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Advances and future directions in the targeting of HER2-positive breast cancer: Implications for the future

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Opinion Statement

The natural history of HER2-positive breast cancer significantly changed in the past 15 years. Form being the most aggressive type of breast cancer, it became a treatable with important cure rates. However, with new and successful drugs, resistance emerges. Progress in research and drug development continues to make available effective anti-HER2 therapies. Our challenge today is to use these tools correctly by looking at the data that supports the indications of each compound, and to continue clinical trial participation.

Keywords

HER2-positive breast cancer; trastuzumab resistance; pertuzumab; T-DM1; PI3K signaling; immunotherapy

Introduction

The ligand-orphan receptor, human epidermal growth factor receptor 2 (HER2), represents a prominent target in breast cancer with approximately 20%–30% of patients with primary invasive breast cancers overexpressing the HER2 receptor.[1,2] Indeed, high levels of HER2 overexpression or gene amplification have been definitively associated with a more aggressive disease phenotype, with a shorter time to relapse after initial treatment and as a significant predictor of survival.[3,4] Understanding the biology of HER2 is fundamental to maximizing its clinical therapeutic efficacy and ultimately deciphering mechanisms of resistance to anti-HER2 therapies. The HER2 gene, a proto-oncogene that maps to chromosome 17q21, encodes the HER2 receptor, a 1255 amino acid, 185kD transmembrane glycoprotein with an intracellular domain with tyrosine kinase catalytic activity.[5] Ligand binding into the receptor produces activation signals leading to the autophosphorylation of a terminal carboxyl segment and heterodimerization of other family members (EGFR, HER3 and HER4) and HER2, leading to a cascade of intracellular events promoting cell growth, proliferation, and metastasis.[6] Indeed the fundamental role of HER2 in receptor signaling cascade has guided the development of anti-HER2 monoclonal antibodies in cancer therapeutics, particular trastuzumab, the humanized monoclonal antibody widely in use against HER2-overexpressing breast cancers. In this review, we will review the impact of

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trastuzumab as the foundation of therapy as well as mechanisms of resistance to trastuzumab and the consequent robust development of novel therapeutics for patients with HER2-positive breast cancers.

Trastuzumab: a success story

The humanized monoclonal antibody trastuzumab (Herceptin; F Hoffmann-La Roche, Basel, Switzerland and Genentech, San Francisco,CA), targets the extracellular domain of the HER2 receptor, and has established clinical benefits in HER2-positive breast cancer in both early disease as well as metastatic settings.[7] Early trials demonstrated the clinical benefit in the metastatic disease setting either as monotherapy or in combination with chemotherapy in the first- or second-line settings. The addition of trastuzumab to standard first-line chemotherapy lead to a longer time to disease progression (TTP), a higher rate of objective response (ORR), a more durable response, better overall survival (OS), and a 20 percent risk reduction of death.[8–12] Dramatic benefits in the metastatic setting led to investigations into the role for trastuzumab as adjuvant treatment concurrently with chemotherapy for patients with patients with HER2-positive early stage breast cancer, defined as stage I to III. Four large randomized phase 3 trials tested various treatment regimens using a trastuzumab-backbone - Herceptin® Adjuvant (HERA), National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31, North Central Cancer Treatment Group (NCCTG) N9831, and Breast Cancer International Research Group (BCIRG) 006. Together these trials investigated 13000 women with HER2-positive early breast cancer and demonstrated a 50% reduction in the 3-year risk of recurrence in this population despite variations in dosing schedules, patient selection as well as concurrent chemotherapy regimens.[13] The largest of these four trials, the HERA trial (Breast International Group 01-01), was an international, multicenter, randomized, open-label, phase III trial comparing therapy with trastuzumab for a period of 1 and 2 years with observation after standard (neo)adjuvant, chemotherapy in patients with HER2-positive early breast cancer.[14] The primary endpoint was disease-free survival (DFS). Overall, 1698 patients randomized to the observation arm and 1703 to the 1-year trastuzumab arm. The planned interim analysis at a 1-year median follow-up time point demonstrated an improved DFS with the addition of trastuzumab after standard adjuvant chemotherapy when compared to chemotherapy alone (hazard ratio [HR] 0·54; 95% CI 0·43–0·67).[14] After this positive first interim analysis, patients in the observation group who had not relapsed and had preserved cardiac function were allowed to cross over to receive trastuzumab. A second analysis at the 2-year median follow-up period again demonstrated an improvement in DFS and OS with the addition of trastuzumab when compared with chemotherapy alone.[15] A more recently updated analysis after a median follow-up of 4 years was reported with particular emphasis on the outcomes of the 885 out of 1698 patients (52%) who crossed over from the chemotherapy alone arm to add trastuzumab to their regimen.[16] The intention-to-treat analysis showed an improved DFS among patients in the 1-year trastuzumab group compared to the observation alone arm (DFS 78·6% vs. 72·2%; HR 0·76; 95% CI 0·66–0·87; $p<0·0001$). The patients in the non-randomized crossover cohort also demonstrated a fewer DFS events than patients remaining in the observation group (adjusted HR 0·68; 95% CI 0·51–0·90; $p=0·0077$), confirming the clinical benefit of treatment with adjuvant trastuzumab for 1 year after chemotherapy. Similarly, the interim analysis from the three other major phase 3 trials (NSABP B-31, NCCTG N9831, BCIRG 006) reinforced the clinical benefit of trastuzumab in the adjuvant setting for early breast cancer with all three studies consistently demonstrating an improved DFS and OS benefit with an acceptable toxicity profile regardless of nodal status, hormone receptor status, tumor size, or age.[15,17,18]

