HER2+ Breast Cancer Targeted Therapy: Neoadjuvant Therapy and New Drugs

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Abstract:

5-20 % of breast cancer patients are diagnosed with overexpression of the HER2 (human epidermal receptor type 2) gene. This amplification is associated with an aggressive type of cancer, poor prognosis, and bad clinical outcomes. Neoadjuvant therapy is a standard treatment used to reduce the size of the tumor in HER2+ breast cancer and make it suitable for surgery. By down-regulating the tumor, neoadjuvant therapy was shown to effectively increase the pathologic complete response (pCR) and to reduce the scope of surgery. This paper reviews different types of drugs that are used as neoadjuvant agents for HER2+ targeted therapy. We will mention many clinical trials that led to the advancement in this treatment and will highlight different combinations between chemotherapy drugs and targeted HER2+ drugs to compare the efficiency and pCR of these combinations. Although chemotherapy plus HER2+ targeted therapy was shown as the most suitable neoadjuvant treatment, Metastatic breast cancer (MBC) is still a pending problem that is not solved by the previous treatments. For this purpose, we will also tackle the most recent drugs (tyrosine kinase inhibitor drugs, monoclonal antibody drugs, and antibody-drug conjugates) that are being tested for their efficiency on MBC. Although FDA-approved medications had improved the efficacy or reduced the side effects of HER2+ therapy in a particular way, neither of these therapies resulted in a major change in the industry and neither led to a final solution for MBC.

Introduction:

Breast cancer is a common prevalent cancer that is the second major cause of death among women (1). HER2+ (human epidermal receptor type 2) breast cancer is one of the most aggressive types of breast cancers that are associated with a higher grade and worse prognosis. HER2 is a part of the epidermal growth factor receptor (EGFR) family that is made up of other receptors like HER1, HER3, HER4, and EGFR. The main function of this family is to regulate the growth, survival, and differentiation of the cells by activating major signaling pathways like Ras/Raf/MEK/MAPK and PI3K/Akt. This activation occurs through homo or hetero-dimerization of HER2 with HER2 or other HER receptor respectively, what leads to phosphorylation of the tyrosine residue and activation of downstream signaling pathways (2,3). In normal cells, the expression of HER2 is low and can be regulated, but when HER2 is overexpressed, it leads to stronger signals, increased responsiveness to growth factors, and the formation of highly malignant tumor cells (3). Among breast cancer tumors, 20% of early, 35% of locally advanced and metastatic, and 40% of inflammatory breast cancer tumors have amplification of HER2 expression (4,5).

Chemotherapy plus HER2+ targeted therapy showed a great advancement in treating HER2+ breast cancer by improving the overall survival (OS) and disease-free survival (DFS) significantly. This also stands true for Neoadjuvant therapy that became a standard treatment for locally advanced, inflammatory, and inoperable HER2+ breast cancer patients (6). Neoadjuvant therapy decreases the tumor size and increases the rate of breast-conserving therapy allowing the conservation of the breast organ and the transition of the surgery from mastectomy to a lumpectomy (7).

Trastuzumab was the first HER2 targeted drug that led to the alteration in the history of early and metastatic breast cancer patients (1). Trastuzumab was followed by other monoclonal antibody drugs (like pertuzumab), tyrosine kinase inhibitor drugs (like lapatinib), and antibody-drug conjugates (like T-DM1) that lead to dramatic changes in the field of HER2+ treatment, and a high proportion of non-metastatic patients being cured. However, metastatic breast cancer is still a major concern due to its resistance to HER2+ drugs. Although many drugs have been approved recently, more clinical trials are still ongoing to find the suitable combination to treat HER2+ metastasis breast cancer (8).

This article will focus on major drugs that were used in neoadjuvant therapy for HER2+ breast cancer treatment and will highlight the most recent drugs that got FDA approval or still ongoing clinical trials.

Neoadjuvant therapy in HER2-positive breast cancer

To test the efficiency of preoperative treatment, and to compare it to post-operational treatment in breast cancer chemotherapy, and National Surgical Adjuvant Breast and Bowel Project B-18 (NSABP B-18) trial was performed. Doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan; AC) chemotherapy was used to determine which treatment leads to more performed lumpectomies, and yields better outcomes. Results showed almost the same efficiency for both pre and post-operational treatment of breast cancer with chemotherapy where post-operational chemotherapy showed more permitted lumpectomies, led to decreased size in breast cancer tumor and was approved as an efficient method to treat patients with stages I and II in the disease (9,10).

Trastuzumab (Herceptin®):

Trastuzumab is the first targeted therapy against HER2 and the first humanized monoclonal antibody for HER2+ breast cancer that took FDA approval in 1998. Trastuzumab functions through two different mechanisms. In the first mechanism, it binds to the extracellular part of H HER2 receptor and leads to its degradation by internalizing it. In the second mechanism, trastuzumab uses its IgG1 Fc region to activate natural killer cells by binding to their FcγRIII/CD16 proteins that are found on its surface. This binding leads to the inhibition of downstream signal that uses RAS/MAPK and PI3K/AKT pathways, which leads to the inhibition of proliferation and cell death . This concept is called antibody-dependent cellular cytotoxicity (ADCC) (11).

Trastuzumab should be a part of neoadjuvant treatment for HER2+ breast cancer according to the international panel on neoadjuvant therapy (12). Clinical Trials that detected the efficiency of trastuzumab are listed below.

As a first randomized trial, patients were randomly assigned to either four cycles of Paclitaxel, fluorouracil, cyclophosphamide, and Epirubicin chemotherapy, or chemotherapy with trastuzumab simultaneously. Pathologic complete response (pCR) was identified for both samples to test the effect of trastuzumab with chemotherapy in neoadjuvant therapy. Even though only 20% improvement in pCR was aimed by the researchers, 66.7% pCR improvement was detected in presence of trastuzumab compared to only 25% in its absence what lead to premature closure of the study due to the unexpected positive results. Although the safety of this approach was not established, no cardiac failure has been detected with the concurrent presence of trastuzumab with anthracycline (13). The NaOH trial then was the first phase III trial to examine the benefits of trastuzumab for HER2+ local advanced and inflammatory breast cancer patients. In this trial, 228 HER2+ patients received neoadjuvant chemotherapy for one year in the

presence or absence of trastuzumab. The addition of trastuzumab in presence of doxorubicin, paclitaxel, cyclophosphamide, methotrexate, and fluorouracil chemotherapy, had improved the overall response rate from 74% to 87% and had shown a significant difference in pCR representing 38% compared to only 19% without trastuzumab. This difference in pCR was translated to 58% vs 43% improvement in event-free survival (EFS) for patients, with no significant effect on the OS that increased from 63% to 74% only in presence of trastuzumab (14).

