REVIEW

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# Advances in Molecular Diagnostics and Targeted Therapeutic Approaches in *HER-2* Positive Breast Cancer

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## Abstract

Human epidermal growth factor receptor 2 (*HER2*) is linked with aggressive tumors in breast carcinoma patients and results in poor outcomes. *HER2* signaling in breast cancer also interacts with phosphoinositide-3-kinase (PI3K)/ Akt signaling route, mitogen-activated protein kinase (MAPK) pathways, and protein kinase C (PKC) activation. Hence, the diagnosis of *HER2*-positive status and targeting of *HER2* in breast cancers is of vital importance as it forms the basis for trastuzumab therapy. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the conventional methods that have been used for the diagnosis of *HER2* status in breast cancer individuals. However, with the advancements in molecular diagnosis techniques like quantitative real-time reverse transcription-PCR (RT-qPCR) have been established with a series of evaluations to complement the diagnosis made through the conventional methods. The advances in molecular diagnosis have paved the way for precision medicine and targeted therapy in *HER2*-positive breast carcinoma. Based on the *HER2* status, the breast cancer patients may be administered with antibodies, antibodies drug conjugates (ADC) or tyrosine kinase inhibitors. Since a significant role of the immune microenvironment has been established, monoclonal antibodies and immunotherapy options are also being evaluated for the management of breast carcinoma. Various clinical trials have highlighted the role of targeted therapy in breast cancer individuals. We present a review on the advances in molecular diagnosis of *HER2* status in breast cancer individuals along with the role of targeted therapy in the management of *HER2*-positive breast carcinoma.

**Keywords:** *HER2* (or *ErbB2*)- Breast cancer- Molecular diagnosis- Precision medicine

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## Introduction

Human epidermal growth factor receptor 2 (*HER2*) also known as ErbB2 is related to aggressive breast tumors and poor outcomes. There were ~276K cases of invasive breast carcinoma that were predicted to be diagnosed in 2020 in the United States alone. Among the overall breast carcinoma cases, 15-20% belong to the category of *HER2* positive [1, 2]. The *HER2*-positive subtype is linked to aggressive clinical phenotype with less survival outcomes and high rates of recurrences [2]. In 2006, it was shown by landmark trials that the administration of trastuzumab and chemotherapy was significantly related to an elevation in progression-free survival and overall survival in both late and early stages of the *HER2*-positive type of breast carcinoma [3]. Since then, the standard of care has been tagged to therapy including trastuzumab. However, despite so many advances in the field, there are still 16-22% of cases in the early phases of breast carcinoma that exhibit relapse [4, 5], and resistance is observed in 22-25% of cases with metastatic forms of *HER2*-positive breast carcinoma [6,7]. Hence, a significant scientific effort is being directed towards overcoming the resistance acquired against the *HER2*-based therapies mainly to improve the outcome in the clinics through targeted therapies. Further, the heterogeneity of breast cancer demands precision medicine in the present era to tailor management of *HER2*-positive breast cancer by including molecular and biological properties of the tumor. The inclusion of genetic and biological factors underlying pathophysiology of breast cancer in the diagnosis of *HER2*-positive breast cancer can aid in moving towards the precision medicine and away from a one-size-fits-all approach.

The amplification of *HER2* in breast cancer patients is of particular concern. This subtype of patients has overexpression of the *HER2* gene or ErbB2 which is

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responsible for the increase in the growth of tumors aggressively and a reduction in clinical outcomes. The protein is associated with the receptor tyrosine kinase family. HER1, HER3 and HER4 are the other proteins belonging to the same family and are referred to as ErbB1, ErbB3, and ErbB4, respectively [8]. There is an extracellular domain in HER receptors that binds to the ligands and contains a cytoplasmic domain, alpha-helical transmembrane domain and 2 cysteine-rich domains [9].

A particular ligand interacts to the HER receptor at the location known as the ligand binding domain, triggering the HER receptors to heterodimerize or homodimerize. After the dimerization of receptor, some residues of tyrosine in the intra-cellular tyrosine kinase domain are auto phosphorylated and trans-phosphorylated. Some of the residues of tyrosine may be auto phosphorylated and may impact the acquisition of adaptor proteins and the subsequent modulation of signaling pathways, contingent upon the ligand and the production of HER dimer. The phosphoinositide-3-kinase (PI3K)/Akt signaling route [10], protein kinase C (PKC) activation [11], and mitogen-activated protein kinase (MAPK) pathway [12,13] effect HER signaling. These play a role in differentiation, cell death, migration, proliferation, and adhesion (Figure 1). The ligand that is involved, the dimer that is created, in addition to the cellular environment, all influence the biological response. Figure 1 shows the *HER2* signaling pathway and *HER2* receptors' recognized ligands. Since *HER2* and HER3 do not have intrinsic tyrosine kinase activity and have no known ligands, respectively, they are favored dimerization associates [14]. The endocytosis of receptors by ligands reduces signaling via HER receptors.

*HER2* is observed to be overexpressed in 20-25% of the cases of breast carcinoma across the world [15]. Investigations have highlighted *HER2* as key target for therapy in the management of breast cancers. Thus, it is important to identify the *HER2* positive breast cancer subtype through molecular analysis for better appreciating the role of *HER2* in breast cancer and its management. In the last decade, techniques like sequencing and microarray have provided better insights into the biology of invasive breast cancers and this has led to development of novel molecular assays for the identification of breast cancer subtypes. These include cost-effective and time saving tools such as RT-qPCR. The detailed explanation of the advances in the molecular diagnosis highlighting tools like RT-qPCR techniques are discussed in the article.

