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Review Article

HER-2 GENE, RECEPTORS AND DRUG TARGET: A SYSTEMATIC REVIEW

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ABSTRACT

The discovery and identification of the human epidermal growth factor receptor 2 (HER's-2) genes, have led to a better understanding of breast tumour biology. A member of the epidermal growth factor receptor family, it is a potent mediator of cellular growth and proliferation in malignant epithelial cells. Amplification or over expression of HER-2 occurs in approximately 15–30 % of breast cancers and 10–30 % of gastric/gastroesophageal cancers and serves as a prognostic and predictive biomarker. Treatment with HER-2 targeted monoclonal antibody, trastuzumab dramatically improved outcome in patients with breast malignancy. This promising approach led to the development of pertuzumab, ado-trastuzumab emtansine and lapatinib. The development of drug resistance and disease progression due to alternate signalling remained a real therapeutic challenge. Several research studies are currently focused on inhibition of onco-proteins, kinases and growth factors linked to HER-2 positive breast cancers. The success of immune checkpoint modulators and vaccines in preliminary studies describes the role of tumour immunity. Apart from its role in the pathogenesis of various cancers, HER-2 directed therapeutic approach has brought about a revolutionary change in terms of time to progression and survival rates in breast cancers.

Keywords: ErbB-2 signalling, Targeted approach, Trastuzumab, Drug resistance

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INTRODUCTION

Breast cancer is the most common invasive cancer diagnosed in women. In spite of early treatment and improved therapy, it is the second leading cause of cancer-related deaths in women after lung cancer. Global incidence of the disease is increasing lately owing to better awareness and early detection. India holds the maximum number of women dying of breast cancer. [1] However death rates dropped during the 1980s when hormonal therapy directed against oestrogen and progesterone positive breast cancer was discovered. Research in the last decades has led to a better understanding of the intricate molecular heterogeneity of this malignancy. One such promising approach was the discovery of HER-2 gene and identification of an antibody against it in the late 1990s.

The success of trastuzumab in the treatment of advanced metastatic breast cancer has not only led scientists in developing newer molecules and targets against HER-2, but also has been looked as a therapeutic option in other malignancies too. A detailed explanation of HER-2 gene expression in cancer, receptor signalling and detection methods have been carried out. This review article also best describes the currently available HER-2 directed therapies, newer molecules and strategies to overcome resistance to trastuzumab therapy.

HER-2/NEU gene

HER-2 gene is expressed in 20%-30% of breast cancers. The oncogene has numerous synonyms like HER-2/Neu/ErbB-2 and p185 depending upon its origin from the cell line and similarity to other genes. Amplification and overexpression of HER-2 has been associated with invasion and progression of gastric and other malignancies also. Since HER-2 activity plays a critical role in the maintenance of breast neoplastic proliferation, HER-2 directed therapies have always been an area of interest for treating malignancies since its discovery [2]. HER-1, the first in the tyrosine kinase family to be discovered by Stanley Cohen *et al.* in 1978. The proto-oncogene Neu was first discovered by Weinberg RA *et al.* in 1982. Later renamed as HER-2 because of its structural similarity to HER-1. It was termed as Neu initially since it was first identified in rodent glioblastoma, a neural tumour. The gene was also termed as ErbB-2 by Yamamoto *et al.* in the year 1984 as they had found it in avian erythroblastosis oncogene B (ErbB) which codes for the human epidermal growth factor. A group of scientists in 1986, Ullrich and Coussens, cloned the Neu oncogene, sequenced and mapped it to human chromosome 17. The gene is also known as p185 as it encodes a phosphoprotein of 185,000 Dalton. Its association with human mammary carcinoma in terms of poor prognosis and survival was evidenced by Slamon *et al.* in 1987 [3].