Taxol Epirubicin Cyclophosphamide Herceptin NeOadjuvant (TECHNO), was another trial of the AGO and GBG study groups that used EC chemotherapy followed by trastuzumab and paclitaxel to evaluate their safety and efficiency for humans with HER2+ breast cancer. In this phase II clinical trial, 217 HER2+ patients received epirubicin and cyclophosphamide for three to four weeks cycles then received another four to three weeks' cycles of paclitaxel and trastuzumab, before the surgery for all except for trastuzumab that continued to be received after the surgery for one year. This trial had resulted in pCR of 39%. It showed, and for the first time, that DFS and OS are strongly correlated to pCR. This was approved by the enhancement of the three-year DFS from 73% to 83%, and the three-year overall survival from 86% to 96% for patients without pCR and with pCR respectively. Only 3.7% of the patients reported cardiac toxicity (15).

The German Breast Group/Gynecologic Oncology Study Group (GeparQuattro), is another significant phase III study where patients with locally advanced or operable HER2+ breast cancer were given four cycles of epirubicin/cyclophosphamide with docetaxel or with docetaxel and capecitabine, or with four cycles of docetaxel then capecitabine with the combination of trastuzumab in all of them. Another reference group composed of HER2- breast cancer patients

was treated with the same treatment but with the absence of trastuzumab. Results showed that pCR of HER2+ breast cancer patients which was 31.7% was higher than that of HER2- patients which were 15,7% in absence of trastuzumab. In HER2+ groups, 32.9%,31,3%, and 34% of the patients reached the pCR when treated with docetaxel alone, decetaxol+ capecitabine, and four docetaxel cycles with capecitabine respectively. The results showed that the addition of capecitabine in the early stages has no effect on the long term and that the addition of trastuzumab increases the pCR rate for HER2+ breast cancer patients (16).

To detect the advantages and disadvantages of using trastuzumab as a treatment for HER2+ breast cancer patients, in addition to neoadjuvant chemotherapy, five trials were done in previous studies. Results showed that the combination of both chemotherapy and trastuzumab leads to a higher pCR compared to the usage of chemotherapy alone. Trastuzumab presence did not show any added toxicity and did not make a difference regarding breast-conserving surgery. It did not show any correlation with the increase of any incident related to neutropenia or any cardiac events (17) .

Although Trastuzumab showed significant advancement in HER2+ breast cancer treatment, it led to resistance, in some patients, that was either de novo resistance (35%) or gained resistance after treatment with trastuzumab (70%). These results suggested the need for further agents and combinations that can result better prognosis in the neoadjuvant treatment for HER2+ breast cancer patients (11).

Combination of neoadjuvant HER2 targeted drugs

Lapatinib (Tykerb®):

Due to the limitation of trastuzumab treatment alone, combinations in neoadjuvant therapy were considered, where dual anti-HER2 blockage was used in addition to other combinations and

applications. Lapatinib is a small, orally active molecule that reversibly leads to dual inhibition of HER1 and HER2 tyrosine kinase receptor. It was approved by FDA in 2007 for the treatment of metastatic breast cancer. The dual inhibition of HER1 and HER2 leads to a decrease in downstream signaling and blockage of PI3K, MAPK, PLCγ, and STAT pathways by decreasing phosphorylation in these pathways. This then leads to apoptosis (18,19). Some studies indicated that lapatinib can also decrease the phosphorylation and cell signaling for HER3 receptors and block their cell growth (20).

As a dual tyrosine kinase inhibitor, the efficiency of Lapatinib as a neoadjuvant agent was tested by many studies, either as a single agent or with the addition of trastuzumab. In the GeparQuinto trial, researchers aimed to compare the efficiency of injecting trastuzumab alone or Lapatinib alone to HER2+ breast cancer patients, 620 patients have received treatment including four cycles of EC plus cyclophosphamide and four cycles of docetaxel with either trastuzumab or Lapatinib. Results showed a higher Pathologic complete response with the usage of trastuzumab (30.3%) compared to the usage of Lapatinib (22.7%) suggesting trastuzumab as a better agent for HER2+ treatment (21).

The dual effect of trastuzumab and lapatinib was then tested by NeoAdjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (*NeoALTTO*) trial. In this phase III study, HER2+ patients with tumor size greater than 2cm were treated for 6 weeks with anti HER2+ therapy alone, and with paclitaxel for 12 weeks, then treated randomly with either lapatinib, trastuzumab, or both lapatinib and trastuzumab simultaneously. Then these patients were treated with the same agents after the surgery for 52 weeks. The combination of trastuzumab and lapatinib yielded a higher pCR rate (51.3%) compared to the monotherapy treatment (27.6% for trastuzumab, 20% for lapatinib). The treatment with trastuzumab showed a slightly better response compared to that of

Lapatinib. Cardiac dysfunctions were not detected, but diarrhea was highly associated with the dual usage of trastuzumab and Lapatinib. Higher toxicity was detected in dual combinations that led to the discontinuation of many patients due to the adverse effects (22) . A 3.8 years' follow-up study for *NeoALTTO* study aimed to collect data for the secondary endpoints: EFS and OS, and to find a relation between these factors and pCR. Results showed no difference in the values of EFS and OS between different treatments but had assured the positive relationship between achieving pCR rate and having a longer OS and EFS (23).

Another study to compare lapatinib and trastuzumab agents was *CHERLOB* (Chemotherapy Plus Lapatinib, Trastuzumab or Both in HER2 Positive Breast Cancer) trial. Patients were divided into 3 arms (A, B, C) after receiving their chemotherapy (paclitaxel (12 weeks), fluorouracil, epirubicin, and cyclophosphamide (four courses every 3 weeks)), where they received trastuzumab, lapatinib, trastuzumab, and lapatinib respectively. Arm C showed the highest pCR rate (47%) compared to arm A and B (25%, 26.3% respectively). Episodes of congestive heart failure were not detected (11). Many other trials to test the efficiency of the usage of lapatinib were done like NSABP B-41, CALGB 40601, and GeparSixto. In NSABP B-41, when HER2+ breast cancer patients were treated with doxorubicin cyclophosphamide + paclitaxel followed by trastuzumab, lapatinib, or both, and results also showed higher pCR for the dual combined treatment (62%) (24). In CALGB 40601, pCR rates varied, where it recorded 56% when paclitaxel was concurrently added with trastuzumab and lapatinib which is higher than the pCR rate when trastuzumab (46%) or lapatinib (32%) given alone (25). GeparSixto showed that carboplatin has no significant effect when added with trastuzumab and lapatinib to treat HER2+ breast cancer patients where it showed a pCR of 32.8% in presence of carboplatin and pCR rate of 36.8% in its absence (26).

All results showed a positive effect of the dual combination of trastuzumab and lapatinib showing an increase in pCR. Six randomized trials from a meta-analysis (18) approved that and showed an increase of 13% in pCR in the dual combination injection. Bria et al.(19) also confirmed these results and showed an increase of 16% for the pCR rate.

Lapatinib did not become a standard agent in neoadjuvant therapy, this might be because it showed some toxicity and side effects like diarrhea, in addition to the absence of its usage in adjuvant therapy (27).