The heterogeneous nature of breast cancer has led to its classification through IHC into various subtypes depending on different surrogate markers. However, there is a need for better understanding of the molecular mechanisms for the development of advanced molecular tools to aid in screening of breast cancer patients [16]. This will help breast cancer patients to have access to precision medicine across various breast cancer subtypes.

The review is aimed at collating information on advanced molecular diagnostics for breast cancers and the targeted therapy that is currently being used for the treatment of *HER2* breast cancer patients.

### *Advancement in Molecular Diagnosis of HER2 Positive breast cancer*

The appropriate diagnosis of *HER2* status in breast cancer individuals is vital from the management point of view since trastuzumab is administered only to individuals with overexpression or amplification of *HER2*. Furthermore, it is important to have highly sensitive diagnostic evaluations as false positive results can lead to ineffective and expensive treatments which may cause side effects that may influence the survival rates [17, 18]. On the other hand, false negative results can deprive the individual of an ideal therapeutic option.

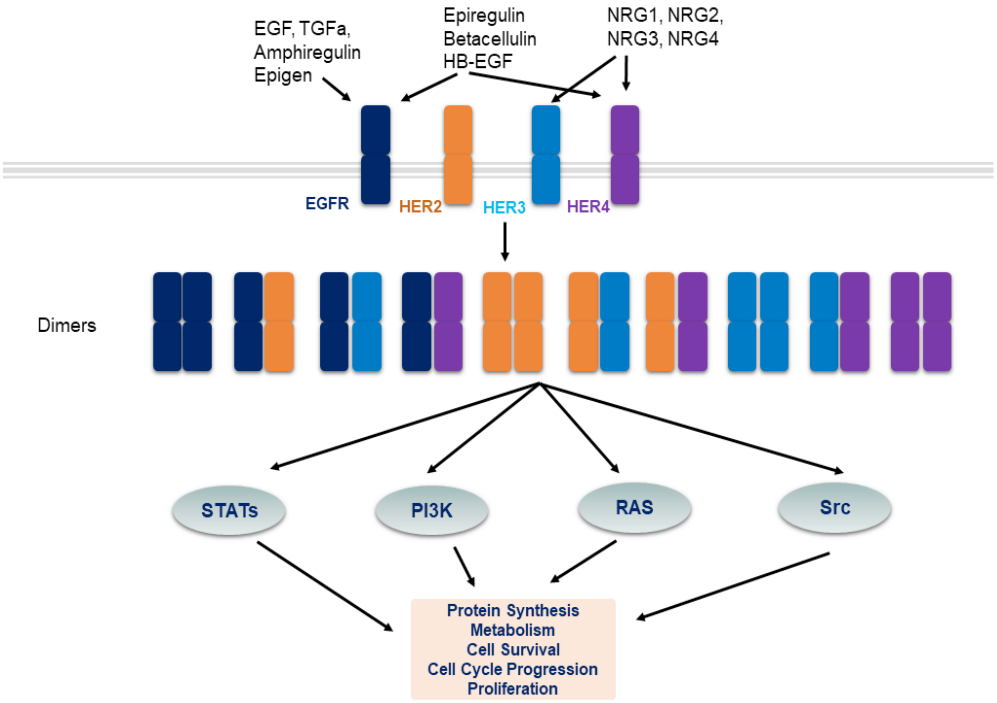


Figure 1. HER Receptor Activation, Downstream Signaling Cascades and Functions

*Present diagnosis strategies for HER2 status in breast cancer*

The amplification of the *HER2* gene is associated with an increase in the expression of mRNA and protein levels. The alterations in *HER2* can be assessed at the level of DNA, RNA, or protein. US Food and Drug Administration have approved the use of immunohistochemistry and fluorescent in situ hybridization for diagnosis the *HER2* status in breast cancer individuals based on the data available from the previous evaluations on the outcome and treatment response to trastuzumab [19]. In case of immunohistochemistry, an antibody is used to assess the protein expression of *HER2* whereas in case of fluorescent in situ hybridization DNA probe is used to determine the copies of *HER2* through a fluorescent detection system [15]. Other methods for the detection of *HER2* status may include enzyme-linked immunosorbent assay, microarray, sequencing, RT-qPCR, and *HER2* testing of circulating tumor cells (Table 1).

*Challenges in diagnosis of HER2 status*

The above-mentioned advanced molecular methods are widely used in various clinical setups across the globe to detect DNA, RNA, or protein levels of *HER2* in breast cancer individuals. Gene expression quantification using RT-PCR is a prominent method for *HER2* detection and can serve as a valuable tool to analyze clinical samples retrospectively. However, there are certain challenges in performing the RT-qPCR which are due to the degradation of RNA from FFPE tissue and choice of endogenous control gene that can significantly affect the results of the technique. Various studies have evaluated the potential for RT-qPCR based analysis for the molecular subtyping of breast cancer as well as detecting the overexpression of *HER2*.