HER-2 dysregulation

Deregulation of HER-2 signalling stimulates cancer cells to develop and spread. These are consequences of two important events. HER-2 gene amplification in cancer cells leads to an excess of the HER-2 protein. HER-2 receptor overexpression on the cell surface is responsible for activation of signalling pathways. The Greater magnitude of membrane receptors also evades endocytic degradation of receptors linking to a pathway of increased potency and signalling [3]. HER-2 protein over expression or gene amplification serves as a guide for chemotherapy choice, antibody therapy, outcome and prognosis in breast carcinoma [4].

HER-2 receptor

Classification: HER-2/neu protein belongs to a four-member family of closely related growth factor receptors. They play a key role in the pathogenesis of several human cancers. The family is composed of four main members: HER-1, HER-2, HER-3 and HER-4, also called ErbB1, ErbB2, ErbB3 and ErbB4 respectively. All four HER receptors are comprised of an extracellular ligand binding site, a lipophilic transmembrane segment and an intracellular domain with tyrosine kinase catalytic activity. HER-1 is activated by epidermal growth factor, transforming growth factor alpha, betacellulin and epiregulin [5].

Heregulins, specific for HER-3 and HER-4 is endogenous ligands falling under a group of proteins called neuregulins which are closely linked to the HER family. They bind and activate receptors responsible for cardiac and neural development during embryogenesis and play an important role in carcinogenesis. [6] However, HER-2 has no known direct activating ligand and may be at an activated state constitutively or become active upon heterodimerization with other family members such as HER-1 and HER-3.

Structure

Understanding the structure of HER-2 enables us to know its involvement in signalling mechanisms and receptor targeted therapies. Each HER-2 receptor is composed of four subdomains. Sub-domain I is in continuous contact with sub-domain III. The permanent interaction between these 2 sub-domains keeps the receptor in an open conformation, exposing sub-domain II. This ensures that sub-domain II (dimerization domain) is always open and ready to dimerize and bind with other receptors in the HER family to initiate downstream signalling. Sub-domain IV stabilizes and locks the receptor in an activated state [5]. In therapies targeting HER-2, there are drugs like pertuzumab and trastuzumab that bind to sub-domain II and IV respectively to interfere with HER-2 signalling [7].

Function

HER receptors exist as monomers on the cell surface. Upon ligand binding to their extracellular domains, HER proteins undergo dimerization, autophosphorylation of tyrosine residues and initiate a variety of signalling pathways. They regulate cell growth, survival and differentiation. Heterodimers containing HER-2 have the high ligand binding capacity and generate more potent signals as HER-2 exists in an open conformation making it the dimerization partner of choice among the family members. HER-2-HER-3 heterodimer is the most potent stimulator of downstream events, particularly the phosphatidylinositol 3-kinase (PI3K)-Akt pathway.

Signaling

Stimulation by growth factors causes phosphorylation of PI3K. Activated PI3K, phosphorylates lipids on the plasma membrane, forming second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). Akt, a serine/threonine kinase resides in the cytosol in an inactive state. With the release of second messenger PIP_3, it is translocated to the plasma membrane where it causes conformational changes and exposure of phosphorylation sites. Cell survival, growth, proliferation, cell migration and angiogenesis are mediated by phosphorylating a range of intracellular proteins like mTOR (mammalian target of rapamycin). Cell survival is potentially mediated through this pathway as there is phosphorylation of pro-apoptotic genes. The PI3K-Akt pathway has many downstream effects and must be clearly regulated. One of the ways the pathway is negatively regulated is by reducing PIP_3 levels. Phosphatase and tensin homolog (PTEN) antagonises PI3K by converting PIP_3 into PIP_2. Loss or mutation of PTEN function common in cancer cells leads to over-activation of Akt. In malignancy, excess PI3K-Akt activity regulates PTEN levels by affecting its transcription and activity. Nuclear factor kappa B (NF-κB) activated by Akt, release tumor necrosis factor α (TNFα) which in turn repress PTEN expression [7]. The RAS/RAF/MAP kinase is another important pathway to be stimulated in HER-2 signalling which is associated predominantly with cell proliferation. RAF kinase activated by RAS phosphorylates and activates a mitogen-activated protein kinase (MAPK). RAF and MAPK are both serine/threonine-selective protein kinases. MAPK governs the activities of several transcription factors like microtubule-associated protein (MAP). A third significant factor in the network is a protein kinase, which is activated by phospholipase C. Protein kinase can activate MAPK pathway bypassing RAS and bring about cell adhesion, migration, proliferation and survival [8, 9].