Pertuzumab (Perjeta®):

Pertuzumab is a humanized monoclonal antibody and a dual inhibitor of HER2-HER3 dimerization. It was approved by FDA in 2012 to be used with trastuzumab and docetaxel. The high efficiency of pertuzumab, when injected with trastuzumab, is because the epitope where pertuzumab binds HER2 extracellular receptor is different from the epitope that trastuzumab binds. It is assumed that pertuzumab prevents ligand-dependent HER2-HER3 hetero-dimerization what leads to suppression of downstream pathways (PI3K, and MAPK) (28,29) .

After lapatinib, pertuzumab was introduced to the field of neoadjuvant therapy.

NeoSphere, a randomized phase two trial, that aimed to study the safety and the efficacy of the combination of trastuzumab or pertuzumab, or both, with docetaxel, and the dual combination of trastuzumab and pertuzumab in the absence of chemotherapy in neoadjuvant therapy. The 417 eligible HER2+ breast cancer patients were divided into four groups to receive four neoadjuvant cycles: 107 were assigned to receive trastuzumab + docetaxel (group A), 107 were assigned to receive pertuzumab + trastuzumab + docetaxel (group B), 107 were assigned to receive pertuzumab and trastuzumab only (group C), and 96 were assigned to receive pertuzumab + docetaxel (group D). The pCR rate was 45.8% for group B, 29% for group A, 24% for group D,

and 16.8% for group C. Serious adverse events like neutropenia, febrile neutropenia, and leucopenia were seen in similar numbers in groups A, B, and D that was higher than that seen in group C (30). As a follow-up on this study, 5 years' analysis of the NeoSphere trial showed an overlap between the results of Progression-free survival and disease-free survival. Progression-free survival results were 81%, 84%, 80%, and 75% for groups A, B, C, and D respectively. This study supported pCR as a primary endpoint and approved that patients who achieved the pCR in all groups had longer progression-free survival (85%) compared to patients who did not achieve pCR (76%). No new safety concerns were detected, and all groups showed the same tolerability (31).

The safety of the dual combination of trastuzumab and pertuzumab was a major concern for Phase II Trastuzumab plus Pertuzumab in Neoadjuvant HER2-Positive Breast Cancer trial (TRYPHAENA), especially cardiac safety. Patients were divided into three arms A, B, and C. Patients in arm A received three cycles of FEC (5-fluorouracil, epirubicin, cyclophosphamide) where trastuzumab (H) + pertuzumab (P) were added with cycle 1 of FEC, followed by three cycles of docetaxel (T). Patients in arm B received also three cycles of FEC and three cycles of T, but with the combination of P+H in the first cycle of docetaxel. Patients in arm C received six cycles of T + carboplatin + H concurrently given. Results of the neoadjuvant therapy for 225 HER2+ breast cancer patients showed pCR rates between 57.3% and 66.2%. Cardiac safety was detected, low rates of left ventricular systolic dysfunction were detected across the patients in the three arms (32).

BERENICE was also another study that aimed to examine the cardiac safety of HER2+ breast cancer patients treated by anthracycline-based chemotherapy in combination with trastuzumab and pertuzumab in neoadjuvant therapy. This phase 2 study approved cardiac safety in standard

and dose-dense treatment. pCR rate was 61.8% for patients receiving dose-dense regime and 60.7%. for patients receiving standard regimens (33).

Another study, GeparSepto, aimed to detect whether nab-paclitaxel can increase the number of HER2+ patients reaching PCR rate if used instead of solvent-based paclitaxel. Epirubicin, cyclophosphamide, trastuzumab+ pertuzumab, plus either nab-paclitaxel or solvent-based paclitaxel were used as neoadjuvant agents in this neoadjuvant therapy. Nab-paclitaxel group reached a pCR rate of 38% which was higher than the pCR reached when the paclitaxel group was used (29%), Results suggested the exchange of solvent-based paclitaxel to nab-paclitaxel especially in triple-negative breast cancer treatment that showed the highest additional benefits (23).

The analysis of the efficiency and safety of the dual combination of trastuzumab and pertuzumab, with or without paclitaxel, was determined by a randomized phase II study done by German investigators from WSG (WSG-ADAPT). 134 HER+ and HER – patients received the dual combination with or without paclitaxel for 12 weeks. Results showed that the presence of Paclitaxel had increased the pCR rate from 34.4% to 90.5% (34).

The TRAIN-2 trial aimed to investigate the effect of adding anthracyclines on pCR when given with the combination of pertuzumab and trastuzumab and to compare it with the results of adding carboplatin-taxane chemo drug with the dual combination. HER2+ patients, in this phase 3 trial, were randomly assigned to receive 5-fluorouracil+ epirubicin+ cyclophosphamide for 3 cycles every three weeks followed by carboplatin and paclitaxel for 6 cycles every 3 weeks or to receive paclitaxel and carboplatin for nine cycles in the presence of pertuzumab and trastuzumab. The pCR rate was recorded high in both groups (68% in absence of anthracycline and 67% in

presence of anthracycline). The more frequent febrile neutropenia in the anthracycline group suggested removing anthracycline from neoadjuvant therapy (35).

The results of many trials like NeoSphere and TRYPHAENA suggest taxane-based therapy in combination with trastuzumab and pertuzumab as a current standard of care for HER2+ breast cancer patients in neoadjuvant therapy. This standard was confirmed by both AGO guidelines 2016 and by NCCN guidelines 2016 (27).

T-DM1 (Kadcyla®):

Ado-trastuzumab emtansine (TDM1) is an antibody-drug conjugate for trastuzumab that directs high doses of chemotherapy directly to cancer cells by linking trastuzumab covalently through thioether linker into chemotherapy agent emtansine (DM1). When T-DM1 binds to the HER2 receptor it is internalized by the cell and DM1 is then released inside it.

T-DM1 was the first FDA drug approved for solid cancer and metastatic HER2+ breast cancer in 2013. It was also approved by FDA for adjuvant usage in 2019 after the results of the KATHERINE Trial. T-DM1 uses trastuzumab for the accurate delivery of Chemotherapy to HER2+ tumor cells. In this way, toxicity is limited on non-malignant cells and more potent chemotherapy is utilized (36).

In the TH3RESA phase 3 trial, 602 patients with progressive advanced HER2+ breast cancer were randomly assigned to either trastuzumab emtansine or to the treatment of physician's choice in this field. All patients had previously received taxane therapy and trastuzumab and lapatinib in an advanced setting where 219 patients (54%) had PFS events compared to 129 (65%) in the other group. Low incidence grade 3 was detected with T-DM1 compared to the other treatment. This study showed the importance of the usage of trastuzumab emtansine for HER2+ patients who previously received trastuzumab plus lapatinib (34).