Mechanisms of resistance to trastuzumab

As trastuzumab emerged as the foundation of therapy for the patient with HER2-positive breast cancer, development of resistance after initial robust response (acquired resistance) or a lack of response from initiation among patients with HER2-overexpressing cancers (*de novo* resistance) were observed – indeed, the modest overall response rates to trastuzumab-based regimen (~26% as monotherapy and 40–60% in combination with chemotherapy) prompted a greater study into both the mechanisms of antitumor activity and therapeutic relapse of trastuzumab, with the aim of developed better rationally designed HER2-targeted therapy.[8,11,19] As a whole, the downstream effects of trastuzumab binding to extracellular domain of HER2 leads to cell proliferation and survival through direct and indirect methods including activating the antibody-dependent cellular cytotoxicity (ADCC) mechanism, preventing formation of a truncated constitutively active form of HER2, blocking ligand-independent HER2 signaling as well as inhibiting HER2-mediated angiogenesis.

Perhaps the most well recognized mechanism of action of trastuzumab has been interference of the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) signaling pathways, where trastuzumab-associated interference of ligand-independent HER2 dimerization leads to uncoupling from PI3K activity, leading to downregulation of proximal and distal AKT signaling via the suppression of Akt phosphorylation.[20] Further suppression of this cascade has been reported by the indirect blocking of Src kinase signaling after trastuzumab binds to HER2, leading to increased levels and activity of the tumor suppressor phosphatase and tensin homolog (PTEN) and a consequent decrease in cell proliferation.[21,22]

Additional antitumor activity has been demonstrated by trastuzumab its effect on inducing the antibody-dependent cellular cytotoxicity (ADCC) mechanism wherein binding of trastuzumab to HER2-overexpressing tumor cells leads to increased recruitment of natural killer (NK) cells by a CD-16 mediated mechanism. Immune testing of tumors treated with trastuzumab in combination with chemotherapy have shown increased presence of cytotoxic proteins and NK cells, leading to a synergistic tumor response particularly when trastuzumab is given in combination with taxanes.[23,24] Alterations in these mechanisms of antitumor activity are proposed as mechanisms of resistance, both de novo and acquired, roughly characterized as the upregulation of other receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-IR), and c-MET leading to activation of PI3K/Akt cascade, and steric hindrance of the receptor-antibody complex from structural alterations to HER2 extracellular domain leading to truncated forms of HER2 that remain constitutively active form and may promote trastuzumab resistance.[25,26]

