"A REVIEW ON HER2-TARGETED THERAPY IN BREAST CANCER"

A PROJECT SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

BY

DESAI SHIPRA N.(16BPH089)

Semester VIII

UNDER THE GUIDANCE OF

Ms. Palak K. Parikh

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APRIL 2020

CERTIFICATE

This is to certify that "A REVIEW ON HER-2 TARGETED THERAPY IN BREAST CANCER" is the bonafide work carried out by DESAI SHIPRA N. (16BPH089), B. Pharm semester VIII under our guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2019-2020. This work is up to my satisfaction.

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CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work entitled "A REVIEW ON HER-2 TARGETED THERAPY IN BREAST CANCER" Submitted by DESAI SHIPRA N. (16BPH089), B.Pharm. Semester VIII is a bonafide review/research work carried out by me at the Institute of Pharmacy, Nirma University under the guidance of "Ms. Palak Parikh". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review/research work carried out by me is not reported anywhere as per best of my Knowledge.

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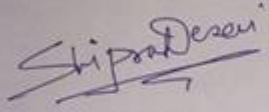
DECLARATION

I, DESAI SHIPRA N. (16BPH089) student of VIIIth Semester of B. Pharm at Institute of Pharmacy, Nirma University, hereby declare that my project entitled "A REVIEW ON HER-2 TARGETED THERAPY IN BREAST CANCER" is a result of culmination of my sincere efforts. I declare that the submitted project is done solely by me and to the best of my knowledge, no such work is done by any other person for the award of degree or diploma or for any other means. I also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.



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I gratefully acknowledged the encouragement and constant guidance received by Ms. PALAK K PARIKH, professor Nirma Institute of Pharmacy, Ahmedabad with my deep sense of gratitude. I also convey my cordial affection to my parents for advocacy & support for the completion of thesis.

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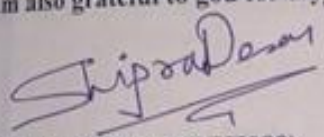

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1. Abstract:

Breast cancer is a form of disease which involves malignant growth of cells in the breast. It is the most common cause of cancer in woman and the highest prevalence of breast cancer is accountable for nearly 26 percent of all women's cancers. Breast cancer has in recent decades been significantly promoted by adding more specific therapies to different sub-types of advanced clinical activity since the discovery of novel treatment aspects related to HER-2 antibody mediated therapy and small molecule inhibitors of HER-2 in breast cancer. The use of HER2 therapies to treat HER2-positive breast cancer patients has led to dramatic survival results in both early and advanced stages of breast cancer. Another approach has focused on effectively inhibiting the HER2 signaling pathway and creating enhanced anti-HER2 therapies such as antibody – drug conjugates. The comprehensive goal of this study was to study the design and synthesis of several small molecule inhibitors of HER-2.

2.Introduction:

- Chronic conditions such as cancer and cardiovascular diseases are the world's main causes of morbidity and death. Cancer is a major cause of death in the world. Mortality rate are predicted to continue to rise with about 12 million deaths per year by 2030(WHO,2011) as stated by the WHO reports.
- Breast cancer is the major reoccurring cause of female cancer and will increase over the consecutive few years. The chance for women to have a lifespan of breast cancer in the USA is around 1/8, Europe 1/12, Asia 1/40.
- As per WHO, breast cancer accounts for 2.09 million cases and 6,27,000 deaths worldwide. It is the most reoccurring cancer in the women in India and results for 14% of all cancers in women. It can occur at any age but the prevalence rates in India set about to advance in the early thirties and peak at ages fifty to sixty-four years. (WHO, 2008).

2.1 ***Cancer***

Cancer is a group of diseases that includes uncontrolled and abnormal cell growth that invades or spreads to one part of the body to other. Cancer is cell proliferation which is uncontrolled and unchecked. Such uncontrolled or fast-growing cells "pile up on top" and form a tumor. The tumor is

called malignant when cells get removed from the tumor and invade surrounding tissue. A tumor whose cells do not invade surrounding tissue is referred to as a benign tumor, and is in most cases harmless.

Cancer can affect all living cells inside the body, at all ages and in both sexes. The cause may be multi-factorial, and cancers at various locations can vary in the disease progression. High levels of cervical and breast cancer have produced an increased prevalence of the most prevailing type of cancer in women as compared to men, and thus these diseases have significant social and family implications.

One of the major reasons of cancer death causes includes intake of narcotic substances consisting of tobacco. Other major factor includes weight gain or obesity, intake of oily and unhealthy food and food products, lack of physical exercises or increase in alcohol intake, spreading of infections, exposure to ionizing radiation etc. The bloodstream or lymphatic network can transport cells of cancer to one part to surrounding parts of the body as a cancerous tumor grows. The cancer cells grow during this cycle, and can evolve into new tumors. This process is called metastasis.

Cancer can affect all living cells inside the body, at all ages and in both sexes. The cause may be multi-factorial, and cancers at various locations can vary in the disease processes. High levels of cervical and breast cancer have produced an increase in prevalence of the deadly disease in women as compared to men, and thus these diseases have significant social and family implications. Cancers are divided on the basis of the tumor cell and are thus known to be the growth of the tumor. Thus, type of disease is originated from different types of cells:

- Carcinoma: Cancer originated from the epithelial cells. This group includes various parts of the human body like prostate, lung, pancreas, colon and breast.
- Sarcoma: Such forms of cancer are originated in the connective tissue (i.e. bone & cartilage) that develops out of mesodermic tissue cells that reside outside to the bone marrow.
- Lymphoma & leukemia: The next two disease types are derived from blood-forming cells that leave the marrow and appear to reside in the lymph nodes and blood.
- Germ cell tumor: This type of tumor is found in testicle or ovary.
- Blastoma: In this class, undeveloped predecessor cells or embryonic tissue create cancer. (www.healthline.com/health/cancer)

2.2 ***Mechanism of Cancer Formation***:

Genes that regulate cell growth need to be disrupted in a particular sequence for the cells to start multiplying abnormally as well as uncontrollably. Proto-oncogenes are the genes that encourage cell growth and mitosis, while genes that suppress tumors inhibit cell development, for the time being terminate cell multiplication to repair DNA.