Another trial was EMILIA, which aimed to test the effect of T-DM1 on progression-free survival (PFS) and OS. 991 HER2+ breast cancer patients participated in this randomized phase 3 study where they were treated with either trastuzumab emtansine or capecitabine + lapatinib. Results showed an OS number of 29.9 months with T-DM1 compared to 25.9 months in control treatment what lead to the transfer of 136 patients from control treatment to T-DM1 treatment. Again, T-DM1 showed low adverse events like thrombocytopenia and anemia compared to control treatment that showed more adverse and grade 3 events (35).

MARIANNE phase 3 trial aimed to assess overall survival (OS) in 1095 HER2+ breast cancer. Patients were randomized to 3 groups where they received either trastuzumab plus a taxane (HT), T-DM1 plus placebo, or T-DM1 plus pertuzumab (T-DM1+P). Results showed an almost similar median of OS in HT (50.9%), T-DM1 (53.7%), and T-DM1+pertuzumab (51.8%). Among groups who achieved objective tumor response (OR) (HT, T-DM1), OS recorded 64.4 months in T-DM1 that was higher than that of HT that recorded 56.3 months of OS. HT treatment recorded the highest rate of adverse events (55.8%) compared to T-DM1 and T-DM1+P treatment (47.1%, 48.6% respectively) (36).

TH3RESA, EMILIA, and MARIANNE trials showed that the usage of T-DM1 had prolonged the PSF by 3 months and had prolonged the overall survival. Their results had supported that T-DM1 is not inferior to T-DM1 plus pertuzumab or trastuzumab plus taxane as a first-line treatment for HER2+ breast cancer(37–39).

T-DM1 usage in neoadjuvant treatment of HER2+ breast cancer

WSG-ADAPT HER2+/HR+ phase II trial was the first trial that tested the efficiency of neoadjuvant dual therapy of T-DM1 with endocrine therapy (ET) in HER2+ breast cancer patients. 380 patients were randomly assigned to receive 12 weeks of either T-DM1 (group A) or

T-DM1+ET (tamoxifen/ aromatase inhibitor) (group B) or trastuzumab + ET (group C) followed by 4 cycles of EC, 12 cycles of paclitaxel weekly, and trastuzumab for one year in all groups after the surgery. Results showed pCR rate of 30.8% for group A, 40.5% for group B, and 6.7% for group C. Results also showed higher benefits for adding T-DM1+ET for premenopausal patients compared to post-menopausal patients (pCR 47.6% in pre and 50% in post) (40).

KRISTINE (NCT02131064) is a phase 3 clinical trial that aimed to compare the efficiency of usage of pertuzumab(P)+ T-DM1 (K) compared to the usage of docetaxel + carboplatin + trastuzumab + Pertuzumab (TCHP) in neoadjuvant therapy for HER2+ early breast cancer. pCR rate was higher with the usage of TCHP (55.7%) compared to the usage of KP therapy (44.4%) and more patients that received TCHP treatment underwent breast-conserving surgery (BCS) (52.6 % compared to 41.7% in KP treatment). Results showed that TCHP led to higher pCR than KP, but KP showed a better safety profile than TCHP (41).

I-SPY 2 TRIAL is another randomized phase 2 trial where breast cancer patients received 12 weekly cycles of either T-DM1+pertuzumab (T-DM1+P) or paclitaxel+ trastuzumab (TH) followed by four cycles of AC (doxorubicin/cyclophosphamide). pCR rates were 52% for T-DM1+P which was higher than that of the control (TH) which was 22% (42).

JBCRG-20 trial is another recent randomized phase 2 clinical trial that enrolled 236 HER2+ breast cancer patients and divided them into 3 arms: Arm A received 6 cycles of docetaxel + carboplatin + trastuzumab + pertuzumab (TCbHP), arm B received 4 cycles of TCbHP followed by 4 cycles of trastuzumab emtansine + pertuzumab (T-DM1+P) and arm C that received 4 cycles of T-DM1+P in addition to hormone therapy if the patient is ER+. In arm C, 21% of the patients did not respond to T-DM1+P and were treated with 4 cycles of 5 fluorouracil + epirubicin + cyclophosphamide (FEC). The highest pCR rate was shown in Arm B (71%)

compared to Arms A and C (57%) where in arm C pCR was higher for patients who responded to T-DM1+P (63%) compared to patients that received FEC (38%). Taxane-based treatments showed higher adverse events compared to T-DM1 based treatments (43).

New drugs for HER2+ breast cancer treatment:

Although HER2+ breast cancer treatment had reached many advancements compared to where it started, drug resistance is still a major problem in clinical trials. This resistance is assumed to be due to many factors like reduction in the activation of the immune system, activation of downstream signals like PI3K/AKT/mTOR pathway, metabolic reprogramming, impaired drug binding, etc. Treatment of metastatic breast cancer is still limited with the usage of trastuzumab, pertuzumab, and T-DM1 (44). The development of new anti-HER2 targeted therapies is a major concern and several trials are being published to tackle this issue. Some of the recent drugs to treat metastasis HER2+ breast cancer will be tackled.

New Tyrosine Kinase inhibitor drugs:

Neratinib (Nerlynx®): Oral, irreversible pan-HER (EGFR/HER1, HER2, HER4) tyrosine kinase inhibitor, that became the second TKI that gets the FDA approval after lapatinib in 2017. Neratinib works by inhibiting downstream pathways (PI3K/AKT and RAS/MAPK) through decreasing the phosphorylation of intracellular HER domain of tyrosine kinase leading to the decrease in phosphorylation of retinoblastoma and expression of cyclin D1. Due to its ability to cross the blood-brain barrier, neratinib had an advantage over monoclonal antibody treatment, where it is being explored to treat the progression of the central nervous system (CNS) in HER2+ breast cancer patients. The dose-limiting toxicity and maximum tolerated dose of neratinib were determined by phase I clinical trial where escalating doses were given. Results showed that 320g,

is the maximum tolerated dose and that diarrhea was the most associated toxicity with neratinib (45).

ExteNET trial was the trial that led to the FDA approval of the usage of neratinib as an adjuvant agent after one year of trastuzumab-based adjuvant therapy. 2840 HER2+ patients who already received neoadjuvant and adjuvant trastuzumab therapy were assigned to receive either neratinib (240g) or a matching placebo. Two years Invasive Disease-free survival rate was 93.3% with the usage of neratinib that was higher compared to the absence of neratinib (91.6%). So the usage of neratinib had increased the Two Years Invasive Disease-free survival in HER2+ breast cancer women when used after adjuvant trastuzumab therapy (46).

Neratinib was tested with vinorelbine chemotherapy for its safety and MTD. Results showed that 240 mg of nercaptin+ 25 mg/m2 vinorelbine is the MTD and that diarrhea, neutropenia, and nausea were the most adverse events with the usage of neratinib (47).

Another trial investigated the efficiency and safety of the usage of T-DM1 and neratinib on HER2+ metastasis breast cancer patients that already received trastuzumab + pertuzumab. Results recommended 3.6 mg/kg of T-DM1 and 160 mg/d neratinib as the suitable dose for this combination and showed that diarrhea and thrombocytopenia were the most grades 3 events (48).