*Advancement in molecular diagnosis*

Park et al. [25] assessed the potential of RT-qPCR as a complementary assay to the available immunohistochemistry and fluorescence in situ hybridization techniques that are time-consuming and require high cost, sensitivity, and accuracy. The tissue samples from breast cancer individuals collected over a period of two years were evaluated. The comparison of the RT-qPCR assay with immunohistochemistry and fluorescence in situ hybridization highlighted specificity and sensitivity of 89.8% and 93%, respectively. Park et al. [25] indicated that mRNA quantification of *HER2* gene may serve as an alternative assay for conventional detection of *HER2* status through immunohistochemistry and fluorescence in situ hybridization.

Wang et al. [26] evaluated a nested RT-qPCR method for the assessment of overexpression of *HER2* mRNA in clinical samples. The patient samples were previously identified through immunohistochemistry or fluorescence in situ hybridization techniques. 92% was the true positive and 2% was the false positive rates observed in the investigation. Further, concordance results highlighted 92.1% and 87.2% overlap between RT-qPCR and fluorescence in situ hybridization and IHC, respectively. The method was proposed to be reliable for *HER2* detection to better utilise the administration of trastuzumab in *HER2* positive breast cancer individuals.

Liable et al., 2016 performed a series of analysis for the evaluation of MammaTyper ® which is an in vitro diagnostic assay for the assessment of mRNA in breast cancer subtypes using RT-qPCR method to address the discrepancy in detection of breast cancer subtypes through immunohistochemistry [27]. The assay involves the detection of *MKI67, PGR, ESR1* and *HER2* expression. In this comprehensive analysis, the assay was found to be linear till 33.5 quantification cycles with minimal variations among two different platforms. There was more than 94% concordance for single marker detection in both platforms. The method did not vary with the change in the isolation procedure of RNA. Liable et al., 2016 were able to validate the MammaTyper® assay through analytical perturbations and highlighted minimal variations in the assessment of the markers. It was highlighted that the MammaTyper® may serve as an advancement in breast cancer assessment for subtyping of breast cancer samples based on molecular analysis.

EL Hadi et al. [28] evaluated the potential of 7 genes that may be utilised as references for the normalization of mRNA from the *HER2* gene in FFPE tissues from breast cancer individuals. EL Hadi et al. [28] in their investigation, further performed a concordance analysis using the between RT-qPCR and immunohistochemistry and fluorescent in situ hybridization. In their analysis, ELHadi et al. [28] evaluated the expression *HER2* in breast cancer cell lines. Further, they evaluated 7 different genes for their potential as reference controls in RT-qPCR reaction. ELHadi et al. [28] stated that RT-qPCR has the potential to be utilised as a valid complementary test to orient the treatment and management of breast cancer.

Wang et al. 2017 performed an investigation to analyse

Table 1. Diagnosis of *HER2*-Positive Breast Barcinoma

| Method | Specimen | Available kits | Target molecule | References |
|---|---|---|---|---|
| Immunohistochemistry | Tissue | Ventana PathwayTM and Dako HercepTestTM | *HER2* protein | [20] |
| Fluorescence In Situ Hybridization | Tissue | PHarmDX, INFORM, and PathVysion™ | *HER2* Gene | [21] |
| Microarray | Tissue | TargetPrin, Mammaprint, and OncotypeDX | *HER2* mRNA | [22] |
| Enzyme-Linked Immunosorbent Assay | Tissue or serum | - | Extracellular domain of *HER2* protein | [23] |
| Testing of circulating tumour | Serum | - | *HER2* mRNA | [24] |

the expression of *HER2* gene in FFPE tissue samples using RT-qPCR technique. They utilized 3 different endogenous control genes to perform relative analysis [29]. The results obtained from RT-qPCR was 96.7% sensitive in comparison to immunochemistry and 92.4% sensitive in comparison to fluorescent in situ hybridization. Wang et al. 2017 further highlighted signification association of overexpression of *HER2* with molecular subtype, PR status, ER status, histological type and TNM stage. It was concluded that RT-qPCR may serve as a complementary tool for screening and identifying overexpression of *HER2* mRNA.

Zoppoli et al. [30] compared immunohistochemistry, fluorescence in situ hybridization, qPCR and RT-qPCR for determination of *HER2* status in breast cancer individuals. They further performed western blot to directly measure the *HER2* protein levels in cases with discordant results. 94.1% was the overall agreement between fluorescence in situ hybridization and qPCR. 90.81% was the overall agreement between RT-qPCR and fluorescence in situ hybridization. It was interesting to note that the analysis of *HER2* proteins was correlating with RT-qPCR more than fluorescence in situ hybridization [30]. Hence, RT-qPCR was shown to outperform fluorescence in situ hybridization in individuals with *HER2* protein overexpression.

Lin et al., 2022 investigated the reproducibility of immunohistochemistry in *HER2* low breast cancer patients and found that the *HER2* mRNA levels were high in cancer patients that were identified from immunohistochemistry as *HER2* low.