There is a need for a number of multiple mutations to these genes before a normal cell becomes a cancer cell. These gene mutations provide signals that uncontrollably begin to separate tumor cells. Yet the uncontrolled division of cancer cells often allows the dividing cell to replace all of its cell components in order to generate two daughter cells. Activation of anaerobic glycolysis, that is not generally triggered by mutations in two major genes including tumor suppressor genes and proto-oncogenes, provides most of the building blocks vital to replicate cell divisional components and is therefore necessary for carcinogenesis. (Koeffler et al., 1991)

3. HER-2 Receptor:

HER-2 receptor is a member of the “Human epidermal growth factor receptor” family. It consists of “ERBB (Erythroblastic Oncogene B)” and “EGFR (Epidermal Growth Factor Receptor)”. In the development of many human cancers, the HER growth factor receptors play a significant role. It controls cell growth, survival, differentiation, and differentiation via multiple pathways for signal transduction. The family consists of mainly 4 members: HER (1,2,3,4), also called ErbB1(1,2,3,4) respectively. It is made up of 1255 amino acid chain, 185 kD glycoprotein transmembrane in the human gene's prolongated arm (chromosome 17). HER2 is present in various tissues and its major function in these tissues is to promote carcinogenesis excess / abnormal development.(Iqbal & Iqbal, 2014)

3.1 Structure Of HER-2 Protein

The structure shown below is of “HER-2/neu” receptors. The displayed domain structure consists of:

- Extracellular domain comprising of “two ligand binding regions (LD1 & LD2)”
- “Intracellular domain comprising of “two cysteine rich regions (CR1 & CR2)”
- A short middle “transmembrane domain”
- A “catalytic tyrosine kinase domain” (TK)
- A terminal carboxy tail (CT)

Different sites with TK and CT domains for tyrosine phosphorylation are identified as P circles.(Moasser, 2007)

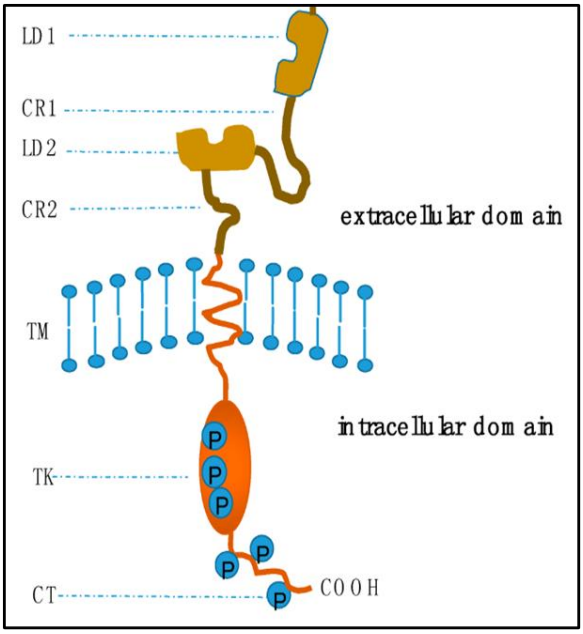


Figure 1: Structure of "Human Epidermal Growth Factor Receptor" (Lv et al., 2016)

3.2 ***Signaling Functions Of HER-2 Receptor:***

HER-2 receptors are a class of proteins that are present on the normal cells as well as the cancerous cells and are responsible for bringing the signals from outside to inside of the cells. Each of the HER receptor family member has to pair up to transmit the signal into the cell interior.

When a growth factor attaches to HER family receptor the receptor dimerizes with the other members HER receptors and undergoes phosphorylation, exception to this process is HER-2 receptor that do not require ligand for its activation and which is always ready to dimerize. The dimerization process leads to phosphorylation of the intercellular segment of the receptors that causes binding of signaling molecules to their respective docking sites. This causes activation of various signaling pathways including the PI3K pathway "phosphatidylinositol 3 kinase" which may avert apoptosis of cancer cells. The HER receptors also impart their effect by activation of various signaling molecules of the MAPK signaling pathway (Mitogen Activated protein kinase pathway) that include RAF, RAS, MAP and ERG. MAPK signaling pathway also lead to increase in the proliferation of cancer cells.(Iqbal & Iqbal, 2014)

3.3 ***Overexpression Of HER-2 Receptor in development of cancer:***

Amplification or over-expression of HER-2 receptor occurs in almost 15 % of the breast cancer patients accompanying increased disease recurrence and poor detection. It is also been proved that HER-2 receptor overexpression is also associated with other types of cancer affecting vital organs of the body including colon, lung etc.

Breast cancer contains up to 25–50 copies of the HER2 gene and an increase in the HER2 protein up to 40–100 fold, resulting in 2 million surface cell tumor receptors. An irregular form of HER2 (called "p95") in some breast cancers that is lacking the extracellular domain oh HER-2 receptor is found.

In the absence of an invasive disease that extends to other sections of the body, the overexpression of HER-2 is found in about half of all in situ ductal carcinomas (DCIS), whereas invasive disorders, nodal metastasis and distant metastasis are sustained throughout growth. Amplified breast cancers HER-2 have increased sensitivity to a wide range of cytotoxic chemotherapies and resistance to other hormonal agents.(Iqbal & Iqbal, 2014)

3.4 ***HER-2 detection tests in Breast cancer:***

There are two different ways to find if the breast cancer in the patient is HER-2 positive or negative. Two of the most common tests used are:

- "ImmunoHisto Chemistry (IHC)" test: To detect the HER2 protein the IHC test uses a chemical dye which stains the cell and evaluates further. It provides a score between 0 to 3+ which detects the amount of HER-2 protein on cell surface in a given test sample of breast cancer tissue. If the score is between 0 to 1 +, then HER2 is considered as negative. If the result of this test is 2+ then the test is considered as borderline. If the result of this test is borderline then the next test that is FISH test is considered that would be performed on the sample of cancer tissue to determine the status HER-2 positive breast cancer. HER2-positive is contemplated to have a score of 3 +.
- "FISH test (Fluorescence in Situ Hybridization)": This test check uses different labels referring to proteins present on HER2 receptor. The special labels have chemicals applied to them and when they bind to HER2 proteins they change color and shine in

the dark. The following way to check the HER-2 status is reliable but it is expensive and the tests can be time consuming. As a result, normally an "IHC" check is the primary check performed to examine whether a it is HER2-positive or not. With the use of "FISH test" the result comprises of a positive or a negative score. (Shah & Chen, 2010)

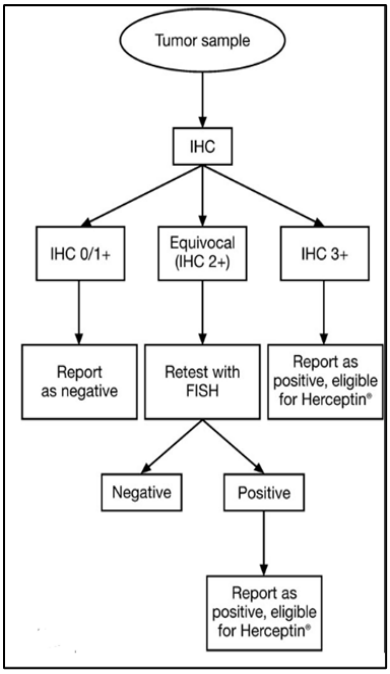


Figure 2: Algorithm for testing HER-2 status in Breast cancer (Wolff et al., 2018)

4. Major Signaling Pathways Of HER-2 Receptor:

Despite our understanding of the intrinsic molecular subtypes of breast cancer, we should know that breast cancer is a heterogeneous disorder. Every molecular subtype has a distinct stimulating advantage over growth. Recent advances in technology and research have given further insight into the cellular process and the pathways involved in development of breast cancer. Different signaling pathways, including cell survival, proliferation, differentiation, migration and apoptosis have been involved in the progression of breast cancer. In order to ensure that breast cancer cells respond adequately to the extracellular growth factors, these signal transduction pathways often cross-talk one

another. Slow and gradual disruption of these signaling pathways provided the cells leading to cancer with the advance in development.(Weigelt et al., 2010)

4.1 ***Downward Signaling of P13K and MAPK pathway:***

"Phosphoinositide 3-kinases (PI3Ks)", also known widely as "phosphatidylinositol 3-kinases", is a member of enzymes involved in cell processes such as cell development, gluconeogenesis, glycogenolysis, cell survival, motility, differentiation, proliferation and intracellular cell trafficking, that results in the occurrence of cancer. Abnormal activation of P13K pathway leads to progression of various types of cancer.