The NEfERT-T Randomized Clinical Trial aimed to compare progression-free survival for HER2+ metastatic breast cancer patients who received either neratinib + paclitaxel or trastuzumab + paclitaxel. Results showed that PFS did not differ between both treatments (12.9 months) showing a preference for neritanib+ paclitaxel for CNS recurrence and highlighting diarrhea as the most common adverse event associated with neratinib (49).

Neratinib monotherapy has also been tested and compared to lapatinib + capecitabine treatment. The PFS and ORR of neratinib monotherapy were 4.5 months and 29% respectively compared to PFS and ORR 6.8 months and 41% for lapatinib + capecitabine treatment(50).

NALA trial is a phase 3 trial that compared the efficiency of treatment using neratinib plus capecitabine (N+C) versus lapatinib plus capecitabine (L+C) in HER2+ MBC. 261 patients that received HER2+ directed therapy were randomly assigned to one of the treatments. Results showed a higher PFS rate with neratinib treatment (5.6 months) compared to lapatinib treatment (5.5 months) and showed improvement in time for intervention for CNS disease with no new adverse events. This trial leads to the FDA approval in 2020 for the usage of neratinib plus capecitabine as a treatment for HER2+ metastatic breast cancer patients who already received targeted HER2 therapy (51,52).

Pyrotinib and Tucatinib: Pyrotinib and Tucatinib are other new tyrosine kinase inhibitors that are used for HER2+ breast cancer treatment. Pyrotinib is a small, irreversible pan-TK inhibitor for HER1, HER2, HER4. It works by inhibiting downstream proliferative signaling and blocking tumor growth in HER breast cancer cells. Pyrotinib has received conditional FDA approval in china for its usage in combination with capecitabine for patients with HER2+ MBC that have already been treated with chemotherapy treatment (taxane or anthracycline-based) (53).

Tucatinib (TUKYSA) is a new selective potent and reversible HER2 inhibitor. Tucatinib works by inhibiting the tyrosine kinase intracellular domain leading to phosphorylation blockage of HER2 and AKT3 (downstream effector of HER2). Tucatinib is a promising treatment for brain metastasis due to its promising ability to cross the blood-brain barrier(54–56). In 2020, FDA approved the usage of Tucatinib in combination with trastuzumab (Herceptin) and capecitabine (Xeloda) for HER2+ breast cancer patients after the treatment with HER2 targeted therapy (57).

New HER monoclonal antibody drugs:

Margetuximab (MGAH22): Margetuximab is a new, chimeric, monoclonal antibody that resembles trastuzumab with its affinity and anti-proliferative effects since it is derived from the same parent antibody, and binds to the same epitope. Through its genetically engineered region, IgG1 Fc region, margetuximab binds to CD16a, a receptor to Fc essential for antibody-dependent cellular cytotoxicity (ADCC), with 6.6 times higher affinity compared to trastuzumab, and binds with 8.5 times less affinity on CD32B receptors that are present on immune effector cells. By doing so, this drug overcomes trastuzumab resistance, leads to better ADCC, and enhances the recognition of cancer cells by the host immune system (58,59). Phase I results, that aimed to identify the safety of this drug, showed the absence of MTD. The significance of the FcγR genotype was then tested using Sophia phase III trial that showed enhancement of PFS (prolonged by 4.3 months) and a trend toward OS (median of 21.6 months with MGAH22 vs 19.8 months with trastuzumab) when used with chemotherapy and compared to trastuzumab+ chemotherapy treatment for HER2+ advanced or metastatic breast cancer patients (60).

ZW25 and PRS-343: ZW25 is a humanized novel bispecific antibody that targets ECD2 and ECD4 (2 different epitopes for HER2). The ability of ZW25 to crosslink many HER receptors leads to clustering of HER2 receptors and improves their internalization. This gives ZW25 a unique characteristic. *In vivo* and *in vitro* studies are still ongoing to test the efficiency of ZW25 in HER2+ metastatic breast cancer (61).

PRS-343 is also a bispecific antibody for HER2+ and CD137 (an immunoreceptor and a part of the TNF superfamily). It works by stimulating tumor antigen-specific T cells where it links T cells that have CD137 receptors on its surface to breast HER2+ tumor cells leading to their

clustering. Recently, ongoing trials are testing the efficiency of PRS-343 alone and in combination with atezolizumab in HER2+ tumors (62) .

Novel antibody-drug conjugates (ADC) for HER2+ breast cancer treatment:

Trastuzumab Deruxtecan (DS-8201or Enhertu®): Trastuzumab Deruxtecan is an ADC that is composed of trastuzumab monoclonal antibodies linked through tetrapeptide linkage to deruxtecan (topoisomerase 1 inhibitor). The linker is stable in the bloodstream and is cleaved when it reaches HER2+ cancer cells that have high concentrations of lysosome protease, thus leading to the distribution of the chemotherapy. Bystander cytotoxic effect might occur on neighbor cells that have a low amount of HER2 receptors due to the release of some of the drug extracellularly. DS-8201 is more permeable to cell membrane compared to T-DM1, what leads to a wider cytotoxic effect in the tumor environment. Trastuzumab Deruxtecan also offers a drug-to-antibody ratio of 8 compared to 3-4 in T-DM1 treatment (63,64). In 2017, DS-8201 received the breakthrough FDA approval for its usage in HER2+ metastasis and early BC patients after the treatment with pertuzumab and trastuzumab when patients are resistant to T-DM1. It then received the accelerated FDA approval for usage on patients with MBC who already received more than one HER2+ targeted therapy in December 2019 (65).

Another novel ADC for HER2+ breast cancer treatment:

ARX788, ZW49, SYD985, RC48, and MEDI4276 are other ADC novel drugs that are being tested to be used to treat HER2+ positive breast cancer patients.

ARX788 is made up of HER2+ monoclonal antibody, monomethyl auristatin F (MMAF), an inhibitor to the microtubule. In vivo and in vitro trials are still ongoing and the first aim in the phase I trial is to identify RP2D (66).

ZW49 is composed of ZW25 + monomethyl auristatin E (MMAE). Ongoing clinical trials are showing the anti-tumor activity of ZW49 in Xenographs and are demonstrating rapid internalization of the HER2 receptor when used compared to trastuzumab (67).

SYD985 (trastuzumab duocarmazine) is an antibody-drug conjugate that uses a monoclonal trastuzumab antibody and the cleavable linker-duocarmycin. Compared to T-DM1, SYD985 preclinical are showing better antitumor effects (68).

RC48 hertuzumab, is a monoclonal antibody that binds to MMAE, using a cleavable linker. It is specific to HER2 and does not bind to any other HER family. Clinical trials are being done on this drug and it did not get FDA approval yet. Finally, MEDI4276 is a bispecific anti-HER2 antibody where it binds to domain 4 of HER2 through the variable fragment of trastuzumab, and to domain 2 through the second monoclonal antibody (39S) (69).