Aberrations to receptor tyrosine kinases and their downstream signaling targets leading to the activation of the PI3K signaling pathway have been demonstrated to be present prior to initiation of therapy or have developed after exposure to trastuzumab, as is the case proposed in acquired resistance.[22,27]*In vitro* models of HER-2 overexpressing breast cancers demonstrate a reduction in trastuzumab-mediated growth arrest in models with increased IGF-IR expression, where trastuzumab sensitivity was reintroduced with IGF-IR activation was blocked with the expression of IGF-BP3.[28] Similarly, the presence of *de novo* has been attributed to the constitutive activation of the PI3K pathway in the setting of PTEN loss or mutations within the *PIK3CA* gene. HER2-overexpressing breast cancer cell lines shown to be resistant to trastuzumab exhibited greater levels of phosphorylated Akt and consequently Akt kinase activity in comparison to parental cells.[29] Moreover, PTEN-deficient HER2-overexpressing breast cancers not only demonstrated an inferior response to trastuzumab-combination therapy but also treatment *in vitro* and *in vivo* of PTEN-deficient cells with

novel PI3K inhibitors overcame trastuzumab resistance, suggesting a role for PTEN as a predictive marker of response to trastuzumab therapy and highlighting a role for PI3K inhibitors in a subset of patients with PTEN loss who develop resistance to trastuzumab.[21]

Additional markers identified within HER2-expressing breast cancers include the oncogene c-MET and its only known ligand hepatocyte growth factor (HGF), which have been shown to be overexpressed in about 25% of HER2-positive breast cancers as well as the tumor stroma and overexpression of c-Met has been associated with a poor prognosis.[30–32] Furthermore, trastuzumab was shown to precipitously upregulate c-MET expression *in vitro* while cellular depletion of c-MET lead to increased cellular sensitivity to trastuzumab, thereby indicating a crucial role for c-MET overexpression in the development of acquired resistance to trastuzumab.[33] Emerging patterns of resistance to trastuzumab prompted developed of alternated therapeutics targeting the HER2 signaling.

Targeting receptor dimerization

Pertuzumab is a first in class of HER2 dimerization inhibitors that block HER2 dimerization with other HER family members; this recombinant humanized monoclonal antibody (2C4) that binds to extracellular dimerization domain II of the HER-2 receptor, thereby inhibiting its ability to dimerize with other ligand-activated HER receptors, most notably HER3.[34] Pertuzumab's binding site lies within domain II where it does not correspond with the subdomain IV of HER-2 that is recognized by trastuzumab.[35] Pertuzumab's inhibition of dimerization with ligand-activated HER receptors works in complement with trastuzumab's blocking of ligand-independent HER2-mediated signaling and HER2 cleavage.[36] Both drugs have been shown to stimulate the antibody-dependent cellular cytotoxicity mechanism.[37]

Early *in vitro* and *in vivo* models demonstrated promising activity of pertuzumab, particularly when used in combination with trastuzumab.[38] Indeed HER2-positive breast cancer xenograft models demonstrated a greater extent of tumor regression with the combination of trastuzumab and pertuzumab than with either agent alone.[37] Furthermore, the xenograft models showed tumor regression with combination therapy even after progression on treatment with single-agent trastuzumab. These findings paved the way for early phase trials with the trastuzumab-pertuzumab doublet in patients with HER2-positive breast cancer who had experienced disease progression after prior therapy with a trastuzumab-based regimen.[39] This multicenter, open-label, single-arm study enrolled 66 patients and reported an objective response rate of 24.2% and a clinical benefit rate of 50% defined as patients who experienced a complete response (n=5, 7.6%), a partial response (n=11, 16.7%), or a prolonged stable disease lasting 6 months or longer (n=17, 25.8%). This work affirmed the findings reported in preclinical models of a synergistic increase in antitumor activity with the addition of pertuzumab to a trastuzumab-backbone after progression to prior trastuzumab therapy and confirmed the clinical efficacy of dual target inhibition of the HER2 signaling pathways.