*Advancement in HER-2 positive BC Therapy*

*Precision or personalized medicine*

Breast cancer represents a diversified illness with many molecular subtypes that have unique clinical manifestations. Six intrinsic sub-types were included in the first molecular subtype categorization of breast cancer proposed by Perou et al. in 2000, which aided in the formulation of efficient treatment plans [31]. Despite unfavorable outcomes, which are partly attributable to imprecise prevailing beliefs and technological limitations, precision medicine is nevertheless a crucial tool for minimizing the side effects of chemotherapy and maximizing the benefits to patients. The precision-medicine approach can find useful biomarkers that forecast how well a given patient population will respond to a tailored therapy [32]. The use of targeted therapy has drawn a great deal of attention from cancer researchers during the past ten years. Endocrine therapy is one of the breast cancer treatments that concentrates on estrogen and progesterone receptors in cases of hormone-receptor-positive breast cancer. Both of the aforementioned medicines are commonly utilized to target hormone receptors that are present on breast cancer cells, and they have transformed the treatment outcomes for patients with breast cancer. The identification of *HER2* overexpression has sparked the creation of numerous *HER2*-targeted drugs as precision therapy for breast cancer advances [33].

*Targeted therapy*

When compared with other forms of breast cancer, *HER-2* positive subtype is indicative of a more aggressive phenotype, which has a poor prognosis leading to a shorter overall and disease-free survival time. *HER2* is not only a significant biomarker, but it is also a therapeutic target that has been thoroughly researched. Patients diagnosed with *HER2*-positive breast cancer have seen a significant increase in their overall survival rate due to treatment directly targeting at *HER2*. The following section elaborates on the targeted-treatments that are presently available for patients with *HER2*-positive breast cancer. These medications primarily include monoclonal antibodies and Tyrosine Kinase inhibitors (TKIs), both of which have significantly improved the natural course of *HER2*-positive breast cancer.

*Monoclonal antibodies*

The typical first-line treatment for individuals with advanced stage breast carcinoma that tests positive for *HER2* should consist of an amalgam of a single-agent chemotherapeutic, pertuzumab and trastuzumab [34, 35] (Table 2). Pertuzumab, a type of monoclonal antibody that interacts with *HER2* extracellular domain II, stops *HER2* from dimerizing with various other receptors, this lead to a stronger signaling inhibition in combination with trastuzumab (Figure 2) [36, 37].

Nearly 50% of the cases in breast carcinoma with *HER2*-positive status express hormone receptors [38]. On the contrary, the hormone receptor-positive cases with *HER2* positivity are related to endocrine resistance [39]. There exists a complex interact between the pathways and aiming *HER2* was observed to restore sensitivity towards hormones [40, 41]. Initial trials namely, eLEcTRA and TANDEM were able to address this notion and provided evidence that trastuzumab monotherapy administration along with aromatase inhibitor in *HER2*-positive individuals and hormone receptor-positive cases led to progression-free survival [42, 43]

In case of a majority of individuals who progress towards chemotherapy based on pertuzumab or trastuzumab in the first line [34, 35], consideration should be given to monotherapy of T-DM1 in the second line of management (Figure 2) [44]. This drug has a cytotoxic activity and is basically an antibody-drug conjugate that delivers the drug specifically on the cells which overexpress *HER2* [45]. It has been shown that T-DM1 substantially advances the progression-free survival and overall survival in individuals with fewer adverse events [45].

*Tyrosine kinase inhibitor*

Tucatinib is a selective tyrosine kinase inhibitor for *HER2* that may have an impact on the toxicity profile of the patients [52]. The individuals with brain metastasis undergoing tucatinib therapy had 7.8 months of progression-free survival [50]. The guidelines from the FDA state that metastatic individuals who have undergone more than one anti-*HER2* therapy can be put on tucatinib therapy. DS-8201a is another drug that is conjugated to an antibody for delivery of the drug directly on *HER2*-positive overexpressing cells and acts as topoisomerase

Table 2. Various Drugs Used for Therapy in *HER2*-Positive Breast Carcinoma

| Therapeutic Drug | Clinical Trial | Arms | Treatment line | Increment in progression-free survival | Increment in overall survival | References |
|---|---|---|---|---|---|---|
| | | Antibodies | | | | |
| Trastuzumab | H0468g | Arm A: AC or paclitaxel + trastuzumab<br>Arm B: AC or paclitaxel | 1 | 2.8 | 4.8 | [46] |
| Pertuzumab | CLEOPATRA | Arm A: docetaxel + trastuzumab + pertuzumab<br>Arm B: docetaxel + trastuzumab + placebo | 1 | 66.3 | 16.3 | [47] |
| | | Tyrosine kinase inhibitors | | | | |
| Tucatinib | HER2CLIMB | Arm A: capecitabine + trastuzumab + tucatinib<br>Arm B: capecitabine + trastuzumab + placebo | 3 | 2.2 | 4.5 | [48] |
| Neratinib | NALA | Arm A: capecitabine + neratinib<br>Arm B: capecitabine + lapatinib | 3 | 2.2 | NS | [49] |
| Lapatinib | EGF100151 | Arm A: capecitabine + lapatinib<br>Arm B: capecitabine | 2 | 1.9 | NS | [50] |
| | | Antibody-drug conjugates | | | | |
| Trastuzumab deruxtecan | EMILIA | Single arm | >2 | NA | NA | [45]. |
| T-DM1 | DESTINY-Breast01 | Arm A: T-DM1<br>Arm B: capecitabine + lapatinib | 2 | 3.2 | 5.8 | [51] |

I inhibitor [53].