HER-2 expression in cancer

HER-2 has been expressed in over 15%-30% of invasive breast cancers. It serves as a prognostic determinant as well as a predictor of therapeutic response. It is correlated to tumour size, tumour stage, lymph node involvement and metastasis. [10]Not only HER-2 positivity is associated with brain metastasis, but it is also an important event occurring very early in the pathogenesis of tumour spread. [11]The risk of recurrence with HER-2 positivity is 9.5 times greater and is a predictor of overall survival rate and time to relapse. [8]HER-2 amplification in tumour cells responds to HER-2 directed therapy. It is associated with increased sensitivity to anthracycline group of chemotherapeutic agents like doxorubicin. HER-2 gene is closely situated near topoisomerase gene which is a target for anthracycline group of drugs. It can up regulate them by a mechanism called as receptor enhanced sensitivity. [12]HER-2 positivity is linked to the absence of oestrogen and progesterone receptor causing resistance to hormonal agents. There exists a bi-directional link between HER-2 and hormone receptor pathway playing an important role in a combination of therapy as well as in the development of resistance. (fig. 1) Drugs which interfere with HER-2 signalling can reverse resistance to tamoxifen. On the other hand, oestrogen can inhibit the transcription of HER-2 promoter genes and decreased expression of HER-2 receptors in mammary tissue [13, 14].

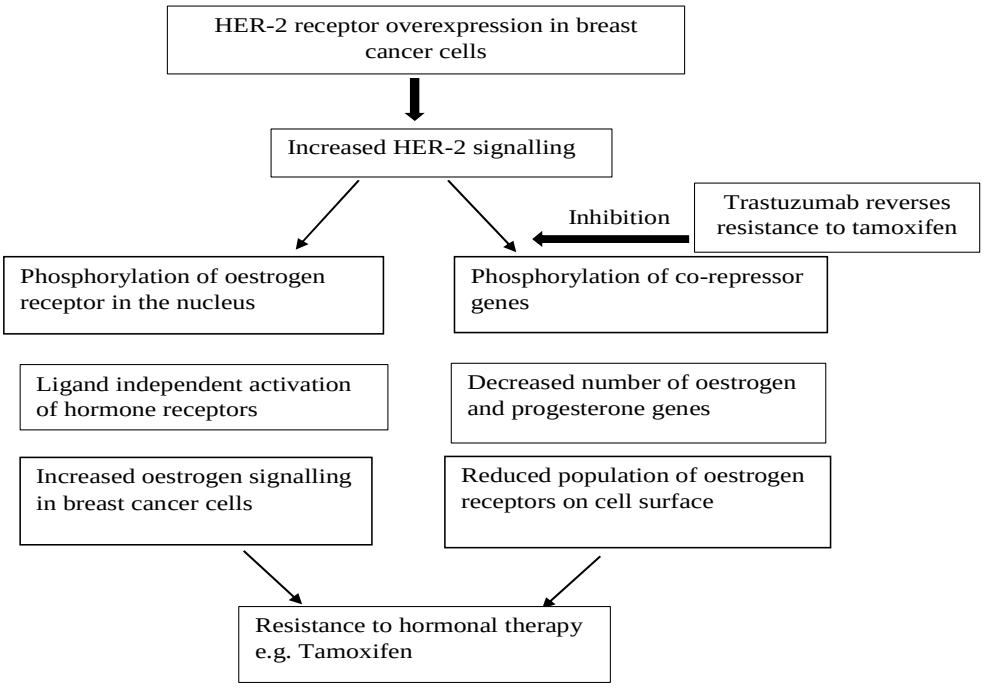

Fig. 1: Bi-directional link between HER-2 and hormone receptor in breast cancer therapy