To identify the role for pertuzumab in the front-line setting of HER2-positive metastatic breast cancer, the Clinical Evaluation of Pertuzumab and Trastuzumab (CLEOPATRA) study was designed to assess the efficacy and safety of pertuzumab plus trastuzumab plus docetaxel, as compared with placebo plus trastuzumab plus docetaxel. The CLEOPATRA study, a randomized, double-blind, placebo-controlled, phase 3 trial, enrolled patients with HER2-positive metastatic breast cancer who were treatment-naïve for their metastatic disease with a primary end point of progression-free survival (PFS) and secondary end points including OS, ORR, and safety.[40] Overall, 808 patients were randomized. At the prespecified interim analysis conducted after 165 events, the pertuzumab arm demonstrated a significantly prolonged PFS (18.5 months vs. 12.4 months), (HR 0.62; 95% CI, 0.51 to

0.75; P<0.001). The improvement in PFS with the addition of pertuzumab therapy was demonstrated across all predefined patient subsets, including age, ethnicity, prior chemotherapy, hormone-receptor status and HER2 status as determined by immunohistochemistry as compared to fluorescence in situ hybridization. Subsequent final analysis conducted at a median follow-up time of 30 months after 267 deaths.[41] The median OS was not reached in the pertuzumab group (95% CI 42.4–not estimable [NE]) as compared to 37.6 months (95% CI 34.3–NE) in the placebo group (HR 0.66, 95% CI 0.52–0.84; P=0.0008).[41] Rates of serious adverse events most commonly neutropenia, diarrhea, febrile neutropenia, pneumonia, and cellulitis were noted at a higher frequency of at least 5% in the pertuzumab groups; however, rates of all adverse events dropped significantly upon discontinuation of docetaxel.

Concurrent efforts to bring pertuzumab to early breast cancer led to the design of the multicenter, open-label, phase II study of neoadjuvant pertuzumab and trastuzumab in women with operable, locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere).[42] Overall 417 patients were randomized to one of four study groups where they would receive four cycles of either trastuzumab plus docetaxel (group A), pertuzumab and trastuzumab plus docetaxel (group B), pertuzumab and trastuzumab (group C), or pertuzumab plus docetaxel (group D), with the primary end-point being pathological complete response (pCR) in the breast. Patients in group B receiving pertuzumab and trastuzumab plus docetaxel had a significantly higher rate of pCR (49 of 107 patients; 45.8%) when compared to group A (trastuzumab plus docetaxel; 31 of 107; pCR rate 29·0%), group D (pertuzumab plus docetaxel, 23 of 96 patients, pCR rate 24·0%) or group C (pertuzumab and trastuzumab, 18 of 107 patients, pCR rate 16·8%) with a favorable adverse events profile. These results substantiate the broadened investigations for pertuzumab and trastuzumab combination in early breast cancer, and the application for approval in the neoadjuvant setting to optimize outcomes for breast cancer. An ongoing randomized, double-blind, placebo-controlled study investigates pertuzumab in addition to chemotherapy plus trastuzumab as adjuvant therapy in patients with operable HER2-positive primary breast cancer.[43] In this study being conducted in collaboration with the Breast International Group (BIG), patients are randomized after surgery to receive either pertuzumab or placebo for one year with 6–8 cycles of chemotherapy and 1 year of trastuzumab with the primary endpoint of invasive disease-free survival (IDFS).