Neratinib, an oral inhibitor of tyrosine kinase, suppresses HER1, *HER2*, and HER4 in an irreversible manner [54]. Neratinib combined with capecitabine were

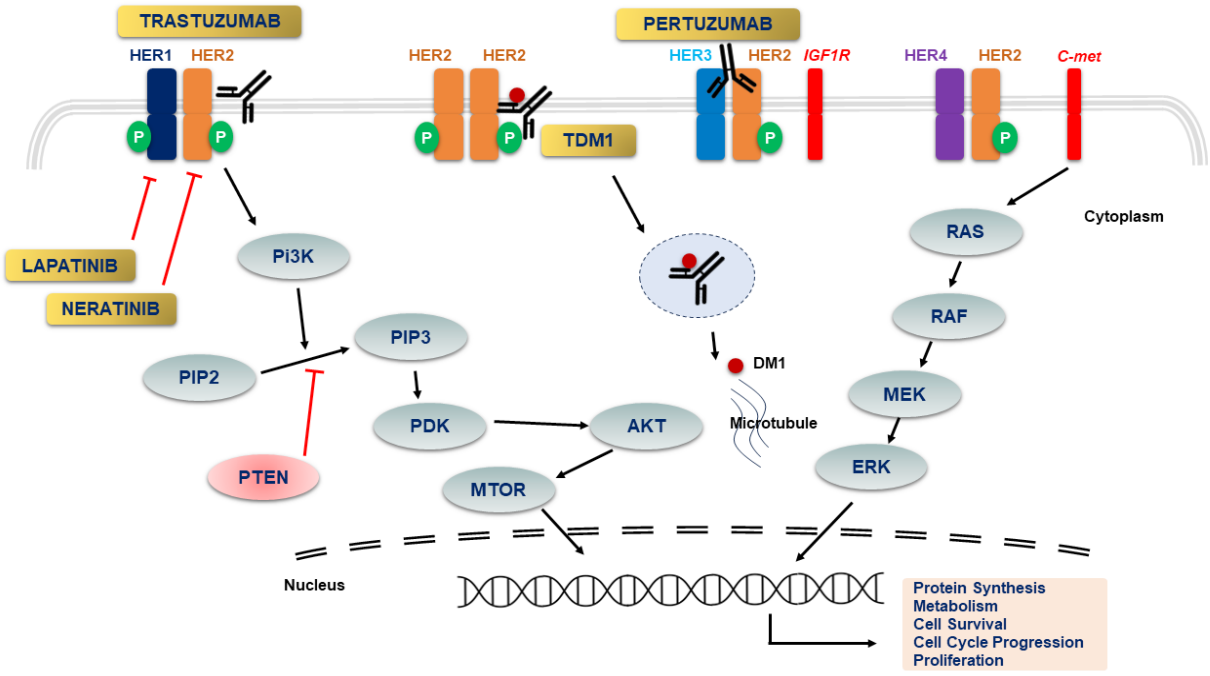


Figure 2. The Mechanism of Action of Therapies Targeting *HER2* Signaling Pathway: This figure shows how therapies target HER2 pathways in cancer. It highlights drugs like Neratinib, Pertuzumab, and TDM1, illustrating their mechanisms in inhibiting pathways like PI3K/AKT/mTOR and RAS/RAF/MEK/ERK, leading to reduced cancer cell survival and proliferation.

authorized by the FDA in 2020 for use in individuals with advanced illness who underwent a minimum of two or more earlier lines of *HER2*-directed treatment [49].

*Other newer approaches*

*HER2*-positive metastatic breast cancer treatment is devoid of a clear therapeutic algorithm [55–57]. Various drugs have been approved in the past for consideration and choosing the drugs in sequence for optimal outcomes (Figure 3). Some of these drugs include tucatinib, DS-8201a, Neratinib, CDK4/6 Inhibitors, immune checkpoint inhibitors, and New ADCs.

A new *HER2*-targeting ADC called trastuzumab duocarmazine (SYD985) harbours a *HER2* antibody which is extremely comparable to trastuzumab coupled to the alkylator duocarmycin through a cleavable linker. High levels of lymphocytes infiltrating the tumor along with increased expression of programmed cell-death protein ligand 1 (*PD-L1*) are typically found in *HER2*-positive breast tumors [58]. Through a synergistic stimulation of CD81 T cells, pre-clinical evidence validates combining the use of inhibitors of immune checkpoint with *HER2*-targeted treatments [59].

Preclinical investigations have confirmed the relationship among *HER2* signalling and control of complexes formed by cyclin D1-CDK [60]. When coupled with trastuzumab in these animals, CDK4/6 inhibitors exhibit attractive anticancer efficacy and could potentially be able to resensitize

