update and Perspectives from Clinical Trials. *Japanese Journal of Clinical Oncology*, 44(3), pp.197-207.

Fink, M. and Chipuk, J. (2013). Survival of HER2-Positive Breast Cancer Cells: Receptor Signaling to Apoptotic Control Centers. *Genes & Cancer*, 4(5-6), pp.187-195.

Finn, R., Bengala, C., Ibrahim, N., Roche, H., Sparano, J., Strauss, L., Fairchild, J., Sy, O. and Goldstein, L. (2011). Dasatinib as a Single Agent in Triple-Negative Breast Cancer: Results of an Open-Label Phase 2 Study. *Clinical Cancer Research*, 17(21), pp.6905-6913.

Gajria, D. and Chandarlapaty, S. (2011). HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. *Expert Review of Anticancer Therapy*, 11(2), pp.263-275.

Gelmon, K. (2006). The Role of Capecitabine in First-Line Treatment for Patients with Metastatic Breast Cancer. *The Oncologist*, 11(suppl_1), pp.42-51.

Gilabert, M., Esterni, B., Bertucci, F., Viens, P. and Goncalves, A. (2010). Capecitabine after anthracycline and taxane exposure in HER2-negative metastatic breast cancer patients: Response, survival, and prognostic factors. *Journal of Clinical Oncology*, 28(15_suppl), pp.2593-2593.

Gogineni, K. and DeMichele, A. (2012). Current approaches to the management of Her2-negative metastatic breast cancer. *Breast Cancer Research*, 14(2).

Gordon, M., Mendelson, D. and Kato, G. (2010). Tumor angiogenesis and novel antiangiogenic strategies. *International Journal of Cancer*, p.NA-NA.

Ha, K., Fiskus, W., Choi, D., Bhaskara, S., Cerchietti, L., Devaraj, S., Shah, B., Sharma, S., Chang, J., Melnick, A., Hiebert, S. and Bhalla, K. (2014). Histone deacetylase inhibitor treatment induces 'BRCAness' and synergistic lethality with PARP inhibitor and cisplatin against human triple negative breast cancer cells. *Oncotarget*, 5(14), pp.5637-5650.

Hielscher, A. and Patel, A. (2015). Angiogenesis Inhibitors in the Treatment of Breast Cancer: Exploring Avenues of New Therapeutic Targets. *Journal of Cancer Prevention & Current Research*, 2(6).

Ibrahim, Y. and Yee, D. (2005). Insulin-Like Growth Factor-I and Breast Cancer Therapy. *Clinical Cancer Research*, 11, pp.944-950.

Joy, A., Ghosh, M., Fernandes, R. and Clemons, M. (2015). Systemic treatment approaches in her2-negative advanced breast cancer—guidance on the guidelines. *Current Oncology*, 22(0), p.29.

Kerbel, R. (2011). Reappraising antiangiogenic therapy for breast cancer. *The Breast*, 20, pp.S56-S60.

Kotake, T. (2016). Impact of Eribulin Monotherapy on Post-Progression Survival in Patients with HER2-Negative Advanced or Metastatic Breast Cancer. *International Journal of Cancer and Clinical Research*, 3(4).

Kümler, I., Knoop, A., Jessing, C., Ejlertsen, B. and Nielsen, D. (2016). Review of hormone-based treatments in postmenopausal patients with advanced breast cancer focusing on aromatase inhibitors and fulvestrant. *ESMO Open*, 1(4), p.e000062.

Lam, S., de Groot, S., Honkoop, A., Jager, A., ten Tije, A., Bos, M., Linn, S., van den Bosch, J., Kroep, J., Braun, J., van Tinteren, H. and Boven, E. (2014). Paclitaxel and bevacizumab with or without capecitabine as first-line treatment for HER2-negative locally recurrent or metastatic breast cancer: A multicentre, open-label, randomised phase 2 trial. *European Journal of Cancer*, 50(18), pp.3077-3088.