The "MAPK/ERK" signaling pathway (commonly referred to as RAS-RAF-ERK pathway) consist of the protein chain within the cell disseminate from a cell surface receptor to the DNA in the cell nucleus. It functions by activating receptors and especially activating k-RAF and b-RAS mutations. This results in increased cell proliferation and apoptosis resistance, apoptosis and chemotherapy resistance, radiation therapy and targeted treatment.

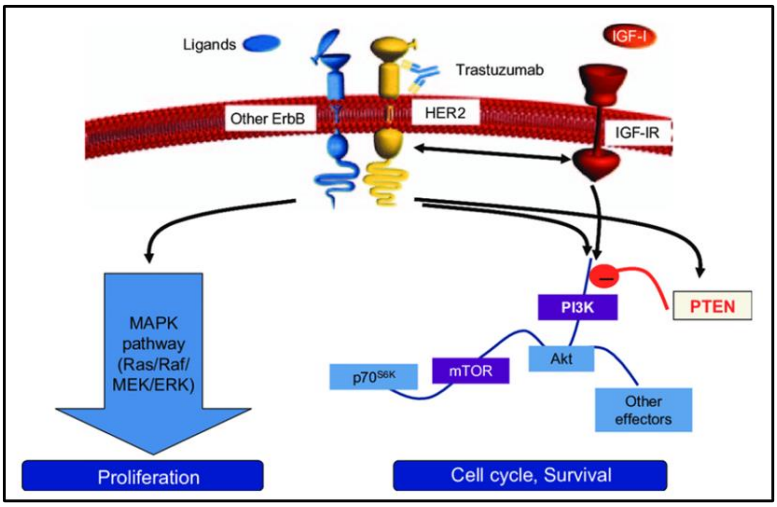


Figure 3: The following figure illustrates the two major pathways elaborated in downstream signaling resulting after HER-2 activation.(Shi et al., 2012)

PTEN is a tumor suppressor phosphate with a tensin homolog (PTEN) that negatively regulates the signals in P13K signaling pathways. PTEN transforms PIP3 back to PIP2, thereby inhibiting the further PI3 K signaling cascade.

In case of breast cancer and targeted resistance to various treatment loss of the gene PTEN is involved, and that leads to activation of P13k signaling which may further stimulate tumorigenesis. The suppression of PTEN gene uses a “tetracycline-regulated short hairpin” [(sh)RNA] in a transgenical prototype of breast cancer cooperates with the HER-2 receptor that results in metastatic diseased with increase in PI3 K signaling and MAPK pathways. For the downregulation of both the PI3 K and MAPK signals, the alteration of tumor suppressive gene PTEN to function is essential and leads to increase in tumor regression. MAPK signals show similar conclusions for PTEN pharmacological inhibition suggesting that MAPK pathway allows advanced breast cancers with Pten losses to be sustained.

The PTEN “phosphatase and tensin homolog” is mutated in a large number of tumor types. PTEN encodes a “phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PIP3)” preventing the functioning of 3-kinase phosphatidylinositol (PI3Ks), or the transmission to downstream mediators such as the serin / threonine-specific proteins AKT family of growth factor signals from receptor tyrosine kinaases is involved. AKT triggers a sequence of downstream effectors that allow the cell to survive and spread of the cells. Hence, loss of tumor suppressive gene PTEN is the main principle mechanism by means of which loss of PTEN contributes to hyperactivation of pathways of PI3 K is thought to be the predominant mechanism. Although the situation becomes complex with the signals of cross-speech and feedback, this molecular mechanism offers clear account for targeting components in PTEN-deficient tumors of PI3 K pathways, and clinical trials are currently underway with several small molecular antagonists.

The deregulation of P13K signaling pathway occurs most frequently in the alpha (PIK3CA) catalytic subunit of P13K pathway obtained due to “phosphatidylinositol-4,5-bisphosphate 3-kinase” (PIP3) mutations in breast cells. PTEN deficiency, on the other hand, was less common to diagnose but has a stronger relation to disease progression. For instance, PTEN inactivation also occurs in “human epidermal growth factor 2 (HER2 / neu)” tumors in HER2 / new HER2 targeting agent resistant patients with oncogenic receptor tyrosine kinase. In a patient also with PIK3CA mutations which established resistance to BYL719, an inhibiter of PI3Kα, PTEN mutations were recently also found. For this reason, in patients with poor prognosis in advanced diseases PTEN inactivation identifies a subtype of breast-cancers where there are unsatisfactory clinical needs.

The value of PI3 K for breast cancer has been confirmed in researches using mice. Transgenic mice which, in combination with HER2/neu show repeated resistance to anti-HER2/new therapies, upregulates mutant PIK3CA in the mammary gland, causes long-term tumors which recover after

removal of the oncogene. While these findings help to understand PI3 K pathways in breast cancer, the findings are based on a prototype in which PIK3CA mutant has been suggested unphysiologically and is a triggering case. Furthermore, experiments in combination with HER2/neu on transgenic model of mice indicate that the de-regulation of endogenous PI3Ks by means of Pten inactivation may promote advanced diseases. However, the issue of whether sustained PTEN inactivation is enough to support advanced cancers remains unanswered. Resolutions to this problem can reveal cell dependencies and inform the clinical application of targeted molecular agents which attack the PTEN network. Though signaling through both of PI3 K and mitogen activated protein kinase (MAPK) is the result of an unexpected Pten loss.(Ebbesen et al., 2016)

5. Breast Cancer

Breast cancer is most commonly prevalent in woman and rarely in men. According to a recent WHO report there is 13.7% of woman who die due to breast cancer annually, In India the cases of breast cancer were almost six lakhs in last few years and it is rising continuously. The extensive amount of cancer-related death occurs due to tumor cell dissemination and metastasis, which disrupts local and systemic physiology.

Breast cancer risk factors may include obesity, lack of physical activity and excessive alcohol consumption, hormonal replacement therapy, preliminary childhood exposure to ionizing radiation, ageing and family history. Roughly 5-10% of women with breast cancer have genes inherited by parents such as BRCA1 and BRCA2, among others.