HER-2 is a key biomarker of gastric cancer expressed in 6% to 33% of cases. HER-2 overexpression is generally found in adenocarcinoma, junctional gastroesophageal tumours and diffuse carcinoma. Its positivity is associated with tumour size, serosal invasion and lymph node metastasis. [15] With increasing understanding of HER-2 science, it has now been acknowledged that HER-2 overexpression occurs in other forms of cancers also such as ovarian, endometrial, colon, bladder, lung, head and neck carcinoma. [8] Apart from its role in the development of diverse cancers, it has also been intensely evaluated as a therapeutic target.

Testing for HER-2

There is a necessity for the accurate determination of HER-2 status for guiding therapy, getting impressive results and to prevent the development of resistance. The available methods are southern blotting, immunohistochemistry (IHC), enzyme immunoassays, polymerase chain reaction (PCR) and fluorescence in situ hybridization technique (FISH). Literature shows that 20% of current HER-2 testing are inaccurate. The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in the year 2007 have set up guidelines for analysis of HER-2 testing. They approved IHC assay and FISH technique for estimation of HER-2 status in order to improve reliability and efficacy and to avoid confusion between test centres. [16] IHC assays determine protein expression. Built on the intensity of membrane staining they can be positive (3+) or negative (0 or 1+) for HER-2.

Cancer specimens with the equivocal (2+) score are submitted for confirmation with the FISH technique. The scoring technique for FISH is based on the number of gene copies and ratio of the receptor (Her-2)-to-centromere probe for chromosome 17 (CEP17). Positive HER-2 amplification are suitable candidates, while if negative they are not considered for HER-2 therapy. Patients with equivocal HER-2 amplification with the FISH technique should undergo further retesting. [17, 18] Gastric cancers are associated with high intra-tumoral heterogeneity and insufficient staining of cancer cells, due to which scoring system used for breast cancer, cannot be considered in this tumour. The national comprehensive cancer network (NCCN) panel has set a separate scoring system based on basolateral membrane staining. [19]

HER-2 targeted therapy

Trastuzumab

Since the identification of HER-2 gene in 1984 and association of HER-2 amplification with human breast carcinoma in 1985, a monoclonal antibody that binds to domain IV of the extracellular segment of the HER-2 receptor was then developed in 1992. The humanized monoclonal antibody, trastuzumab received its approval in 1998 for the treatment of metastatic breast cancer. In 2005, it was demonstrated that trastuzumab reduced the risk of recurrence in early stage cancer. Thus in 2006, it received its approval as an adjuvant therapy regimen with doxorubicin, cyclophosphamide and paclitaxel for women with node-positive, early stage HER-2 expressing breast cancer for a period of one year. Results of meta-analyses have revealed the efficacy of an adjuvant treatment of HER-2 directed therapy in terms of improved disease-free survival rate, decrease the incidence of local and distant metastasis as well as superior in terms of reducing overall mortality. [20, 21] In 2010, it was also approved in combination with cisplatin and a fluoropyrimidine (either capecitabine or 5-fluorouracil) for HER-2 expressing metastatic gastric or gastroesophageal junction adenocarcinoma. The results were based on the TOGA (Trastuzumab for gastric cancer) trial where overall median survival rate was 13.8 mo in the trastuzumab arm compared with 11.1 mo in the chemotherapy alone arm. Improvements were seen in terms of tumor response, time to progression and duration of response to trastuzumab in gastric cancer. [22]

Proposed mechanisms of actions include [23, 24]

- Inhibition of HER-2 shedding, where the extracellular segment is cleaved off and a p95HER-2 truncated active form of the receptor is viewed.
- Antibody-dependent cellular cytotoxicity (ADCC) is an important immune mechanism of tumour lysis owing to infiltration of natural killer cells around the drug.
- Inhibit dimerization of HER-2 receptors with HER-3 or HER-1 and inactivate the pathway.
- Negatively regulates PI3K-AKT pathway by inhibiting phosphorylation of PTEN a tumour suppressor gene.
- Inhibits tumour angiogenesis by decreasing vascular endothelial growth factor.