Lavaud, P. and Andre, F. (2014). Strategies to overcome trastuzumab resistance in HER2-overexpressing breast cancers: focus on new data from clinical trials. *BMC Medicine*, 12(1).

Lee, J., Kim, H., Ro, S. and Yang, J. (2017). Capecitabine and cisplatin (XP) combination systemic chemotherapy in heavily pre-treated HER2 negative metastatic breast cancer. *PLOS ONE*, 12(2), p.e0171605.

Lee, J., Loh, K. and Yap, Y. (2015). PI3K/Akt/mTOR inhibitors in breast cancer'. 12, pp.342-354.

Leone, J. and Leone, B. (2015). Breast cancer brain metastases: the last frontier. *Experimental Hematology & Oncology*, 4(1).

Li, L. (2013). Targeted therapy in HER2-positive breast cancer (Review). *Biomedical Reports*.

Liu, M. (2012). Chemotherapy Options for Metastatic HER2-Negative Breast Cancer after Prior Exposure to Anthracyclines and Taxanes. *Oncology & Hematology Review (US)*, 08(01), p.21.

Liu, T., Yacoub, R., Taliaferro-Smith, L., Sun, S., Graham, T., Dolan, R., Lobo, C., Tighiouart, M., Yang, L., Adams, A. and O'Regan, R. (2011). Combinatorial Effects of Lapatinib and Rapamycin in Triple-Negative Breast Cancer Cells. *Molecular Cancer Therapeutics*, 10(8), pp.1460-1469.

Livraghi, L. and Garber, J. (2015). PARP inhibitors in the management of breast cancer: current data and future prospects. *BMC Medicine*, 13(1).

LoRusso, P. (2013). Mammalian Target of Rapamycin as a Rational Therapeutic Target for Breast Cancer Treatment. *Oncology*, 84(1), pp.43-56.

Lorusso, V. (2008). Bevacizumab in the treatment of HER2-negative breast cancer. *Biologics: Targets & Therapy*, p.813.

Ma, C. (2017). PI3K Pathway as a Therapeutic Target in Breast Cancer. *BREAST CANCER*, 11(3).

Malumbres, M. (2016). CDK4/6 Inhibitors resTORe Therapeutic Sensitivity in HER2 + Breast Cancer. *Cancer Cell*, 29(3), pp.243-244.

McArthur, H. and Page, D. (2016). 'Immunotherapy for the treatment of breast cancer: checkpoint blockade, cancer vacccines and future directions in combination immunotherapy'. *Clinical Advances in Hematology & Oncology*, 14(11).

Miller, K., Cortes, J., Hurvitz, S., Krop, I., Tripathy, D., Verma, S., Riahi, K., Reynolds, J., Wickham, T., Molnar, I. and Yardley, D. (2016). HERMIONE: a randomized Phase 2 trial of MM-302 plus trastuzumab versus chemotherapy of physician's choice plus trastuzumab in patients with previously treated, anthracycline-naïve, HER2-positive, locally advanced/metastatic breast cancer. *BMC Cancer*, 16(1).

Minckwitz, G. and Rezai, M. (2012). Neoadjuvant Chemotherapy and Bevacizumab for HER2-Negative Breast Cancer. *The New England Journal of Medicine*, 366(4), pp.299-309.

Monero-Aspitia, A. and Perez, E. (2009). Treatment options for breast cancer resistant to anthracyclin and taxanes'. 84(6), pp.533-545.

Morabito, A., Piccirillo, M., Monaco, K., Pacilio, C., Nuzzo, F., Chiodini, P., Gallo, C., de Matteis, A. and Perrone, F. (2007). First-Line Chemotherapy for HER-2 Negative Metastatic Breast Cancer Patients Who Received Anthracyclines as Adjuvant Treatment. *The Oncologist*, 12(11), pp.1288-1298.

Moyer JD et al, (1997) [online] Available at: 4. http://www.medscape.org/viewarticle/504206_3 [Accessed 17 Jan. 2017].