Antibody-drug conjugates

Ado-trastuzumab-DM1 (T-DM1, Kadcyla, Genentech) represents a novel class of anti-HER2 targeting agents which are conjugates of antibody and drug (ADC), where a highly potent cytotoxic agent is selectively delivered to antigen-expressing cells with the intention of improving the drug's therapeutic index.[44] T-DM1 is a conjugate of the HER2-targeting trastuzumab with DM1, a potent derivative of the antimicrotubule agent maytansine with a 100- to 1000-fold higher cytotoxic potency than clinically used anticancer drugs.[45] Preclinical and *in vivo* models of ADCs utilizing synthesized maytansinoids demonstrate a high antigen-specific cytotoxicity, low systemic toxicity, and suitable pharmacokinetic behavior.[44] The first-in-human phase I study of T-DM1 enrolled patients with advanced HER2-positive breast cancer and demonstrated a mild, reversible toxicity with a noteworthy clinical activity in the heavily pretreated patients.[46] Of the 15 patients on study, the clinical benefit rate (objective response plus stable disease at 6 months) was 73% with a confirmed response rate of 44% among the 9 patients with measurable disease treated at the maximum tolerated dose (MTD). The subsequent single-arm phase II study (TDM4258g) enrolled patients with HER2-positive metastatic breast cancer who were previously treated with disease progression on anti-HER2 therapy and chemotherapy.[47] The 112 treated patients received intravenous T-DM1 every 3 weeks to a median follow-up of at least 12 months.

The study demonstrated an the objective response rate of 25.9% (95% CI, 18.4% to 34.4%) and a median PFS of 4.6 months (95% CI, 3.9 to 8.6 months) while the median duration of response was not reached (lower limit of 95% CI, 6.2 months).

Similarly, a second, single-arm, phase II study of T-DM1 administered every 3 weeks assessed patients with HER2-positive metastatic breast cancer with prior exposure to trastuzumab, lapatinib, an anthracycline, a taxane, and capecitabine; the primary objectives were safety and overall response rate.[48] The study enrolled 110 heavily pretreated patients who received a median of 7 prior agents in the metastatic setting and demonstrated an ORR of 34.5% (95% CI, 26.1% to 43.9%) and a median PFS of 6.9 months (95% CI, 4.2 to 8.4 months); the median duration of response was 7.2 months (95% CI, 4.6 months to not estimable). The ORR was higher among patients who had confirmatory testing of their HER2 positivity by retrospective central testing – among this subset, the response rate was 41.3% (95% CI, 30.4% to 52.8%), and median PFS was 7.3 months (95% CI, 4.6 to 12.3 months).

To explore the role for single-agent T-DM1 as first-line treatment of metastatic HER2 positive breast cancer, a randomized, phase II study enrolled 137 patients with HER2-positive MBC or recurrent locally advanced breast cancer.[49] Patients were treated until disease progression or unacceptable toxicity with the primary end points being PFS and safety and secondary end points including OS, objective response rate ORR, duration of objective response, and quality of life. Patients receiving first-line T-DM1 monotherapy demonstrated a significantly improved PFS (14.2 months vs. 9.2 months, HR, 0.59; 95% CI, 0.36 to 0.97) and an improved ORR (64.2% vs. 58.0%) when compared to trastuzumab and docetaxel. Patients on the T-DM1 arm also have fewer grade 3 or greater adverse events (46.4% vs. 90.9%) and a lower rate of adverse events leading to treatment discontinuation (7.2% vs. 40.9%), and serious adverse events (20.3% vs. 25.8%). Preliminary survival analysis demonstrated a comparable OS in both treatment arms. The promising clinical benefit observed in these phase II trials prompted the design of several ongoing phase III studies. EMILIA (Emtansine vs. Capecitabine+ Lapatinib in Patients with HER2-Positive Locally Advanced or Metastatic Breast Cancer) randomly assigned 991 patients with HER2-positive, unresectable, locally advanced or metastatic breast cancer, previously treated with trastuzumab and a taxane to two treatment arms: T-DM1 vs. lapatinib plus capecitabine, with the primary end points of independently-assessed PFS, OS, and safety.[50] Pre-specified secondary end points included investigator-assessed PFS, the ORR, the duration of response, and the time to symptom progression. Overall, patient treated with T-DM1 demonstrated a significantly improved, independently assessed median PFS of 9.6 months compared to 6.4 months in the lapatinib plus capecitabine arm (HR 0.65; 95% CI, 0.55 to 0.77; $P<0.001$) with an higher ORR of 43.6% with T-DM1 compared to 30.8% with lapatinib plus capecitabine ($P<0.001$). Furthermore, data for all additional secondary end points supported a better clinical benefit profile for T-DM1. The success of the EMILIA trial has prompted further promising efforts to improve the efficacy and potency of HER2-targeting therapies.