Signs and symptoms of breast-cancer consist of breast swelling or thickening of tissues that are different from the tissue surrounding and newly formed, pain in the breast, red skin pitting over the complete area of breast, inflammatory skin on part of the breast, scaling, peeling, and skin flaking on a nipple or breast, sudden unexploded bloody discharge from your nipple additional to breast milk, sudden and change in the structure or size of patient' breast, a lump or bulge in patient's armpit. (www.healthline.com/health/breast-cancer)

5.1 ***Development of Breast Cancer:***

As we know the cells in the human body are continually rising, dividing and replicating and dying after a certain time span. The progenitor cells form new cells. Breast cancer occurs as a result of breast abnormality in cell growth which results in the change in functioning of breast tissue. This abnormality in the breast tissue occurs in the inner lining of milk ducts and lobules. It also occurs in tumor form when breast cell proliferation is uncontrolled. When tumor cells invade near tissues and organs, the tumor is malignant and the cells divide very quickly. Without proper treatment malignant breast cancer becomes irreversible and the patient becomes fatal in early stages.(Faculty, 2010)

5.2 ***Classification of Breast Cancer:***

The two major classes of breast cancer are invasive and non-invasive or in-situ. Invasive breast cancer spreads to surrounding areas of the breast tissue from ducts or glands, whereas a non-invasive breast cancer does not invade the surrounding tissue. Invasive breast cancer is malignant and progresses more often to metastasis. Further different classes are listed below:

- "Ductal carcinoma in-situ (DCIS)": It consists of non-invasive condition in which the cells affected by cancer are confined in milk ducts and this type of cancer later develops into the invasive form of cancer.
- "Lobular carcinoma in-situ (LCIS)": Similar to DCIS, they also belong to the non-invasive condition but the cancer cells remain confined in the milk creating glands of the breast.
- "Invasive (Infiltrating) ductal Carcinoma": It is the most common type of cancer of the breast. This breast cancer category begins in the milk conduct of your breast and then invades the tissue around the breast and spreads further to the lymph node and then eventually the organs and tissue surrounding it.
- "Invasive (Infiltrating) lobular carcinoma": The following form of cancer grows in the lobules and spreads to the surrounding tissue on the other side of the lobules.
- "Inflammatory Breast Cancer": This type of cancer occurs less frequently in 1% to 3% of the breast cancer patient. There is no formation of tumor. It looks red and wet

to the skin of your breast. It can also make the skin look thick and pitched and make the skin look brown. The breast may become bigger, tough, tender or itchy.

- "Paget's disease of the nipple": The breast cancer group begins in the nipples' canals, but as it develops, the skin and areola of the nipple become important. (www.healthline.com/health/breast-cancer)

5.3 ***Pathophysiology of Breast Cancer:***

As compared to other cancers, breast cancer arises due to an association linking a genetically prone host and an environmental cause. Cells of human regularly multiply and reproduce, and ultimately die. They remain confined in the tissue and further attach themselves to surrounding cells. Further the cells lose the ability to divide when they become cancerous and bind themselves to other cells, remain where they belong, and die at particular time. When no longer required, normal cells perform cell suicide. The several protein clusters and routes guard against suicide in the cells. "PI3K / AKT pathway" is one of the defensive pathways while the other is "RAS / MEK / ERK pathway". The genes in these defense pathways are also altered so that they are "on" permanently so that the cell cannot commit suicide when no longer needed. This is one of the measures in conjunction with other mutations that cause cancer. Eventually the P13K pathway is switched "on" position and further the tensin homologue shuts off the following pathway the PTEN protein is mutated and the cancer cells does not perform suicide. Exposure to estrogen has been experimentally linked with mutations that can lead to breast cancer. The G-protein coupled estrogen receptors were related to multiple breast cancer cancers in the female reproductive system. (en.wikipedia.org/wiki/Breast cancer # Pathophysiology)

5.4 ***Diagnosis of Breast cancer:***

In most cases of breast cancer, it can be easily diagnosed by microscopic analysis of a sample or biopsy of the breast area examined by it. There are also classes of breast cancer requiring specialist laboratory tests. The two most commonly used screening methods, a healthcare provider's physical examination of the breasts and mammography, can offer an estimated likelihood of a lump being cancer, and can also determine some other lesions, equivalent to a simple cyst. When these exams are without any result, a health care provider can extract a fluid

sample in the lump for further microscopic analysis. Many biopsy options include a core biopsy or vacuum assisted breast biopsy, which are procedures that remove a part of the breast lump; or an excisional biopsy that removes the whole lump. (www.mayoclinic.org/diseases-conditions/breast-cancer/diagnosis-treatment)

5.5 ***Prevention Of Breast Cancer***

Breast cancer is triggered by various risk factors. Some of the risk factors cannot be avoided because of age and because of gender. Government has initiated many programs for awareness and prevention methods for reducing the onset of breast cancer. Prevention offers the most efficacious plan in cost long-term cancer control plan. WHO's trying hard to raise awareness in women around the world. Disease information, proper diet, and physical activity are given in the local language and free women's dispensaries in poor countries.(Faculty, 2010)

6. HER-2 antibody mediated therapy for Breast Cancer

Overexpression of HER-2 gene is prevalent in about 25-30 % of the patient suffering from breast cancer. HER-2 is a driver of breast cancer development. As “Human epidermal growth factor receptor-2” is upregulated in the “HER- positive breast cancer” subtypes, it has become a biological biomarker for successful treatment of breast cancer. There are three different categories to target HER-2:

- Monoclonal Antibodies such as “transtuzumab”, “pertuzumab”.
- Small molecule of tyrosine kinase inhibitors such as “Lapatinib”.
- Antibody Drug Conjugates such as “TDM-1”

6.1 ***Transtuzumab***

Trastuzumab considered as a monoclonal antibody which is used to cure metastatic breast cancer and stomach cancer, and is sold under the brand name Herceptin. In 1998 Trastuzumab was approved in the United States for medicinal purpose to treat breast cancer. It is on the World Health Organization's list of necessary medicines, the safest and most effective medicines needed in a

health care system. It can be used alone or in conjunction with other chemotherapy drugs, hormone blockers, lapatinib. Trastuzumab is administered gradually inserted into a vein and inserted just below the eye.(Boekhout et al., 2011)

6.1.1 ***Mechanism of Action of Transtuzumab:***

The mechanisms of trastuzumab's operation are not completely understood. Relevant in vitro and in vivo evidence describing multiple action mechanisms indicate multifactorial and complex mode of action. Determining which pathways lead to the therapeutic gain of individual patients is currently not possible. Some of which are following:

- Extracellular domain IV of HER-2 can be isolated for preventing the dimerization of HER-2 and inhibition of HER-2 mediated signal transduction and inhibition of HER-2 mediated signal transduction and then blocks downstream of “P13K/Akt” and “Ras/Raf/MAPK” pathways