The normal course of breast cancers that are positive for *HER2* has been altered by *HER2* targeted treatment. The majority of patients should undergo adjuvant or neoadjuvant trastuzumab and pertuzumab in combination to chemotherapy based on taxane along with or without anthracyclines if they have a tumor greater than 2 cm or at least one positive lymph node [61, 62]. A pCR rate of 65%-70% and enhanced EFS and DFS were obtained with neoadjuvant docetaxel with dual *HER2* blockage with trastuzumab and pertuzumab, indicating the safety and efficacy of anthracycline-free protocols in the era of strong anti-*HER2* treatment [63,64]. For patients with *HER2*-positive, operative breast cancer, combining pertuzumab to trastuzumab and chemotherapy dramatically increased the IDFS rate, particularly for those with node-positive disease [64]. The mainstay of therapy for patients with relatively low risk, stage I malignancies that are *HER2*-positive is paclitaxel with trastuzumab. This relies on data from the non-randomized APT study (NCT00542431), which showed that patients who received weekly paclitaxel for a period of twelve weeks and trastuzumab for a year had a 7-year DFS rate of 93% (95% CI = 90%–93%) [65,66]. According to the ExteNET research [67,68], adjuvant neratinib therapy for an extra year following trastuzumab therapy can enhance IDFS in high-risk *HER2*-positive breast cancer patients. The inclusion of neratinib may reduce the chance of a central nervous system recurrence, according to this study.

The development of breast cancer involves an intricate microenvironment that includes a variety of benign cell types and the extracellular matrix (which supports the tumor mechanically and permits paracrine cellular communication). Fibroblasts associated with cancer are among the most prevalent cell types, but the breast cancer microenvironment additionally includes cells of the leukocyte lineage, the majority of which are engaged in the immune response (Figure 4) [69].

These cells include macrophages, lymphocytes, and myeloid-derived stromal cells. Among the various molecular subtypes of breast cancer, TNBC and *HER2*-positive tumors have the highest immunogenicity, whereas luminal A and luminal B subtypes have the lowest immunogenicity [70,71]. Additionally, the overall number of tumor-infiltrating lymphocytes, which represents the strength of the immune response inside the tumor bed, has a favorable impact on the prognosis of breast cancer and the response to neoadjuvant therapy [58 ,72]. Immune editing and immune surveillance concepts explain how the immunological milieu affects the growth and spread of breast cancer. Through the cytokine milieu produced by activated CD8+ and CD4+ T cells, the immune microenvironment predominantly has an anti-tumor effect during the early stages of carcinogenesis. In contrast, as a tumor spreads, the microenvironment's cell makeup, particularly its cytokine and cancer-associated fibroblast populations, become tumor-promoting, having been "hacked" by breast cancer cells [73–75].

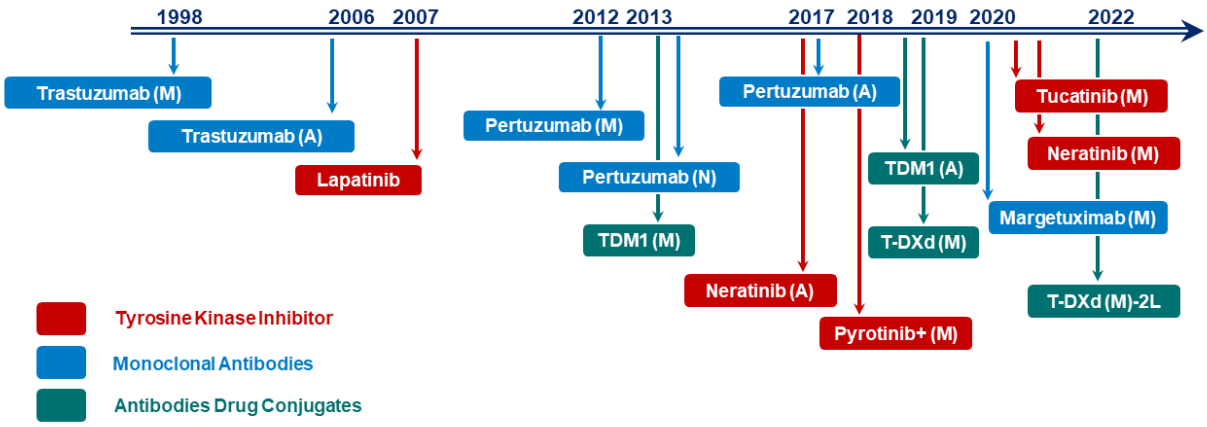


Figure 3. Timeline of Approved Anti-*HER2* Therapies. A, adjuvant setting; M, metastatic setting; N, neoadjuvant setting