An initial loading dose of 4 mg/kg, followed 2 mg/kg weekly is regarded as the standard treatment dose for breast cancer. However, a higher dosage is required for gastric cancer. Taken from the experiences of TOGA trial, a 3 w schedule (loading dose of 8 mg/kg followed by 6 mg/kg weekly) or weekly schedule (loading dose of 4 mg/kg, 2 mg/kg weekly) is currently recommended for HER-2 positive gastric/gastroesophageal cancer. Increased drug clearance, advanced tumor stage and pharmacologic resistance are causes of high dose requirement. [25] Apart from the development of resistance, the major problem is heart dysfunction with trastuzumab therapy. Fever, nausea, vomiting, infusion reactions, myalgia and infections are the most common adverse events. However, these adverse events are not of major concern. But the development of cardiotoxicity is the most severe life-threatening adverse reaction of trastuzumab. It can be asymptomatic with low LVEF, abnormal ECG or can present with new onset myocardial infarction, cardiomyopathy and signs of heart failure. It is extremely important to monitor cardiac function before as well as post chemotherapy. It is a possibility that HER-2 protein is essential for cardiac development at the time of embryogenesis, and immune-mediated destruction occurs with trastuzumab therapy. The risk is also increased if underlying cardiac dysfunction pre-exists or when other chemotherapeutic cardiotoxic drugs like doxorubicin, cyclophosphamides are co-administered. [26] Recovery of cardiotoxicity is reversible with withdrawal and timely management. However, 2/3rd of patients do not recover and evaluation of Troponin I predicts recovery from cardiac dysfunction. [27]

Lapatinib

To overcome the resistance with trastuzumab, a new drug targeting HER-1 in addition to HER-2 was identified. This dual tyrosine kinase inhibitor Lapatinib received its approval in 2007 as a combination therapy with capecitabine for HER-2 overexpressing metastatic breast cancer patients, who have already received therapy previously with trastuzumab. Approval was based on the delay in progression of the disease. Unlike trastuzumab, it is orally active, dual acting and binds to the inner segment of the receptor, leading to complete inhibition of downstream signalling pathways [28]. It is administered at a dose of 1250 mg orally for 21 d along with capecitabine. The drug crosses the blood-brain barrier thereby having a promising role in brain metastasis. Lapatinib was also approved in 2010 as combination therapy with letrozole in postmenopausal women. The advantage was observed in terms of greater progression-free survival and overall response rate [29].

Lapatinib is comparatively a safer drug, with the incidence of cardiac toxicity being low. Some widespread adverse effects are diarrhoea, rash, nausea and anaemia. Most of them are mild to moderate. Severe ones include hand-foot syndrome, liver dysfunction and cardiac dysfunction in the form ECG changes or asymptomatic low LVEF [30]. Even though lapatinib offers an advantage in several ways, confusion still exists on switching the therapy to lapatinib or prolonging treatment with trastuzumab. However, p95HER-2 form of trastuzumab and cells with a mutation PTEN gene responds to lapatinib in-vitro and *in-vivo* [31].

Pertuzumab

After the introduction of two HER-2 antagonists, for almost half a decade no drug directing HER-2 came into the market. The discovery of two monoclonal antibodies has brought about a revolutionary change in the treatment of HER-2 positive breast cancer. The new molecule pertuzumab is a humanised monoclonal antibody, binds