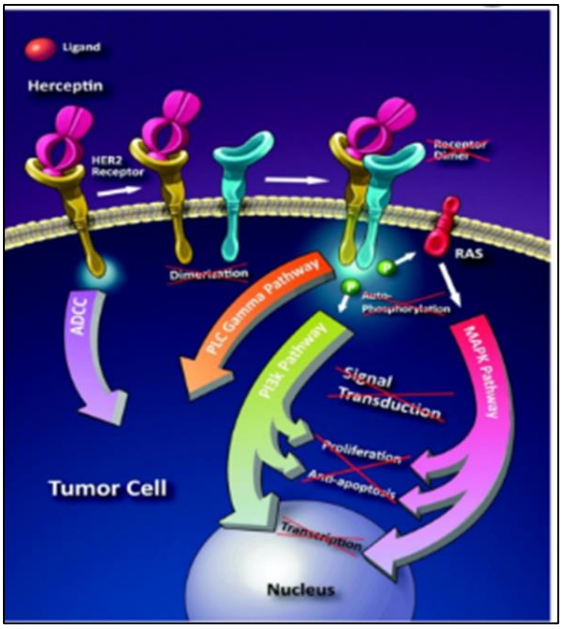


Figure 4: The figure below illustrates the function of transtuzumab which binds to HER-2 receptor and causes inhibition of signal transduction, apoptosis and cell proliferation (Gemmete & Mukherji, 2011)

- The suppression of angiogenesis is another process that appears to be utilized by trastuzumab. Angiogenesis is important for continued tumors growth beyond a few millimeters and for metastasis. Angiogenesis is caused by a variety of growth factors and regulatory molecules, such as the vascular endothelial growth factor (VEGF). HER2 overexpression is strictly linked with increased VEGF expression in tumor cells in humans. In mice overexpression of HER-2 causes breast cancer tumors, trastuzumab therapy resulted in normalization and regression of the vasculature, as well as reduced expression of VEGF which normally act to promote angiogenesis. Additionally, blood vessels in the trastuzumab treated tumours more closely resemble those of a normal phenotype.

- It is also involved in inhibition of P13K/AKT pathway. HER2 overexpression results in the development of excessive HER2 homo- and heterodimers that activate multiple cells signaling pathways, including the 3-kinase (PI3 K) phosphoinositide pathway, partly responsible for cell growth and cell survival. Receptors undergo dimerization and then as a consequence of its phosphorylation of tyrosine kinase domain occurs, that has a site of docking of adaptor proteins and begins cascade signaling. The PI3 K starts in the cell membrane after receptor activation, where messenger proteins, such as pyruvate dehydrogenase kinase 1 (PDK1) and acetamine, are regulated by phosphorylation of “phosphatidylinositol 4,5-bisphosphate (PIP2)” to “phosphatidylinositol (3,4,5)-trisphosphate (PIP3)”. Akt stop the activation and obstruct cell survival, growth and proliferation processes when triggered. The PTEN gene negatively adjusts the pathway of PI3 K / Akt. The non-receptor tyrosine kinase Src is also used to suppress phosphorylated tyrosines and inhibiting the PTEN growth. Researchers have shown that when HER-2 and Src undergo interaction the resulting response is disrupted and Src is inactivated and gradually results in PTEN activation. Increased PTEN activity causes rapid dephosphorylation of the Akt, which inhibits cell proliferation.

- It also plays an important in Antibody Dependent Cell Mediated Cytotoxicity. (Vu & Claret, 2012)

6.1.2 ***Clinical Trials of Transtuzumab:***

The clinical trials were conducted on transtuzumab in three phases. In Phase 1 of clinical trials were conducted primarily to assess the safety and pharmacokinetics of i.v. trastuzumab issued. The doses are administered as single dose or once a month. Gradually a two-phase II clinical trials of fixed-dose trastuzumab was administered in 46 and 39 patients in a single agent or in combination with cisplatin, resulting in overall response levels of 11.6% respectively and 24.3% respectively. When a series of phase II clinical trial were performed, trastuzumab was administered as a single agent on a bodyweight-adjusted basis to 222 patients with metastatic cancer that was HER2-positive who had weaken following one or two previous chemotherapy regimens. When the randomized phase III trials were performed researchers concluded that combination of transtuzumab and anthracyclines caused heart failure and cardiac dysfunction resulting in 27 percent of patients with metastatic breast cancer as in contrast with less than 7 percent of anthracyclines treated patients. Further results showed occurrence of trastuzumab-induced cardiac dysfunctions in adjuvant settings concluded that around one in four women who experience left ventricular systolic dysfunction after trastuzumab administration. For most clinical trials, a higher amount of cardiac toxicity was found for combined anthracyclines and trastuzumab treatment. The design of later clinical trials, which replace combined treatment by trastuzumab and anthracyclines with competitor therapy, were changed in these studies with unexplained high cardiac dysfunction levels.(Shah & Chen, 2010)

6.1.3 ***Resistant Mechanism of Transtuzumab:***

Various resistant mechanisms against trastuzumab were identified in preclinical environment. For early and advanced clinical trials, some of these have been tested as definite variables and others associated with diagnosis. The lack of positive therapeutic reaction or progression of disease after inceptive therapeutic gain may demonstrate resistance to a drug. Mechanisms of trastuzumab resistance evolve before therapy is applied. Some are associated with an inactive target receptor, for instance the docked HER2 receptor that is absent from an extracellular trastuzumab binding domain. Most compounds bind to an inactivated target receptor or PI3K / Akt / mTOR signaling pathway that are modified to the target of the downstream components. Owing to changes in the target signal frequency, the acquired resistance requires an active target receiver. This group includes other TKRs or their ligands. Nevertheless, in both groups certain mechanisms were found. Several mechanisms were not found in each group.

General mechanisms of trastuzumab resistance most extensively researched are:

- Overexpression of HER2 downstream signaling pathways Especially in P13K/AKT pathway, a natural inhibitor called PTEN, the function of which is to restrict cell proliferation and help prevent cancer, PTEN-deficient breast cancer had considerably worse responses than those with typical PTEN to trastuzumab-based care.
- Signaling by alternative pathways. For example, a transmembrane tyrosin kinase receptor linked to developing cell proliferation and metastases is an insulin-like growth factor – the IGF-IR receptor. Higher IGF activity tends to interfere with the effectiveness of Trastuzumab in the lines over-expressing HER2 of breast cancer cells. (Vu & Claret, 2012)

6.2 ***Pertuzumab***

The humanized monoclonal antibody Pertuzumab (Perjeta) was recently approved for HER- positive early breast cancer for adjuvant therapy and neoadjuvant treatment. The humanized, recombinant monoclonal antibody binds to HER-2, domain II of extracellular dimerization. As pertuzumab and transtuzumab are related to different areas of extracellular dimerization of HER-2, pertuzumab and transtuzumab combination therapy is successful in the treatment of metastatic cancer, advanced local cancer and early cancer via a dual blockage of HER-2 receptor.(Hubalek et al., 2012)

6.2.1 ***Mechanism of Action Pertuzumab:***

As studied previously there is a HER2 receptor which is an extracellular receptor, a receptor tyrosine-kinase, which, when activated, can cause abnormal growth through several pathways to stimulate the cell proliferation and development of cell. The amplified ERBB2 gene induces positive breast cancer HER2, which contributes to over-expression of the HER2 in about 15-30 percent of breast cancer tumors. Its unique feature is that it binds to second HER2 and that works as a homodimer and heterodimerize with different HER2, receptor. Like other HER2, this mechanism typically combines another protein for work (a process called dimerization). The HER2/HER3 signaling path is the most efficient dimer. The HER2 receptor binds to HER3, that further prevents HER2 / HER3 dimers from being formed and blocks dimer. This receptor prevents the dimer from

forming. Trastuzumab is another monoclonal HER2 antimicrobial, its receptor being the domain for HER2.(Hubalek et al., 2012)