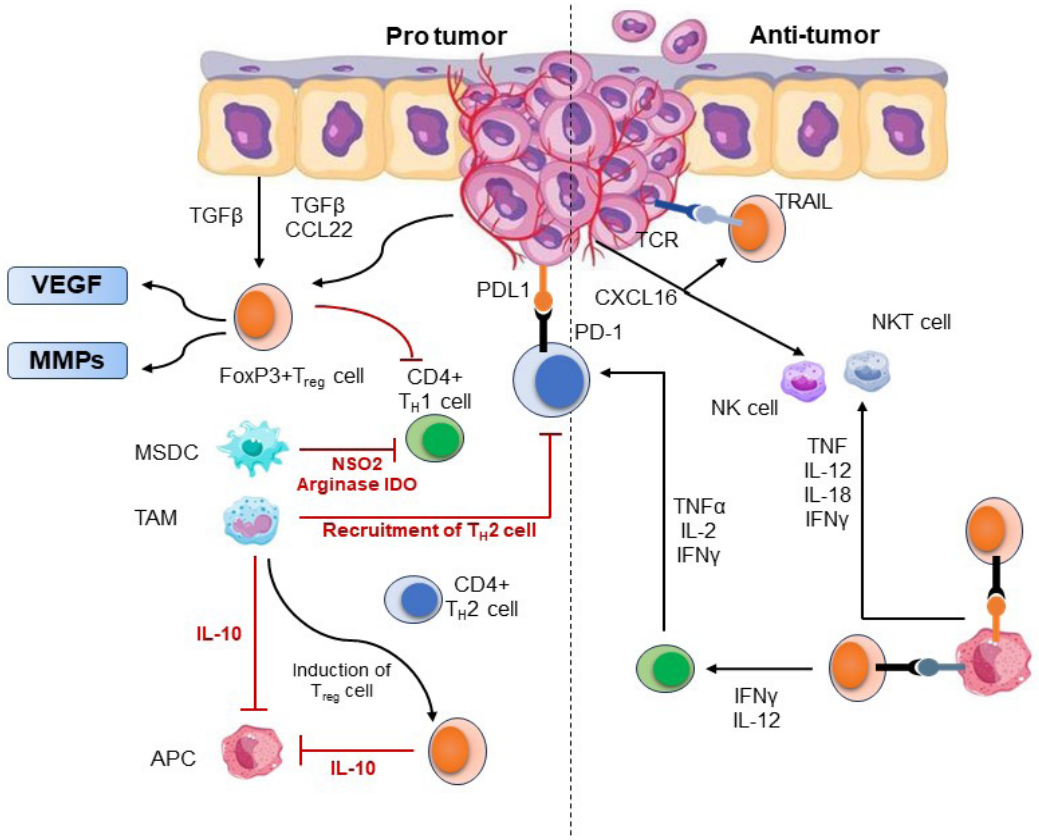


Figure 4. Immune Crosstalk in Breast Cancer: It shows the interactions between pro-tumor and anti-tumor immune responses within the breast cancer microenvironment, highlighting key factors such as *VEGF, MMPs, TGFβ, IL-10, PD-1/PD-L1, NK* cells, cytokines, and the balance determining the immune response.

*Trastuzumab resistance: A new challenge to HER-2 positive BC therapy*

Nearly 70% of cases with *HER2* positive status develop resistance to therapy despite showing response at early stage of trastuzumab treatment [76]. This highlights that although targeted therapy using trastuzumab is an advancement in the management of breast cancer, resistance developed to trastuzumab limits its advantages in the cancer individuals. This suggests that there is a need for identifying potential targets that can be targeted through dual or multiple therapies. The solution lies in better appreciating the underlying mechanisms for acquired resistance that can enable formulation of targeted solutions to overcome the resistance observed in cancer individuals.

In this pursuit, various factors have been identified that play a role in the resistance to trastuzumab through signaling pathways and may form potential targets for overcoming resistance. These are presented in Table 3.

Overall, breast cancer is a highly heterogenous in nature which means that various mutations can lead to the disease in different individuals. This creates difficulty in identifying a single target for effective treatment. This implies that further research is required to identify novel targets before precision medicine can be brought to clinics. Furthermore, the finances involved in genetic evaluations

Table 3. Biomarkers Correlating with Resistance to Trastuzumab

| Reference s | Biomarkers | Pathway involved | Correlation with drug resistance |
|---|---|---|---|
| [77] | STAT3 | IL6-pSTAT3-PTEN | Direct |
| [78] | ARID1A | ErbB/PI3K/AKT | Inverse |
| [79] | PKCα | Jagged-1/Notch-1 | Inverse |
| [80] | MUC4 | NF-κB/MUC4 | Direct |
| [82] | CDK12 | WNT/β-catenin/-TCF; IRS1/ErbB/PI3K/AKT | Direct |
| [83] | ANKRD44 | TAK1/Akt/NF-kβ | Inverse |
| [83] | EPHA5 | Notch1; PTEN/AKT | Inverse |
| [84] | Cullin7 | IRS-1/ErbB/PI3K/AKT | Direct |
| [85] | NCAPG | JAK/STAT3 | Direct |
| [86] | YAP1 | Hippo/YAP1/TEAD | Direct |

Table 4. The Recent Update on the Trials for Targeted Therapy in *HER2* Positive Breast Carcinoma

| References | Identifier | Therapeutic agent | Overall response | Progression Free Survival | Overall Survival |
|---|---|---|---|---|---|
| [95] | HER2CLIMB | Tucatinib/Trastuzumab | 47.3 | 9.9 | 21.6 |
| | | Trastuzumab | 20 | 4.2 | 12.5 |
| [51] | DESTINY Breast-01 | T-DXd | 58.3 | 18.1 | NR |
| [100] | KAMILLA | T-DM1 | 20.5 | 3 | NR |
| [49] | NALA | Neratinib | 28.6 | 5.6 | 13.9 |
| | | Lapatinib | 28.2 | 4.3 | 12.4 |
| [96] | TBCRC 022 | Neratinib (lapatinib naive) | 49 | 5.5 | 13.3 |
| | | Neratinib (prior lapatinib) | 33 | 3.1 | 15.1 |
| [94] | DESTINY Breast-03 | T-DXd | 67.4 | 15 | NR |
| [99] | PATRICIA | Pertuzumab + High-dose Trastuzumab | 11 | NR | NR |
| [101] | PERMEATE | Pyrotinib (RT-naïve) | 74.6 | 11.3 | NR |
| | | Pyrotinib (RT-progressive) | 42.1 | 5.6 | NR |
| NCT04334330[1] | NCT04334330 | Fulvestrant + trastuzumab + pyrotinib + palbociclib | N/A | N/A | N/A |
| NCT03765983[2] | NCT03765983 | Trastuzumab + GDC-0084 (PI3K inhibitor) | N/A | N/A | N/A |
| NCT03975647[3] | NCT03975647 | T-DM1 + Tucatinib | N/A | N/A | N/A |