Conclusion:

Since the initial approval of trastuzumab in 1998, plenty of drugs have been introduced and many therapeutic options have been changed to treat HER2+ breast cancer patients. Each FDA-approved drug contributed differently either by improving the efficiency or by decreasing the adverse effects of HER2+ treatment, but none of these treatments led to a huge shift in this field. Neoadjuvant therapy showed its significance in increasing the pCR and decreasing the extent of surgery by downregulating the tumor growth. It was affirmed that Chemotherapy in addition to dual HER2 targeted therapy was the most efficient neoadjuvant therapy option to treat HER2+ breast cancer patients. Recently, Neoadjuvant therapy is being tested for new drugs, and advancements in this therapy are being done.

Many new HER2+ BC therapies currently are on the horizon. Three drugs got FDA approval recently in 2020 (neratinib, DS-8201, and tucatinib) and other promising drugs are on their way.

Perhaps, the main concern for the next years will be to find the best sequencing of these medications that can lead to the most efficient outcome with the least adverse effects and toxicity. Recent studies show optimism that HER2+ MBC will be finally treated. Next decades will prove whether this optimism is valid, or whether drug resistance will stay a challenge to treat HER2+ MBC (8).

References:

1. *de Melo Gagliato D, Leonardo Fontes Jardim D, Marchesi MSP, Hortobagyi GN. Mechanisms of resistance and sensitivity to anti-HER2 therapies in HER2+ breast cancer. Oncotarget. 2016 Sep 27;7(39):64431–46.*

2. *Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol. 1996 Oct;16(10):5276–87.*

3. *Rubin I, Yarden Y. The basic biology of HER2. Ann Oncol. 2001;12:S3–8.*

4. *Zell JA, Tsang WY, Taylor TH, Mehta RS, Anton-Culver H. Prognostic impact of human epidermal growth factor-like receptor 2 and hormone receptor status in inflammatory breast cancer (IBC): analysis of 2,014 IBC patient cases from the California Cancer Registry. Breast Cancer Res. 2009 Jun;11(1):R9.*

5. *Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 Receptor and Breast Cancer: Ten Years of Targeted Anti–HER-2 Therapy and Personalized Medicine. The Oncologist. 2009 Apr;14(4):320–68.*

6. *Moja L, Tagliabue L, Balduzzi S, Parmelli E, Pistotti V, Guarneri V, et al. Trastuzumab containing regimens for early breast cancer. Cochrane Breast Cancer Group, editor. Cochrane Database Syst Rev [Internet]. 2012 Apr 18 [cited 2021 May 10]; Available from: http://doi.wiley.com/10.1002/14651858.CD006243.pub2*

7. *The I-SPY 1 TRIAL and ACRIN 6657 Investigators, Mukhtar RA, Yau C, Rosen M, Tandon VJ, Hylton N, et al. Clinically Meaningful Tumor Reduction Rates Vary by Prechemotherapy MRI Phenotype and Tumor Subtype in the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Ann Surg Oncol. 2013 Nov;20(12):3823–30.*

8. *Kunte S, Abraham J, Montero AJ. Novel HER2–targeted therapies for HER2–positive metastatic breast cancer. Cancer. 2020 Oct;126(19):4278–88.*

9. *Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol. 1997 Jul;15(7):2483–93.*

10. *Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol. 1998 Aug;16(8):2672–85.*

11. *Guarneri V, Frassoldati A, Bottini A, Cagossi K, Bisagni G, Sarti S, et al. Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: results of the randomized phase II CHER-LOB study. J Clin Oncol Off J Am Soc Clin Oncol. 2012 Jun 1;30(16):1989–95.*

12. *Kaufmann M, von Minckwitz G, Bear HD, Buzdar A, McGale P, Bonnefoi H, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic*

treatment of operable breast cancer: new perspectives 2006. Ann Oncol. 2007 Dec;18(12):1927–34.

13. *Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, et al. Significantly Higher Pathologic Complete Remission Rate After Neoadjuvant Therapy With Trastuzumab, Paclitaxel, and Epirubicin Chemotherapy: Results of a Randomized Trial in Human Epidermal Growth Factor Receptor 2–Positive Operable Breast Cancer. J Clin Oncol. 2005 Jun 1;23(16):3676–85.*

14. *Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. The Lancet. 2010 Jan;375(9712):377–84.*

15. *Untch M, Fasching PA, Konecny GE, Hasmüller S, Lebeau A, Kreienberg R, et al. Pathologic Complete Response After Neoadjuvant Chemotherapy Plus Trastuzumab Predicts Favorable Survival in Human Epidermal Growth Factor Receptor 2–Overexpressing Breast Cancer: Results From the TECHNO Trial of the AGO and GBG Study Groups. J Clin Oncol. 2011 Sep 1;29(25):3351–7.*

16. *Untch M, Rezai M, Loibl S, Fasching PA, Huober J, Tesch H, et al. Neoadjuvant Treatment With Trastuzumab in HER2-Positive Breast Cancer: Results From the GeparQuattro Study. J Clin Oncol. 2010 Apr 20;28(12):2024–31.*

17. *Valachis A, Mauri D, Polyzos NP, Chlouverakis G, Mavroudis D, Georgoulias V. Trastuzumab combined to neoadjuvant chemotherapy in patients with HER2-positive breast cancer: A systematic review and meta-analysis. The Breast. 2011 Dec;20(6):485–90.*

18. *Clavarezza M, Puntoni M, Gennari A, Paleari L, Provinciali N, D'Amico M, et al. Dual Block with Lapatinib and Trastuzumab Versus Single-Agent Trastuzumab Combined with Chemotherapy as Neoadjuvant Treatment of HER2-Positive Breast Cancer: A Meta-analysis of Randomized Trials. Clin Cancer Res. 2016 Sep 15;22(18):4594–603.*

19. *Bria E, Carbognin L, Furlanetto J, Pilotto S, Bonomi M, Guarneri V, et al. Impact of neoadjuvant single or dual HER2 inhibition and chemotherapy backbone upon pathological complete response in operable and locally advanced breast cancer: Sensitivity analysis of randomized trials. Cancer Treat Rev. 2014 Aug;40(7):847–56.*

20. *Amin DN, Sergina N, Ahuja D, McMahon M, Blair JA, Wang D, et al. Resiliency and Vulnerability in the HER2-HER3 Tumorigenic Driver. Sci Transl Med. 2010 Jan 27;2(16):16ra7-16ra7.*

21. *Untch M, Loibl S, Bischoff J, Eidtmann H, Kaufmann M, Blohmer J-U, et al. Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. Lancet Oncol. 2012 Feb;13(2):135–44.*

22. *Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. The Lancet. 2012 Feb;379(9816):633–40.*

23. *Untch M, Jackisch C, Schneeweiss A, Conrad B, Aktas B, Denkert C, et al. Nab-paclitaxel versus solvent-based paclitaxel in neoadjuvant chemotherapy for early breast cancer (GeparSepto—GBG 69): a randomised, phase 3 trial. Lancet Oncol. 2016 Mar;17(3):345–56.*