News.isncc.org. (2017). *ISNCC Blog – Incidence of Breast Cancer in Bangladesh*. [online] Available at: http://news.isncc.org/incidence-of-breast-cancer-in-bangladesh/ [Accessed 17 Jan. 2017].

Nielsen, D., Andersson, M., Andersen, J. and Kamby, C. (2010). Antiangiogenic therapy for breast cancer. *Breast Cancer Research*, 12(5).

Nolan, L., Johnson, P., Ganesan, A., Packham, G. and Crabb, S. (2008). Will histone deacetylase inhibitors require combination with other agents to fulfil their therapeutic potential?. *British Journal of Cancer*, 99(5), pp.689-694.

Nye, L. and Mutonga, M. (2016). Management of Metastatic HER2-Positive Breast Cancer: Where are we and Where Do We Go From Here?. *CancerNetwork*.

Pegram, M. (2011). Challenges in HER2-Positive Breast Cancer. *American Soceity of Clinical Oncology*, 10.

Lee, H., Shields, A., Siegel, B., Miller, K., Krop, I., Ma, C., LoRusso, P., Munster, P., Campbell, K., Gaddy, D., Leonard, S., Geretti, E., Blocker, S., Kirpotin, D., Moyo, V., Wickham, T. and Hendriks, B. (2017). 64 Cu-MM-302 Positron Emission Tomography Quantifies Variability of Enhanced Permeability and Retention of Nanoparticles in Relation to Treatment Response in Patients with Metastatic Breast Cancer. *Clinical Cancer Research*.

Perera, N. and Hergovich, A. (2017). The promise of using histone deacetylase inhibitors in combination treatment against breast cancer and other solid tumors. *Chinese Clinical Oncology*, 6(1), pp.9-9.

Perey, L., Paridaens, R., Hawle, H., Zaman, K., Nole, F., Wildiers, H., Fiche, M., Dietrich, D., Clement, P., Koberle, D., Goldhirsch, A. and Thurlimann, B. (2006). Clinical benefit of fulvestrant in postmenopausal women with advanced breast cancer and primary or acquired resistance to aromatase inhibitors: final results of phase II Swiss Group for Clinical Cancer Research Trial (SAKK 21/00). *Annals of Oncology*, 18(1), pp.64-69.

Perez, E., Patel, T. and Moreno-Aspitia, A. (2010). Efficacy of ixabepilone in ER/PR/HER2-negative (triple-negative) breast cancer. *Breast Cancer Research and Treatment*, 121(2), pp.261-271.

Perez, E., Romond, E., Suman, V., Jeong, J., Davidson, N., Geyer, C., Martino, S., Mamounas, E., Kaufman, P. and Wolmark, N. (2011). Four-Year Follow-Up of Trastuzumab Plus Adjuvant Chemotherapy for Operable Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer: Joint Analysis of Data From NCCTG N9831 and NSABP B-31. *Journal of Clinical Oncology*, 29(25), pp.3366-3373.

Piccart-Gebhart, M. (2005). Moving Away From the “One Shoe Fits All” Strategy: The Key to Future Progress in Chemotherapy. *Journal of Clinical Oncology*, 23(8), pp.1611-1613.

Polk, A., Kolmos, I., Kümler, I. and Nielsen, D. (2017). Specific CDK4/6 inhibition in breast cancer: a systematic review of current clinical evidence. *ESMO Open*, 1(6), p.e000093.

Rexer, B., Chanthaphaychith, S., Dahlman, K. and Arteaga, C. (2014). Direct inhibition of PI3K in combination with dual HER2 inhibitors is required for optimal antitumor activity in HER2+ breast cancer cells. *Breast Cancer Research*, 16(1).

Paplomata, E. and O'Regan, R. (2014). The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. *Therapeutic Advances in Medical Oncology*, 6(4), pp.154-166.