[1], Clinical trial NCT04334330: A multi-center, prospective study evaluating the combination of Palbociclib, Trastuzumab, Pyrotinib, and Fulvestrant in patients with brain metastases from ER/PR-positive, *HER2*-positive breast cancer. [2], Clinical trial NCT03765983: A Phase II study evaluating the combination of GDC-0084 and Trastuzumab in patients with *HER2*-positive breast cancer brain metastases. [3], clinical trial NCT03975647: A Phase III, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of tucatinib in combination with ado-trastuzumab emtansine (T-DM1) in patients with unresectable locally advanced or metastatic *HER2*-positive breast cancer who have previously received a taxane and trastuzumab.

and personalized medicine may impose burden on patients and families [87, 88]. Various treatments in the field of personalized medicine are underway. This implies that the data is being generated with respect to newer drugs and the true value of the treatment cannot be determined for the disease.

*Future directions*

Although it is advantageous to sustain *HER2* pathway repression, *HER2*-targeted treatment should be provided for metastatic breast cancer that is *HER2*-positive after progression.

At present, dual *HER2* blocking with trastuzumab and pertuzumab in conjunction with chemotherapy (ideally taxanes) is advised as first-line treatment [89, 90]. The option for patients with malignancies that are both HR- and *HER2*-positive is to get ET in conjunction with *HER2*-targeted treatment [91–93]. Trastuzumab deruxtecan (T-DXd), T-DM1, and pyrotinib (authorized in China) are the second-line choices. A cytotoxic topoisomerase I inhibitor and an anti-*HER2* antibody are combined to form the next-generation antibody-drug combination known as trastuzumab deruxtecan [51]. T-DXd shown a substantial improvement in PFS against T-DM1 in the phase III DESTINYBreast03 study (hazard ratio = 0.2840; P = 7.8 1022) for second-line therapy with *HER2*-positive unresectable or MBC [94]. Another study, DESTINY-Breast09, is now taking place to evaluate T-DXd to the first-line standard-of-care combination of trastuzumab and pertuzumab combined with docetaxel. In *HER2*-positive MBC after prior trastuzumab and taxanes, a phase III randomized PHOEBE trial found that the combined administration of capecitabine and pyrotinib—a non-reversible pan-ErbB receptor tyrosine kinase inhibitor—significantly prolonged progression-free survival (12.5 months vs. 6.8 months; hazard ratio = 0.39, 95% CI = 0.27-0.56). Neratinib and capecitabine were better to lapatinib and capecitabine as second-line treatments [49]. According to the *HER2*CLIMB study, patients with brain metastases who had received pretreatment with trastuzumab, pertuzumab, and T-DM1 had significantly improved PFS and OS when treated with the exceptionally selective anti-*HER2* tyrosine kinase inhibitor tucatinib in comparison to placebo in addition to capecitabine and trastuzumab [95].

In *HER2*-positive patients with brain metastases, pyrotinib and T-DXd also shown remarkable effectiveness (Table 4) [96–101]. The number of options for treating *HER2*-positive breast cancer patients, for whom ongoing anti-*HER2* treatment is crucial, will be widened by the discovery of new anti-*HER2* medicines.

*Conclusion*

There is now research being done on expanding the therapeutic advantages of *HER2*-targeted therapy from breast cancer to other solid tumors that are positive for protein. Despite being extremely promising medications, trastuzumab, pertuzumab, lapatinib, and neratinib, some patients may not respond to treatment or grow resistant to them. The introduction of several new medications

offers a fresh perspective on combination *HER2* therapy techniques. Future clinical research should focus on investigating novel clinical trial techniques, particularly those that focus on gene-level study. The *HER2* detection methods will be improved during the next ten years, and the findings will be more precise. It is important to do more studies regarding the molecular biology of breast cancer to identify the important genes that influence the growth and spread of breast cancer cells.

The customized diagnosis and treatment of *HER2*-positive breast cancer will be influenced by more practical and reliable prognostic factor prediction. Our relentless endeavor in the fight for the ultimate cure and better survival advantages should be to unveil the law of effectiveness and safety to identify reasonable administration of regimens.

## Author Contribution Statement

BA carried out most of the experiments. HE helped in performing experiments. HE, AM participated in the design of the study, performed the statistical analysis and helped in the drafted the manuscript. BY participated in the design of the study and were involved with revising the manuscript critically. MK and AB provided and managed the patient's samples and revised the manuscript critically and conducted the external validation of the test. AM conceived, designed, coordinated the study and drafted the paper. All authors read and approved the final manuscript.

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*How the ethical issue was handled (name the ethical committee that approved the research)*

Ethical approval for the use of patient's samples has been obtained from the Casablanca medical school ethic committee

*Any conflict of interest*

The authors declare that they have no competing interests.

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