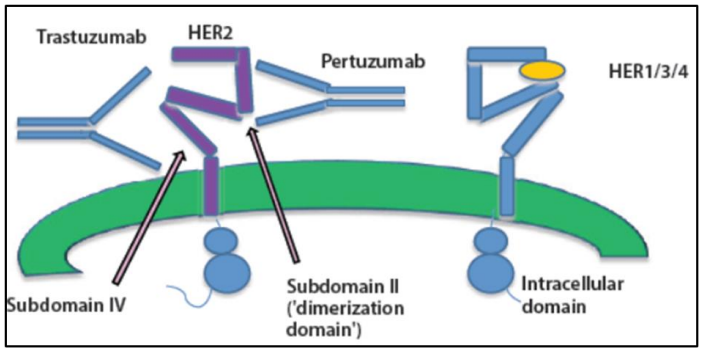


Figure 5: represents that transtuzumab acts on extracellular domain IV of the HER-2 receptor where as pertuzumab acts on extracellular domain II of HER-2 receptor. (www.cancernetwork.com/oncology-journal/pertuzumab-and-its-accelerated-approval-evolving-treatment-paradigms-and-new-challenges-management)

6.2.2 ***Clinical Trials of Pertuzumab:***

When pertuzumab was used alone or in combination to transtuzumab in various preclinical studies it showed synergistic antitumor activity in lung, prostate, ovarian, gastric or breast cancer xenograft models. Pertuzumab has been developed in phase I (with trastuzumab), cytotoxic drugs (docetaxel and capecitabine) and selective therapies (Trastuzumab-DM1, EGFR-inhibitors) and targeted therapies (trastuzumab-DM1, EGFR inhibitors), based on this test.

Gradually further, five phase II trials in random prostate, non-small cell, ovarian and breast cancer patients exhibit moderate pertuzumab single-agent clinical activity. In general, the pertuzumab was well tolerated. The number of adverse conditions is degree 1-2. Diarrhea, rash, asthenia, throat, nausea and abdominal pain were the most repeated symptoms. In a paneling study of 598 patients in 14 trials pertuzumab was found to have a heart defense at least comparable to trastuzumab in combination or with capecitabine, docetaxel, gemcitabine, carboplatin, paclitaxel or trastuzumab. The only weak phosphorylation suppression with a single or double drug medicinal drug therapy occurred in the tumor tissue four days after triple drug therapies started was a sharp reduction of phosphorylation in HER2, EGFR, in HER3, extracellular signal-controlling kinase (ERK). Histopathological studies examined that triple-drug treatment has increased apoptosis after docetaxel-induced arrest at the mitotic phase and mitotic arrest

In the Phase III trial of HER-2 positive breast cancer (the CLEOPATRA study) the three-drug combination arm of (pertuzumab + transtuzumab + docetaxel) demonstrated considerably longer progression less survivors and general survival than the transtuzumab plus docetaxel arm did. We

explored the mechanism of action of three-drug combination therapy in vivo during this study. In this regard we developed a mouse xenograft model based on KPL-4, the HER-2 positive line of human breast cancer in which a triple drug combination therapy induced a regression to tumors similar to double drug.

Indeed, combined triple-drug therapy has induced the mononuclear cells in the tumor cells to invade the cells. These results suggested that the underlying mechanism for effectiveness of the three-drug combination was due, in part, to enhanced HER2-HER3-AKT signaling inhibition promoted docetaxel mediated apoptosis and was also responsible for enhanced docetaxel-induced intratumor infiltration of mononuclear anti-HER2 cells.(Osako et al., 2015)

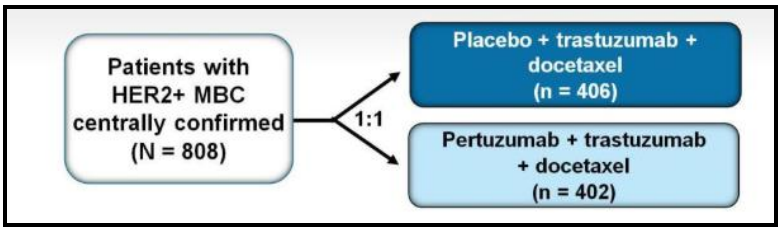


Figure 6 : The above figure shows CLEOPATRA design in which randomization was carried out by geographic region and prior treatment status(nonadjuvant chemotherapy received or not).Study dosing (>= 6 cycles recommended) untill disease progression:
Pertuzumab: loading dose-840 mg and maintainance dose-420 mg; Transtuzumab: loading dose-8 mg / kg and the maintenance dose-6 mg / kg; Docetexal: 75 mg / m to 100 mg / m if tolerated;<6 cycles allow for inacceptable toxicity or worsening of disease;>6 cycles required at the discretion of the investigator. (Herold, 2016)

6.3 ***Antibody Drug Conjugate (TDM-1)***

Trastuzumab emtansine consist of the humanized monoclonal antibody, known as Adotrastuzumab emtansine, marketed under the trade name Kadcyla. This mixture has been combined with a wide range of factor agents consisting of toxins to improve the effectiveness of the antibody guided therapy.

The humanized monoclonal antibody, TDM-1 is covalently coupled with DM1. This led to the development of an antibody medicinal drug ADC Transtuzumab emtansine TDM 1, consisting of a transtuzumab anti-HER 2, which is connected by the thioether linker to a strong maytensine cytototoxic antimicrotubule (DM 1). (Balasubramaniam, 2013)

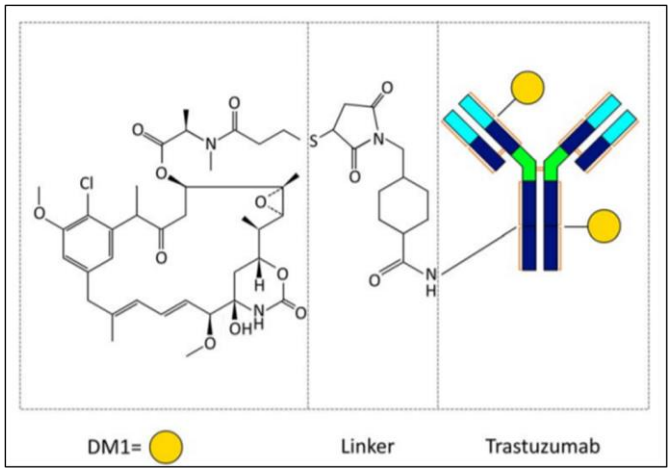


Figure 7: The figure represents Antibody drug conjugate ADC transtuzumab emtansine TDM 1 consisting of Anti-HER 2 antibody transtuzumab, potent antimicrotubule cytotoxic agent Maytensine (DM 1) and Thioether linker. (Conte et al., 2018)