Romond, E. and Perez, E. (2005). Trastuzumab Plus Adjuvant Chemotherapy for Operable HER2-Positive Breast Cancer. *The new England journal of medicine*, 356(16).

Santa-Maria, C. and Gradishar, W. (2015). Changing Treatment Paradigms in Metastatic Breast Cancer. *JAMA Oncology*, 1(4), p.528.

Schroeder, R., Stevens, C. and Sridhar, J. (2014). Small Molecule Tyrosine Kinase Inhibitors of ErbB2/HER2/Neu in the Treatment of Aggressive Breast Cancer. *Molecules*, 19(9), pp.15196-15212.

Serkan, A., Taner, B., Furkan, S. and Kadri, A. (2014). A novel targeted therapy in breast cancer: Cyclin dependent kinase inhibitors. *JBUON*, 19(1)(1107-0625), pp.42-46.

Sevcikova, K., Vertakova-Krakovska, B. and Spanik, S. (2013). Neoadjuvant Treatment in Patients with HER2-Positive Breast Cancer. *ISRN Oncology*, 2013, pp.1-8.

Shim, J., Rao, R., Beebe, K., Neckers, L., Han, I., Nahta, R. and Liu, J. (2012). Selective Inhibition of HER2-Positive Breast Cancer Cells by the HIV Protease Inhibitor Nelfinavir. *JNCI: Journal of the National Cancer Institute*, 104(20), pp.1576-1590.

Singh, J., Jhaveri, K. and Esteva, F. (2014). HER2-positive advanced breast cancer: optimizing patient outcomes and opportunities for drug development. *British Journal of Cancer*, 111(10), pp.1888-1898.

Sini, V., Cassano, A., Corsi, D., De Laurentiis, M., Gamucci, T., Mauri, M., Naso, G., Roselli, M., Ruggeri, E., Tonini, G., Vici, P., Zampa, G. and Marchetti, P. (2016). Bevacizumab as first-line treatment in HER2-negative advanced breast cancer: pros and cons. *Tumori Journal*, 102(5), pp.472-480.

Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M., Pinter, T., Valero, V., Liu, M., Sauter, G., von Minckwitz, G., Visco, F., Bee, V., Buyse, M., Bendahmane, B., Tabah-Fisch, I.,

Lindsay, M., Riva, A. and Crown, J. (2011). Adjuvant Trastuzumab in HER2-Positive Breast Cancer. *New England Journal of Medicine*, 365(14), pp.1273-1283.

Song, C., Park, S., Eom, K., Kim, J., Kim, S., Kim, J. and Kim, I. (2010). Potential prognostic value of heat-shock protein 90 in the presence of phosphatidylinositol-3-kinase overexpression or loss of PTEN, in invasive breast cancers. *Breast Cancer Research*, 12(2).

Subbiah, I. and Gonzalez-Angulo, A. (2013). Advances and Future Directions in the Targeting of HER2-positive Breast Cancer: Implications for the Future. *Current Treatment Options in Oncology*, 15(1), pp.41-54.

Tanei, T., Choi, D., Rodriguez, A., Liang, D., Dobrolecki, L., Ghosh, M., Landis, M. and Chang, J. (2016). Antitumor activity of Cetuximab in combination with Ixabepilone on triple negative breast cancer stem cells. *Breast Cancer Research*, 18(1).

Telli, M. and Ford, J. (2010). PARP Inhibitors in Breast Cancer. *Clinical Advances in Hematology and Oncology*, 8(9).

Thomas, E., Gomez, H., Li, R., Chung, H., Fein, L., Chan, V., Jassem, J., Pivot, X., Klimovsky, J., de Mendoza, F., Xu, B., Campone, M., Lerzo, G., Peck, R., Mukhopadhyay, P., Vahdat, L. and Roché, H. (2007). Ixabepilone Plus Capecitabine for Metastatic Breast Cancer Progressing After Anthracycline and Taxane Treatment. *Journal of Clinical Oncology*, 25(33), pp.5210-5217.