subdomain II of the extracellular segment of receptor and inhibits ligand-dependent HER-2 dimerization and signalling. Like trastuzumb it activates ADCC but differs from it in terms of the binding site. Even though their binding site is different, they have a similar and complementary mechanism of action [32]. It was approved in 2012, in combination with trastuzumab and docetaxel in HER-2 positive metastatic breast cancer patients previously not treated with hormone therapy or chemotherapy. The approval was based on the results of Cleopatra trial which showed an increase in progression-free survival rate and fewer side effects [33]. Pertuzumab is given every 3 w at a dose of 840 mg followed by 420 mg as IV infusion for one hour. It can also be administered as a fixed dose of 1050 mg, as pharmacokinetic parameters do not vary much. Common adverse effects include alopecia, diarrhoea, nausea and neutropenia. The drug is comparatively safer with the incidence of asymptomatic left ventricular dysfunction being 0.3%. Sometimes infusion-related reactions and anaphylaxis can be dangerous [34].

Ado-trastuzumab emtansine (T-DM1)

Trastuzumab's role in breast cancer was well established in terms of disease progression and survival. A synergistic combination of a monoclonal antibody with a chemotherapeutic drug emtansine (DM1) was a novel approach to overcome trastuzumab resistance and improved cytotoxic killing of tumour cells. T-DM1 is the first antibody-directed chemotherapy approved for HER-2 positive breast cancer in 2013, as a single agent who has already received trastuzumab and a taxane either separately or in combination. Approval was based on improvement in overall survival rate and fewer side effects seen in EMILIA trial, where ado-trastuzumab was compared to lapatinib plus capecitabine. [35]Emtansine is a product of isolated plant product maytansine. It is a highly potent microtubule inhibitor. Clinical use is restricted by toxicity. However, it is covalently linked to trastuzumab via a hetero bifunctional stable linker which restricts the systemic spread and reduces toxicity. Maximum tolerated dose is 3.6 mg/kg as an infusion every 3 w. Cases of thrombocytopenia, liver dysfunction, were observed in clinical studies. At therapeutic doses fatigue, nausea, musculoskeletal pain and constipation were observed. Keeping the doses at a lower range, grade 3 or 4 peripheral neuropathy can be prevented [36].

Problems of HER-2 therapies

HER-2 directed therapies have made its mark in the field of breast cancer and has brought about a revolution in terms of treatment and outcome. Until recently, issues on HER-2 testing were the principal concern.

However, after the implementation of guidelines, it has become clear that therapy needs to be initiated if tested positive. Accurate testing is necessary to avoid false negative results, where patients will be deprived of HER-2 therapy which might bring out a dramatic change in terms of outcome and survival. At the same time, it is also important to identify false positive cases, where the patients will be subjected to unnecessary medications, cost and toxicity.

It is more than a decade since trastuzumab has been approved and provided excellent results in millions of women suffering from one of the most common cancers-breast malignancy. Fastest research is occurring in the field of HER-2, and more molecules targeting HER-2 pathways are being subjected to *in-vitro* and *in vivo* studies. This is of utmost importance, where the real challenge faced in clinical set up is the development of primary and acquired resistance seen in 66%-88% of cases. Either therapy needs to be changed to another drug targeting HER-2 or combined with other drugs to supplement its action and overcome resistance [37, 38].

Numerous mechanisms have been postulated in preclinical studies [39, 40]. Knowledge of the underlying mechanisms will guide in the identification of novel molecules, new targets and alternate pathway inhibitors for HER-2 in breast cancer.

Table 1: Mechanisms of Trastuzumab resistance in the treatment of HER-2 positive breast cancers

Mechanisms	Alterations	Consequence
Interference of antigen-antibody interaction	Proteolytic cleavage of extracellular domain of HER-2	Mutated active truncated p95HER-2 isoform
Epitome masking	Expression of glycopeptide Mucin-4 and CD44, a transmembrane receptor for hyaluronan	Masking of antigen binding site on HER-2 receptor
Alternate HER family pathway signalling	Overexpression of HER-1, HER-3 and HER-4	Overcome trastuzumab-mediated inhibition of HER-2 signalling
Compensatory signalling of tyrosine kinase family	Expression of Insulin-like growth factor-1R, tyrosine kinase *c-Met* gene	Synergism with HER-2 and stimulate PI3K/AKT pathway
Intracellular alteration	Phosphatase and tensin homolog deficiency/mutation	Active PI3K/AKT pathway, by loss inhibiting hydrolysis of PIP3
Activating mutations	PIK3CA mutation	Inherent and acquired resistance to trastuzumab