24. *Robidoux A, Tang G, Rastogi P, Geyer CE, Azar CA, Atkins JN, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. Lancet Oncol. 2013 Nov;14(12):1183–92.*

25. *Carey LA, Berry DA, Cirrincione CT, Barry WT, Pitcher BN, Harris LN, et al. Molecular Heterogeneity and Response to Neoadjuvant Human Epidermal Growth Factor Receptor 2 Targeting in CALGB 40601, a Randomized Phase III Trial of Paclitaxel Plus Trastuzumab With or Without Lapatinib. J Clin Oncol. 2016 Feb 20;34(6):542–9.*

26. *von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol. 2014 Jun;15(7):747–56.*

27. *Wuerstlein R, Harbeck N. Neoadjuvant Therapy for HER2-positive Breast Cancer. Rev Recent Clin Trials. 2017 May 9;12(2):81–92.*

28. *Yamashita-Kashima Y, Shu S, Yorozu K, Moriya Y, Harada N. Mode of action of pertuzumab in combination with trastuzumab plus docetaxel therapy in a HER2-positive breast cancer xenograft model. Oncol Lett. 2017 Oct;14(4):4197–205.*

29. *Nami B, Maadi H, Wang Z. Mechanisms Underlying the Action and Synergism of Trastuzumab and Pertuzumab in Targeting HER2-Positive Breast Cancer. Cancers. 2018 Sep 20;10(10):342.*

30. *Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol. 2012 Jan;13(1):25–32.*

31. *Gianni L, Pienkowski T, Im Y-H, Tseng L-M, Liu M-C, Lluch A, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. Lancet Oncol. 2016 Jun;17(6):791–800.*

32. *Schneeweiss A, Chia S, Hickish T, Harvey V, Eniu A, Hegg R, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). Ann Oncol. 2013 Sep;24(9):2278–84.*

33. *Swain SM, Ewer MS, Viale G, Delaloge S, Ferrero J-M, Verrill M, et al. Pertuzumab, trastuzumab, and standard anthracycline- and taxane-based chemotherapy for the neoadjuvant treatment of patients with HER2-positive localized breast cancer (BERENICE): a phase II, open-label, multicenter, multinational cardiac safety study. Ann Oncol. 2018 Mar;29(3):646–53.*

34. *Nitz U, Gluz O, Christgen M, Grischke E-M, Augustin D, Kümmel S, et al. Final analysis of WSG-ADAPT HER2+/HR- trial: Efficacy, safety, and predictive markers for 12-weeks of neoadjuvant dual blockade with trastuzumab + pertuzumab ± weekly paclitaxel in HER2+/HR- early breast cancer (EBC). J Clin Oncol. 2016 May 20;34(15_suppl):518–518.*

35. *van Ramshorst MS, van der Voort A, van Werkhoven ED, Mandjes IA, Kemper I, Dezentjé VO, et al. Neoadjuvant chemotherapy with or without anthracyclines in the presence of dual HER2 blockade for HER2-positive breast cancer (TRAIN-2): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2018 Dec;19(12):1630–40.*

36. *Oostra D, Macrae E. Role of trastuzumab emtansine in the treatment of HER2-positive breast cancer. Breast Cancer Targets Ther. 2014 Jul;103.*

37. *Krop IE, Kim S-B, González-Martín A, LoRusso PM, Ferrero J-M, Smitt M, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. Lancet Oncol. 2014 Jun;15(7):689–99.*

38. *Perez EA, Barrios C, Eiermann W, Toi M, Im Y, Conte P, et al. Trastuzumab emtansine with or without pertuzumab versus trastuzumab with taxane for human epidermal growth factor receptor 2–positive advanced breast cancer: Final results from MARIANNE. Cancer. 2019 Nov 15;125(22):3974–84.*

39. *Diéras V, Miles D, Verma S, Pegram M, Welslau M, Baselga J, et al. Trastuzumab emtansine versus capecitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer (EMILIA): a descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. Lancet Oncol. 2017 Jun;18(6):732–42.*

40. *Harbeck N, Gluz O, Christgen M, Braun MW, Kummel S, Potenberg J, et al. Efficacy of 12-weeks of neoadjuvant TDM1 with or without endocrine therapy in HER2-positive hormone-receptor-positive early breast cancer: WSG-ADAPT HER2+/HR+ phase II trial. J Clin Oncol. 2015 May 20;33(15_suppl):506–506.*

41. *Hurvitz SA, Martin M, Symmans WF, Jung KH, Huang C-S, Thompson AM, et al. Pathologic complete response (pCR) rates after neoadjuvant trastuzumab emtansine (T-DM1 [K]) + pertuzumab (P) vs docetaxel + carboplatin + trastuzumab + P (TCHP) treatment in patients with HER2-positive (HER2+) early breast cancer (EBC) (KRISTINE). J Clin Oncol. 2016 May 20;34(15_suppl):500–500.*

42. *DeMichele AM, Moulder S, Buxton M, Yee D, Wallace A, Chien J, et al. Abstract CT042: Efficacy of T-DM1+pertuzumab over standard therapy for HER2+ breast cancer: Results from the neoadjuvant I-SPY 2 TRIAL. In: Clinical Trials [Internet]. American Association for Cancer Research; 2016 [cited 2021 May 10]. p. CT042–CT042. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/1538-7445.AM2016-CT042*

43. *Masuda N, Ohtani S, Takano T, Inoue K, Suzuki E, Nakamura R, et al. A randomized, 3-arm, neoadjuvant, phase 2 study comparing docetaxel + carboplatin + trastuzumab + pertuzumab (TCbHP), TCbHP followed by trastuzumab emtansine and pertuzumab (T-DM1+P), and T-DM1+P in HER2-positive primary breast cancer. Breast Cancer Res Treat. 2020 Feb;180(1):135–46.*

44. *Vernieri C, Milano M, Brambilla M, Mennitto A, Maggi C, Cona MS, et al. Resistance mechanisms to anti-HER2 therapies in HER2-positive breast cancer: Current knowledge, new research directions and therapeutic perspectives. Crit Rev Oncol Hematol. 2019 Jul;139:53–66.*

45. *Wong K-K, Fracasso PM, Bukowski RM, Lynch TJ, Munster PN, Shapiro GI, et al. A Phase I Study with Neratinib (HKI-272), an Irreversible Pan ErbB Receptor Tyrosine Kinase Inhibitor, in Patients with Solid Tumors. Clin Cancer Res. 2009 Apr 1;15(7):2552–8.*

46. *Chan A, Delaloge S, Holmes FA, Moy B, Iwata H, Harvey VJ, et al. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2016 Mar;17(3):367–77.*

47. *Awada A, Dirix L, Manso Sanchez L, Xu B, Luu T, Diéras V, et al. Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. Ann Oncol. 2013 Jan;24(1):109–16.*