6.3.1 ***Mechanism of action of TDM-1:***

TDM-1 is used to find highly active HER-2 positive tube cells specifically for transtuzumab. The part of ADC transtuzumab binds to the receptor HER-2 and is internalized into the cancer cells. In summation to transtuzumab- triggered anti-tumor activity after cancer cells internalize, transtuzumab and connector degradation by lysosomal degradation and DM 1, triggering cell cycle arrest and programmed cell death by direct inhibition of microtubular assemblage and cancer cell polymerisation. Linker was engineered to avoid transtuzumab detachment of DM 1, which suggests a premature decrease in tumor size.(Barok et al., 2014)

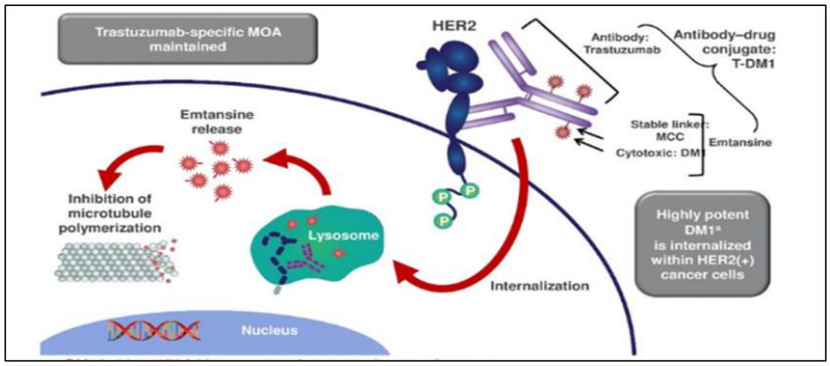


Figure 8: This figure represents the mechanism of TDM-1 as discussed above. (www.slideshare.net/DanaFarber/metastatic-breast-cancer-highlights-by-dr-erica-mayer)

6.3.2 ***Mechanism of Resistance of TDM-1***

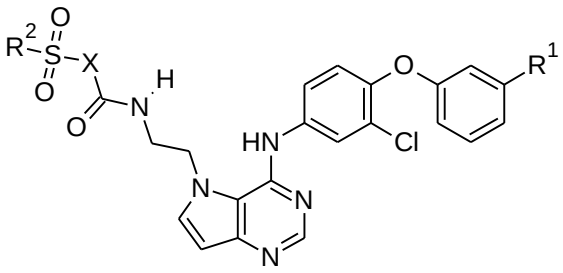
The resistance mechanisms are unclear, but trastuzumab binding mechanisms can be limited to cancer cells. A lack of internalization of the HER2-T-DM1 complex into carcinoma cells or a compromised trastuzumab and intracellular lysosome degradation of HER2, may affect the cytotoxic effect of T-DM1. The impact of T-DM1 can also be undermined by the multi-medicine-resistant proteins which cancer cell pump DM1. In the course of the study we are investigating T-DM1 mechanisms of action, T-DM1 combination with other cytotoxic and anti-HER medicines and T-DM1 combinations with other cytotoxic and anti-HER medicines.(Peddi & Hurvitz, 2014)

7. Small molecule inhibitors of HER-2:

In recent years, researchers have been interested in studying the molecular selective carcinogenic therapies, which have more efficient and safe goals and modes of action than traditional cytotoxic chemotherapy. Ligand stimulation, such as “EGF”, allows erbB receptors to homodimerize or heterodimerize with other erbB receptors and thus to increase the activity of tyrosine kinases. These events activate downstream signal pathways and eventually promote tumor cell growth. HER2 / EGFR inhibitors can inhibit tyrosine kinase phosphorylation and can cause a loss of tumor controlling function by preventing high intracellular signaling pathways in cancer cells.(Schroeder et al., 2014)

7.1 ***Design and synthesis of novel HER-2/ EGFR dual inhibitors consisting of a pyrrolo[3,2-d] pyrimidine scaffold:***

During various experiments on a novel dual inhibitor of HER2/EGFR (TAK-285), Ishikawa T et. Al. discovered, as shown in figure 9, a substitute effective “pyrrolo[3,2-d] pyrimidine” compound (1a) To improve this compound's pharmacokinetic (PK) profile, enhancing A – B permeability and strong cLogD and FISA they performed various changes in its chemical structure to its N-5 side chain and transformed then synthetically altered compounds into salts.



1a: R1 = CF3, R2 = CH3, X = CH2

2a: R1 = Cl, X= C(Me)2

Figure 9: Synthetic strategies to enhance membrane permeability and PK in pyrrolo[3,2-d] pyrimidine scaffold

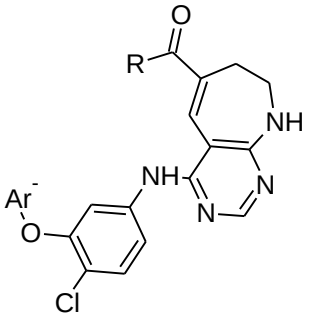
Further physiochemical properties of compound 2a was studies by reacting it with reagent (a)benzenesulfonic acid monohydrate, (b) p-toluenesulfonic acid monohydrate, and the salt form of 2ab. Among these, 2ab, compound 2a tosylate salt, exhibited effective HER2/EGFR kinase inhibitory activity (IC (50): 11/11 nM) and growth of cell inhibitory activity (BT-474 cell GI (50): 56 nM) with strong metabolism of drug and profile PK (DMPK). In addition, 2ab demonstrated that in both mouse and rat xenograft models with transplanted in vivo, major efficacy of the antitumor represented 4-1ST gastric cancer cell lines (mouse, T / C=0 percent, 2ab po 100 mg / kg bid; rat, T / C: -1 percent, 2ab po 25 mg / kg bidding).(Kawakita et al., 2012)

7.2 *Design and synthesis of novel pyrimido[4,5-b] azepine derivatives as HER-2/EGFR dual inhibitors:*

Researches such as Y. Kawakita et. al. discovered new structure consisting of 7,6 fused bicyclic scaffolds, “pyrimido[4,5-b] azepine” that has been engineered to fit into the HER2/EGFR protein binding site for ATP. Intermolecular Claisen-type condensation was used for the synthesis of this scaffold. The results of the optimization resulted in formation of the 4-anilino and 6-functional groups and the 6-substituted amide derivative 1b, which has a 1-benzothiope-4-yloxy group, have already been discovered.