Overcome resistance

Tyrosine kinase inhibitors

Introduction of drugs lapatinib and pertuzumab was to overcome resistance with trastuzumab. The involvement of other receptors in the HER family in addition to HER2 signalling led to the discovery of these molecules. Lapatinib also is HER-1 family inhibitor along with HER-2, whereas pertuzumab inhibits dimerization with HER-3 receptor by binding to the dimerization domain, thereby controlling escape pathways of activation. Based on the same principle, two different tyrosine kinase inhibitors are being studied in phase 3 trials, showing promising results in terms of response and survival [41].

Neratinib, like lapatinib, is an oral tyrosine kinase inhibitor directing towards EGFR/HER-1 and HER-2. However, covalent binding to the receptor makes it irreversible. It is under evaluation for locally advanced and metastatic breast cancer. The benefit was seen in terms of improvement in progression-free survival rate in naive trastuzumab patients and previously treated patients. The incidence of cardiotoxicity is less. Maximum tolerated dose is 340 mg with severe diarrhoea being the dose-limiting toxicity requiring discontinuation or conservative treatment. Afatinib is another orally active irreversible inhibitor like neratinib, inhibiting EGFR/HER-1, HER-2 and HER-4 family of tyrosine kinases. It is also being studied in phase 3 trials for advanced breast cancer along with vinorelbine in comparison to trastuzumab [42, 43].

PI3K/Akt/mTOR pathway inhibitors

In breast cancer, until now the monoclonal antibodies and tyrosine kinase inhibitors are approved and those under phase 3 trials target the HER receptor per se, giving a chance for alternating signals and oncogene to stimulate PI3K/Akt/mTOR pathway. To overcome resistance, molecules are developed so as to target directly the pathway without involvement of receptor. Several PI3K/Akt inhibitors to target PI3K/Akt mutations are in preclinical studies [44]. The most promising drug is everolimus, a mTOR inhibitor being investigated in a phase 3 trial along with trastuzumab and vinorelbine for breast cancer patients refractory to trastuzumab. Everolimus arm had greater time to progression of disease [45].

Heat shock protein 90 (HSP90) inhibitors

A novel therapeutic approach is degradation of HER-2 receptors by using HSP90 inhibitors. Overexpression of HSP90 is associated with

treatment resistance and worse prognosis in breast malignancy. Heat shock proteins are a group of molecular chaperones involved in evolution, stability and folding of oncoproteins. At the time of stress, malignant cells depend on HSP for signal transduction, cell cycle regulation and apoptosis. [46] Inhibitors of HSP like tanespimycin, ganetespib as monotherapy or combination therapy have shown modest clinical benefit in phase 2 studies. Hepatotoxicity was the dose-limiting toxicity with these agents. Ganetespib is more potent with fewer side effects [47].

Vascular endothelial growth factor (VEGF) inhibitors

Anti-angiogenic drugs have always had a crucial role to play in any cancer. Growth factors of neovascularization associate with HER-2 in the pathogenesis, survival and spread of tumour cells. [48] Anti-VEGF antibody-like bevacizumab, are being evaluated in phase 3 studies as add-on therapy for breast cancer. Bevacizumab arm has shown improvement in progression-free survival rate. Cardiotoxicity in terms of hypertension and heart failure is a major limiting factor. Sunitinib an orally effective tyrosine kinase inhibitor against VEGF was also tried in HER-2 positive cancers. Numerous phase 3 studies showed only increased cases of dermatological toxicity and poorer therapeutic outcome [49].