Several ongoing late phase trials are aiming to identify the exact role for T-DM1 in the treatment algorithm for HER2-positive breast cancers both in the metastatic and in the adjuvant settings. One such trial, the KATHERINE study, was developed as a collaboration between the National Surgical Adjuvant Breast and Bowel Project (NSABP) and the German Breast Group.[51] This trial randomizes patients with HER2-positive breast cancer with residual tumor in the breast or axillary lymph nodes following preoperative therapy to receive T-DM1 versus trastuzumab as adjuvant therapy every 3 weeks for 14 cycles.

The completed MARIANNE trial is a randomized, three arm, phase III study to evaluate T-DM1 plus pertuzumab placebo (blinded for pertuzumab) versus T-DM1 plus pertuzumab

versus trastuzumab plus a taxane (docetaxel or paclitaxel) in patients with HER2-positive locally advanced, recurrent or previously untreated metastatic breast cancer as front-line therapy.[52] Patients will be randomized to 1 of 3 treatment arms (Arms A, B or C). Arm A will be open-label, whereas Arms B and C will be blinded. The results from these phase III studies remain eagerly anticipated as the development of T-DM1 continues not only as single agent therapy in metastatic breast cancer but also an eventual role in the management of early-stage breast cancer. A randomized, multicenter, two-arm, open-label study (named TH3RESA) will evaluate the efficacy and safety of T-DM1 in in the third-line of therapy in comparison with treatment of the physician's choice in patients with metastatic or unresectable locally advanced/recurrent HER2-positive breast cancer who have received at least two prior regimens targeting HER2.[53]

Targeting PI3K and MAPK signaling pathways

With the advent of novel therapies targeting aberrant cell signaling downstream the HER2 receptors, several inhibitors of the oncogenic singling within the MAPK and PI3K cascades have emerged as potential drugs of interest in HER2-postive breast cancers. The impact of HER2 inhibition using trastuzumab on these alternate signaling pathways has been implicated as both a mechanism of response, but also resistance to therapy with trastuzumab. Consequently the prospect of dual inhibition of HER2 and the PI3K pathways with combination agents has drawn interest.

A phase I, dose escalation study combined everolimus, an inhibitor of mammalian target of rapamycin (mTOR), with paclitaxel and trastuzumab in patients with HER2-overexpressing metastatic breast cancer previously treated with trastuzumab.[54] Overall, therapy was generally well tolerated with ORR of 44% and disease control for 6 months or more seen in 74% of patients. This early evidence of antitumor activity is particularly promising in these patients, most of whom (32 of the 33 enrolled patients) were deemed resistant to trastuzumab.

A pooled analysis combined results from two trials running concurrently at 3 institutions and included 47 patients with HER2-overexpressing metastatic breast cancer who had progressed on trastuzumab-based therapy.[55] Patients received trastuzumab every 3 weeks in combination with everolimus daily. Of 47 patients treated on study, seven (15%) demonstrated a partial response to the combination while nine patients (19%) had a prolonged stable disease lasting 6 months or longer, with an overall clinical benefit rate of 34% and a median PFS of 4.1 months. Early subset analysis using molecular markers showed that patients with PTEN loss had a shower overall survival (P=.048).