Tian-wen, C. and Ya-nan, L. (2013). metformin inhibits proliferation and promotes apoptosis of HER2 positive breast cancer cells by downregulating HSP90'. 18(1), pp.51-56.

Tinoco, G., Warsch, S., Glück, S., Avancha, K. and Montero, A. (2013). Treating Breast Cancer in the 21st Century: Emerging Biological Therapies. *Journal of Cancer*, 4(2), pp.117-132.

Tkaczuk and Tkaczuk (2011). Ixabepilone as Monotherapy or in Combination with Capecitabine for the Treatment of Advanced Breast Cancer. *Breast Cancer: Basic and Clinical Research*, p.1.

Truong, T. and Munster, P. (2013). Histone deacetylase inhibitors for the treatment of breast cancer: recent trial data. *Clinical Investigation*, 3(6), pp.557-569.

Untch, M., Rezai, M., Loibl, S., Fasching, P., Huober, J., Tesch, H., Bauerfeind, I., Hilfrich, J., Eidtmann, H., Gerber, B., Hanusch, C., Kühn, T., du Bois, A., Blohmer, J., Thomssen, C., Dan Costa, S., Jackisch, C., Kaufmann, M., Mehta, K. and von Minckwitz, G. (2010). Neoadjuvant Treatment With Trastuzumab in HER2-Positive Breast Cancer: Results From the GeparQuattro Study. *Journal of Clinical Oncology*, 28(12), pp.2024-2031.

Viani, G., Afonso, S., Stefano, E., De Fendi, L. and Soares, F. (2007). Adjuvant trastuzumab in the treatment of her-2-positive early breast cancer: a meta-analysis of published randomized trials. *BMC Cancer*, 7(1).

Viswanath, S. and Pathak, A. (2016). Management of HER2-positive breast cancer: A review. *Journal of Dr. NTR University of Health Sciences*, 5(2), p.87.

Voudouri, K., Berdiaki, A., Tzardi, M., Tzanakakis, G. and Nikitovic, D. (2015). Insulin-Like Growth Factor and Epidermal Growth Factor Signaling in Breast Cancer Cell Growth: Focus on Endocrine Resistant Disease. *Analytical Cellular Pathology*, 2015, pp.1-10.

Voutsadakis, I. (2016). Immune Blockade Inhibition in Breast Cancer. *Anticancer Research*, 36(11), pp.5607-5622.

Watanabe, J. (2015). Eribulin monotherapy improved survivals in patients with ER-positive HER2-negative metastatic breast cancer in the real world: a single institutional review. *SpringerPlus*, 4(1).

Wilks, S. (2015). Potential of overcoming resistance to HER2-targeted therapies through the PI3K/Akt/mTOR pathway. *The Breast*, 24(5), pp.548-555.

Yarden & Sliwkowski, (2001) [online] Available at: 1. http://slideplayer.com/slide/8625727/ [Accessed 17 Jan. 2017].

Yardley, D., Dickson, N., Drosick, D., Earwood, C., Inhorn, R., Murphy, P. and Hainsworth, J. (2016). Sorafenib Plus Ixabepilone as First-Line Treatment of Metastatic HER2-Negative Breast Cancer: A Sarah Cannon Research Institute Phase I/II Trial. *Clinical Breast Cancer*, 16(3), pp.180-187.

Yee, D. (2006). Targeting insulin-like growth factor pathways. *British Journal of Cancer*, 94(4), pp.465-468.

Zagouri, F., Bournakis, E., Koutsoukos, K. and Papadimitriou, C. (2012). Heat Shock Protein 90 (Hsp90) Expression and Breast Cancer. *Pharmaceuticals*, 5(12), pp.1008-1020.

Zhang, B. and Hurvitz, S. (2016). Long-term Outcomes of Neoadjuvant Treatment of HER2-Positive Breast Cancer. *Clinical Advances in Hematology & Oncology*, 14(7).