The “pyrimido[4,5-b]” has azepine N-1 & N-3 nitrogen scaffolds have a one-b(1b) X-ray co-crystalline structure and EGFR, respectively, hydrogen-bonding interconnect with the Met793 NH main chain & the Thr854 lateral chain through a hydrogen-mediated bond network. The NH proton in nine positions also allows for a further hydrogen link to the Met793 carbonyl group, as they predicted. Composure 1b has shown solid, pseudo-irreversible (PI) profiles for hemotoxic activity in

HER2 / EGFR kinase (IC50: 24/36 nM) and BT474 cell growth (GI 50: 18 nM).(Kawakita et al., 2013)



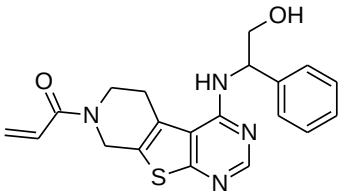
1a: R = -NHCH2CH2OH

1b: R = -NHCH2CH2OH

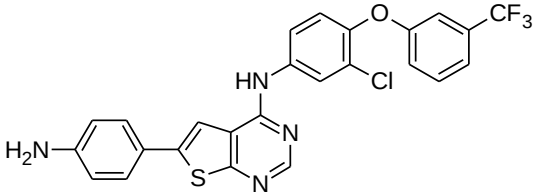
Figure 10: Pyrimido [4,5-b] azepine derivative

7.3 *Design and synthesis of thieno[2,3-d] pyrimidine derivatives as dual EGFR/HER-2 inhibitors:*

Thienopyrimidine scaffold is as fused ring structure that can be constructively represented as adenine, the purine base contained both in RNA and DNA-bioisosteres. Three distinct Thienopyrimidine isomer groups exist. Researchers are encouraged to study the relationship of SAR and is associated synthetic energy through a range of medical applications such as anti-cancer, anti-inflammatory, anti-microbial and CNS protective agents. In this review, the synthetic approaches to thieno[2,3-d] derivatives that are being prepared are briefly summarized.



Compound 1



Compound 2

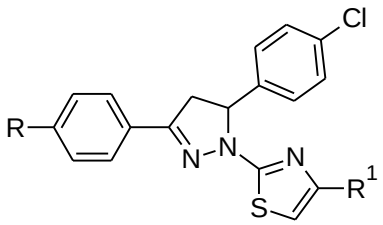
Figure 11: Thieno[2,3-d] pyrimidine derivatives as anti EGFR compounds

The pyrimidine nucleus thieno[2,3-d] is an important pharmacophore found in many anti-carcinogenic drugs, including EGFR and HER2-TKI.Researchers have developed the compound 1 and further synthesized the ATOP competitive inhibitors interacting with the active site of EGFR-TK using a knowledge-based design strategy. Another example is thieno [2,3-d] pyrimidines, of the IC50

value of 13.9 uM versus the NCI-H1975 cell line and anti-EGFR and HER2 TK activity. (Elmetwally et al., 2019)

7.4 Design, synthesis and biological evaluation of anew series of thiazolyl-pyrazolines as dual EGFR and HER-2 inhibitors:

Halil l. Cifti et. al. synthesized and screened new derivatives of thiazolyl-pyrazoline, for its cytotoxic impact on human adenocarcinoma A459, human breast adenocarcinoma MCF-7 and human melanoma cell lines A375, based upon the importance of pyrazoline and thiazole scaffold in the discovery of powerful anticancer molecules. 1-(4-(4-Fluorophenyl)thiazol-2-yl)-3-(4-morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (3c),1-(4-(4-cyanophenyl)thiazol-2-yl)-3-(4-morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (3f) and 1-(4-(4-cyanophenyl)thiazol-2-yl)-3-(4-piperidinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (3q) were found as the most potent anticancer agents against A549 and MCF-7 cell lines compared to erlotinib .



1a: R = Morpholino, R1 = 4 fluorophenyl

1b: R = Morpholino, R1 = 4 cyanophenyl

1c: R = Piperidino, R1 = 4 cyanophenyl

Figure 12: Thiazolyl-pyrazoline derivatives

Compound 1c also showed mild cytotoxicity to cell line A375. These compounds have also been targeted by carcinogenicity against the Jurkat cell line, which does not cause toxicity to peripheral mononuclear blood cells (PBMCs). Compounds 1a, 1b and 1c were tested for 8 various RTKs, consist of EGFR and HER2, compared to erlotinib for their apoptotic impacts on cell lines A549 and MCF-7 and inhibitory potentials so as to illuminate the mechanism of action underlying anti-cancer activity.

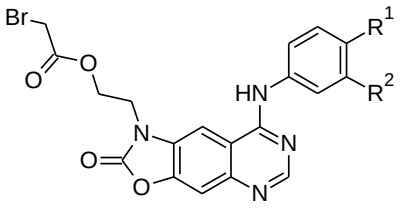
The studies revealed that compounds 1b and 1c induced programmed cell death in these cell lines and displayed substantial inhibitory activity of EGFR with IC50 values of 4.34 ± 0.66 mM and 4.71 ± 0.84 mM as compared to erlotinib (IC50 1⁄4 0.05 ± 0.01 mM) respectively. In addition, 3f also

inhibited HER2 with an IC50 value of 2.28 ± 0.53 mM rendering it a coupled inhibitor of EGFR and HER2. Molecular docking studies carried out in aid with vitro experiments have shown a high affinity with the EGFR and HER2 ATP binding sites in compound 1b.(Sever et al., 2019)

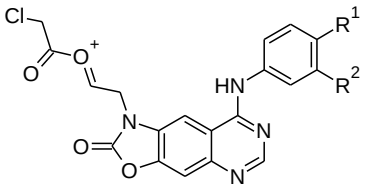
7.5 ***Design and synthesis of novel EGFR/HER2 dual inhibitors bearing an oxazolo[4,5-g] quinazolin-2(1H)-one scaffold***:

Two new series of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives as EGFR/HER2 dual inhibitors introducing two 2-(2-bromoacetyl)ethyl and 2-(2-chloroacetoxy)ethyl group of electrophiles as side-chain at 1-position respectively and evaluated their EGFR and HER2 inhibition activity and toxicity comparing with Lapatinib. All these compounds were evaluated by EGFR and HER2 kinase inhibition and two anti-proliferation assays in vitro.

Most of the designed compounds exhibited moderate to high inhibition activity against EGFR and HER2. The compound shown below exhibition to excellent anti-proliferation activity against human lung adenocarcinoma cell line (A549) and human breast cancer cell line (SK-Br3), and also exhibited the lowest toxicity against human embryonic lung fibroblast cell line (HELF) cell. Finally, compound has significant remarkably higher inhibition efficacy towards tumor growth than Lapatinib in a mouse lewis lung cancer (LLC) xenograft model.



1a: R1 = Cl, R2 = Oxazole



2a: R1 = Cl, R2 = Oxazole

Figure 13: Oxazolo [4,5-g] quinazolin-2(1H) derivatives

Most of the compounds developed were moderately to strongly inhibitory to EGFR and HER2. The high inhibition of EGFR and HER2, in particular, was seen in compounds 1a and 2a as shown in figure 13. Compounds 1a and 2b also demonstrated excellent anti-proliferation activity against human cell lines (A549) and cell lines of human breast cancer (SK-Br3) and 12f also showed the lowest degree of toxicity to human embryonic cell lines (HELF). Compound 2b also had a considerably higher inhibition efficacy in xenograft model of mouse lewis lung cancer (LLC) than lapatinib.(Yin et al., 2016)

8. Conclusion:

Treatment specifically aimed at HER2 in patients with HER2-positive breast cancer has increased survival over the past decade. Resistance, however, remains a challenge particularly in the metastatic stages. Researchers developed several novel EGFR/HER-2 dual inhibitors bearing different scaffolds for improving the treatment of HER-2 positive metastatic breast cancer. All the novel compounds developed showed good inhibition of EGFR and HER-2 and showed good IC50 values.

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