Insulin-like growth factor-1 (IGF-1) inhibitors

IGF-1 and HER-2 are always a linked pathway in PI3K/AKT signalling in breast cancer, especially where IGF-1 is associated with the mechanism of resistance. IGF-1 can cause phosphorylation of HER-2 in malignancy even when an anti-HER-2 drug like trastuzumab is administered. Inhibition of IGF-1 signalling along with HER-2 inhibition in combination therapy can overcome resistance to trastuzumab [50]. As per the results of pre-clinical studies, Cixutumumab, an IGF-1 inhibitor has been considered for clinical trials in breast malignancy [51].

Immune checkpoint modulators

Modulating the negative regulators of the immune system by developing antibodies directed towards them have shown a promising approach in the field of cancer therapy. Programmed cell death protein 1 (PD 1) and Cytotoxic T-lymphocyte-associated antigen 4 (CTLA 4) are immune checkpoint proteins, down regulating the immune system. PD 1 suppresses autoimmunity and activation of T cells in the periphery. CTLA 4 causes downregulation of helper T cells and up regulates immuno-suppressive regulatory T cell. The immune system plays a major role in the degradation of tumour cells by activation of T cells. The similar mechanism underlies the action of trastuzumab by induction antibody dependent cytotoxicity. It was noted that higher the infiltration of lymphocytes around the malignant cells, better the tumour responded to trastuzumab in in-vitro studies [52]. By targeting antibodies against these immune checkpoint modulators, the immune response against tumour cells can be upgraded and finally assist in the treatment of breast malignancy. Anti-CTLA4 antibody (ipilimumab) and anti-PD1 antibodies are in various stages of a clinical trial for breast cancers [53].

HER-2 vaccines

Induction of humoral and cellular response against cancer cells through antibody-directed HER-2 therapy has already proven its efficacy and results in breast malignancy. The same concept was applied in the development of HER-2 vaccines to generate anti-HER-2 immune responses against HER-2 positive breast tumours [54]. Immune responses are induced in patients utilizing HER-2 antigen protein or DNA. E75 a peptide-based vaccine was investigated in HER-2 positive breast cancer, which is the extracellular portion of the receptor. Results revealed 2 y survival rates of 86% in comparison to just 60% in those without vaccination. HLA restriction and immunological tolerance were the limitations. Whole tumour cell vaccines developed can be autologous or allogenic. Vector-mediated dendritic cell vaccines are also available. Poor efficacy and autoimmune reactions were few limitations [55].

CONCLUSION

HER-2 gene has been expressed in 15-30% of breast cancer, serving as an important biomarker, in terms of therapy and outcome. HER-2 directed therapy in patients with gastro-esophageal cancers has provided remarkable results in terms of survival. Although HER-2 expression was also found in other malignancies, therapy with HER-2 antagonists has failed to provide substantial results, where further research is needed. HER-2 testing is routinely recommended in breast and gastric malignancies. Monoclonal antibodies, tyrosine kinase inhibitors and drug conjugates have been developed and approved for HER-2 positive malignancies. The first monoclonal antibody introduced was trastuzumab against early and metastatic breast cancer, followed by lapatinib for patients non-responsive to trastuzumb. Two drug entities pertuzumb and T-DM1, are examples of antibody and cytotoxic therapy to overcome resistance with trastuzumab. One of the real challenges with HER-2 therapy is the development of resistance. Newer tyrosine kinase inhibitors target additional HER family and mTOR inhibitors interfering the pathway has shown promising results. Stimulation of the immune system to attack cancer cells via vaccine or immune checkpoint modulators can also be a promising approach. This review highlights the newer molecules and targets against HER-2 signalling developed and studied for overcoming resistance. The basic concept of introducing new targets is based on recognising the underlying mechanisms of resistance. The exact role of newer drugs like HSP-90 inhibitors, IGF-1 inhibitors and VEGF inhibitors in the field of breast cancer cannot be commented in view of monotherapy; however they can be considered as an option for combination with trastuzumab for breast cancer chemotherapy in the near future.

CONFLICT OF INTERESTS

Declare none

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