This benefit for mTOR-based therapies in HER2-positive disease was further explored with 2 large, phase III studies, BOLERO-1 and BOLERO-3. BOLERO-1, a randomized, phase III, double-blind, placebo-controlled multicenter trial, randomized women with HER2-positive, locally advanced or metastatic breast cancer to receiving everolimus in combination with trastuzumab and paclitaxel as first-line therapy to assess the efficacy of adding the mTOR inhibitor to first-line standard therapy in HER2-positive advanced disease.[56] Similarly, BOLERO-3, a phase III, double-blind, placebo-controlled multinational study, assesses the combination everolimus, vinorelbine, and trastuzumab compared to the combination placebo, vinorelbine and trastuzumab in HER2/neu positive women with locally advanced or metastatic breast cancer who are resistant to trastuzumab and have been pre-treated with a taxane.[57] The study met its primary endpoint of improvement in PFS with the addition of everolimus to the standard regimen of vinorelbine and trastuzumab, reducing the risk of disease progression by 22% (HR 0.78, 95% CI, 0.65 to 0.95, p<0.01). The median

time to progression was 7.0 months in the everolimus combination arm as compared to 5.8 months in the placebo combination arm. Definitive analysis for OS is ongoing.

Immunotherapeutics targeting HER2

HER2's selective over-expressivity on a specific subset of breast cancer tumors makes it a suitable attractive target against which to induce a potent immune response – indeed passive immunity through the use of anti-HER2 monoclonal antibodies forms the mainstay of therapy for these patients. The most in depth studies to date have investigated the role for HER2-derived peptide vaccines, with encouraging early evidence of inducing durable immunologic responses in these small clinical trials. One such vaccine employs the HLA class-I peptide E75 constructed from the HER2 extracellular domain to stimulates cytotoxic T lymphocytes (CTLs).[58] A series of small phase I trials using E75 as monotherapy or in combination with other immune adjuvants established its safety and early evidence of durable immunity inducing peptide-specific response in the CTLs when used in the locally advanced or metastatic setting.[59–61] To assess the clinical activity of HER2 peptide vaccines in the adjuvant setting, two phase II trials studied 186 women with HER-2 positive disease after completion of a standard course of surgery, chemotherapy and radiation, with one trial enrolled patients with node-positive disease and the second node-negative.[62] The combined analyses reports on 101 HLA-A2 and HLA-A3 patients who were vaccinated and 85 patients who were followed prospectively as controls. A dose-dependent immunologic response to the vaccine was reported with the preplanned interim analysis at a median follow-up time of 20 months demonstrating a 5.6% recurrence rate in the vaccinated patients in comparison to 14.2% in the controls (P=0.04). This study also noted a diminishing vaccine-specific immunity over time particularly after the risk of recurrence lost significance after 2 years of follow-up, suggesting that future prospective trials should adopt intermittent booster dose(s) to decrease the risk of recurrence.

Beyond vaccine based therapies, recent rapid development in immune-based therapies have emphasized the role for regulating the T cell activation and tolerance by targeting signaling through the T cell's costimulatory and coinhibitory receptors. Specifically, the programmed death-1 (PD-1), a member of the CD28/CTLA-4 family of costimulatory receptors, its ligand (PD-L1) along with the cytotoxic T-lymphocyte antigen 4 (CTLA-4) function as inhibitory signal to the T cell. Recent trials particularly in melanoma, non-small cell lung cancer and renal cell carcinoma reveal the promising roles for targeting PD-1, PD-L1 and CTLA-4 in release their inhibitory signaling on the T lymphocytes, thereby activating the immune system and producing durable responses.[63,64]

Greater efforts are underway to characterize the presence and clinical significance of immune infiltrates in breast cancer in an effort to highlight the importance of PD-1/PD-L1 signaling pathway. Preclinical studies demonstrate increased expression of PD-1 in up to 70% of tumor infiltrating lymphocytes (TIL) when compared to the noncancerous breast tissue where the expression level was 30%.[65] Additional data has revealed correlates between the presence of TIL expressing PD-1 with histologic grade, and hormone receptor status. Furthermore, a subset of breast cancer specimen with HER2 positivity demonstrated a significantly higher intratumor expression of PD-L1, thereby reducing the immunogenicity of tumor-reactive T lymphocytes.[66] Taken together, the preclinical data supports a role for the activation of the PD-1/PD-L1 signaling pathway in the breast cancer and highlights the potential impact for antibody therapies targeting this cascade in this disease.

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