48. *Abraham J, Montero AJ, Jankowitz RC, Salkeni MA, Beumer JH, Kiesel BF, et al. Safety and Efficacy of T-DM1 Plus Neratinib in Patients With Metastatic HER2-Positive Breast Cancer: NSABP Foundation Trial FB-10. J Clin Oncol. 2019 Oct 10;37(29):2601–9.*

49. *Freedman RA, Gelman RS, Anders CK, Melisko ME, Parsons HA, Cropp AM, et al. TBCRC 022: A Phase II Trial of Neratinib and Capecitabine for Patients With Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer and Brain Metastases. J Clin Oncol. 2019 May 1;37(13):1081–9.*

50. *Martin M, Bonneterre J, Geyer CE, Ito Y, Ro J, Lang I, et al. A phase two randomised trial of neratinib monotherapy versus lapatinib plus capecitabine combination therapy in patients with HER2+ advanced breast cancer. Eur J Cancer. 2013 Dec;49(18):3763–72.*

51. *Saura C, Oliveira M, Feng Y-H, Dai M-S, Chen S-W, Hurvitz SA, et al. Neratinib Plus Capecitabine Versus Lapatinib Plus Capecitabine in HER2-Positive Metastatic Breast Cancer Previously Treated With ≥ 2 HER2-Directed Regimens: Phase III NALA Trial. J Clin Oncol. 2020 Sep 20;38(27):3138–49.*

52. *Research C for DE and. FDA approves neratinib for metastatic HER2-positive breast cancer. FDA [Internet]. 2020 Feb 26 [cited 2021 May 10]; Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-neratinib-metastatic-her2-positive-breast-cancer*

53. *Blair HA. Pyrotinib: First Global Approval. Drugs. 2018 Nov;78(16):1751–5.*

54. *Moulder SL, Borges VF, Baetz T, Mcspadden T, Fernetich G, Murthy RK, et al. Phase I Study of ONT-380, a HER2 Inhibitor, in Patients with HER2 $^{+}$ -Advanced Solid Tumors, with an Expansion Cohort in HER2 $^{+}$ Metastatic Breast Cancer (MBC). Clin Cancer Res. 2017 Jul 15;23(14):3529–36.*

55. *Murthy RK, Hamilton EP, Ferrario C, Aucoin N, Falkson CI, Chamberlain MC, et al. Clinical benefit of tucatinib after isolated brain progression: A retrospective pooled analysis of tucatinib phase 1b studies in HER2+ breast cancer. J Clin Oncol. 2018 May 20;36(15_suppl):1015–1015.*

56. *Pheneger T, Bouhana K, Anderson D, Garrus J, Ahrendt K, Allen S, et al. Abstract #1795: In Vitro and in vivo activity of ARRY-380: A potent, small molecule inhibitor of ErbB2. Cancer Res. 2009 May 1;69(9 Supplement):1795–1795.*

57. *Research C for DE and. FDA approves tucatinib for patients with HER2-positive metastatic breast cancer. FDA [Internet]. 2020 Apr 20 [cited 2021 May 10]; Available from:*

https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-tucatinib-patients-her2-positive-metastatic-breast-cancer

58. *Bang YJ, Giaccone G, Im SA, Oh DY, Bauer TM, Nordstrom JL, et al. First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. Ann Oncol. 2017 Apr;28(4):855–61.*

59. *Nordstrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcγ receptor binding properties. Breast Cancer Res. 2011 Dec;13(6):R123.*

60. *Rugo HS, Im S-A, Wright GLS, Escriva-de-Romani S, DeLaurentiis M, Cortes J, et al. SOPHIA primary analysis: A phase 3 (P3) study of margetuximab (M) + chemotherapy (C) versus trastuzumab (T) + C in patients (pts) with HER2+ metastatic (met) breast cancer (MBC) after prior anti-HER2 therapies (Tx). J Clin Oncol. 2019 May 20;37(15_suppl):1000–1000.*

61. *Weisser N, Wickman G, Davies R, Rowse G. Abstract 31: Preclinical development of a novel biparatopic HER2 antibody with activity in low to high HER2 expressing cancers. In: Experimental and Molecular Therapeutics [Internet]. American Association for Cancer Research; 2017 [cited 2021 May 10]. p. 31–31. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/1538-7445.AM2017-31*

62. *Hinner MJ, Aiba RSB, Jaquin TJ, Berger S, Dürr MC, Schlosser C, et al. Tumor-Localized Costimulatory T-Cell Engagement by the 4-1BB/HER2 Bispecific Antibody-Anticalin Fusion PRS-343. Clin Cancer Res Off J Am Assoc Cancer Res. 2019 Oct 1;25(19):5878–89.*

63. *Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer. N Engl J Med. 2020 Feb 13;382(7):610–21.*

64. *Rinnerthaler G, Gampenrieder S, Greil R. HER2 Directed Antibody-Drug-Conjugates beyond T-DM1 in Breast Cancer. Int J Mol Sci. 2019 Mar 5;20(5):1115.*

65. *Commissioner O of the. FDA approves new treatment option for patients with HER2-positive breast cancer who have progressed on available therapies [Internet]. FDA. FDA; 2020 [cited 2021 May 10]. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-option-patients-her2-positive-breast-cancer-who-have-progressed-available*

66. *Humphreys RC, Kirtely J, Hewit A, Biroc S, Knudsen N, Skidmore L, et al. Abstract 639: Site specific conjugation of ARX-788, an antibody drug conjugate (ADC) targeting HER2, generates a potent and stable targeted therapeutic for multiple cancers. In: Experimental and Molecular Therapeutics [Internet]. American Association for Cancer Research; 2015 [cited 2021 May 10]. p. 639–639. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/1538-7445.AM2015-639*

67. *Hamblett KJ, Hammond PW, Barnscher SD, Fung VK, Davies RH, Wickman GR, et al. Abstract 3914: ZW49, a HER2-targeted biparatopic antibody-drug conjugate for the treatment of HER2-expressing cancers. In: Experimental and Molecular Therapeutics [Internet]. American Association for Cancer Research; 2018 [cited 2021 May 10]. p. 3914–3914. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/1538-7445.AM2018-3914*

68. *Dokter W, Ubink R, van der Lee M, van der Vleuten M, van Achterberg T, Jacobs D, et al. Preclinical Profile of the HER2-Targeting ADC SYD983/SYD985: Introduction of a New Duocarmycin-Based Linker-Drug Platform. Mol Cancer Ther. 2014 Nov;13(11):2618–29.*

69. *Li J, Toader D, Perry SR, Muniz-Medina V, Wetzel L, Rebelatto MC, et al. Abstract 2970: MEDI4276, a HER2-targeting antibody tubulysin conjugate, displays potent in vitro and in vivo activity in preclinical studies. In: Experimental and Molecular Therapeutics [Internet]. American Association for Cancer Research; 2016 [cited 2021 May 10]. p. 2970–2970. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/1538-7445.AM2016-2970*