Breast Cancer Therapeutics and Biomarkers: Past, Present, and Future Approaches

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ABSTRACT: Breast cancer (BC) is the leading cause of cancer death in women and the second-most common cancer. An estimated 281 550 new cases of invasive BC will be diagnosed in women in the United States, and about 43 600 will die during 2021. Continual research has shed light on all disease areas, including tumor classification and biomarkers for diagnosis/prognosis. As research investigations evolve, new classes of drugs are emerging with potential benefits in BC treatment that are covered in this manuscript. The initial sections present updated classification and terminology used for diagnosis and prognosis, which leads to the following topics, discussing the past and present treatments available for BC. Our review will generate interest in exploring the complexity of the cell cycle and its association with cancer biology as part of the plethora of target factors toward developing newer drugs and effective therapeutic management of BC.

KEYWORDS: Breast cancer, anti-cancer therapy, clinical trial, hormone receptor, anti-cancer drug resistance, FDA

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Introduction

Breast cancer (BC) is the leading cause of cancer death in women and the second-most common cancer overall, with 2 million new cases diagnosed in 2018 and more than 600 000 deaths attributed to the disease.[1]

In the United States, an estimated 281 550 new cases of invasive BC will be diagnosed in women, and about 43 600 will die during 2021.[2] This scenario is prompting continual research into all areas of the disease—including tumor classification and proposed therapeutic interventions.

Historically, BC was classified into histological groups, with the 2 most common subtypes being invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), respectively.[3] This classification has since become of secondary importance to molecular approaches.

Molecular classifications of BC have greater utility for prognosis and guiding therapeutic strategies. The implementation of staining for hormone receptor (HR) status, specifically estrogen receptor (ER) and progesterone receptor (PR), has led to a semistandard method to select patients for endocrine therapies involving selective ER modulators (SERM), aromatase inhibitors (AIs), and anthracycline and taxane-based chemotherapy.[4] The other commonly tested protein in BC biopsies is the human epidermal growth factor receptor 2 (HER2),[5] which, among other considerations is used to select for therapies including monoclonal antibodies (mAbs),[6] antibody-drug conjugates (ADCs),[7] tyrosine kinase inhibitors (TKI), poly ADP-ribose polymerase (PARP) inhibitors, and cyclin-dependent kinase (CDK) 4/6 inhibitors.[8] Further information is discussed along with the text within the respective topics.

The latest drugs/classes approved by the US Food and Drug Administration (FDA) exhibiting positive results are ADCs,[9] PARP inhibitors,[10,11] and programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) inhibitors for selected patients.[12] Recently the gamma delta (γδ) *T* lymphocytes have come to light as a potential BC immunotherapy due to their unique biology and established role in cancer immunosurveillance.[13,14] Histone deacetylase inhibitors (HDACi) are another promising class,[15] but not currently approved. An ongoing phase-III trial with the HDACi tucidinostat combined with exemestane has evaluated progression-free survival (PFS) data. It is expected to have mature overall survival (OS) data in early 2021.[16]

This review covers relevant past and current treatments available for BC and biomarkers as targets that show promising results for future treatments. We first present the classification and terminology for diagnosis and prognosis, which leads to the next topics, which describe the drugs' mechanism, main clinical indications, and adverse effects.

Classification and Terminology for Diagnosis and Prognosis

According to the World Health Organization (WHO), based on histomorphology and growth patterns alone, there are 21 histological types of BC that differ in risk factors, presentation, response to treatment, and outcomes.[17] A component that is always included in a pathology report and has been a cornerstone in the determination of BC prognosis is histological classification and grade.[18] The first major division is in situ versus invasive carcinoma. Invasive carcinoma is then broken down

into multiple subtypes, including the most common infiltrating ductal carcinoma and invasive lobular.[19] Determination of HR positivity through immunohistochemistry (IHC) is used in conjunction with histology as a starting point for determining therapeutic management. Tumors can then be classified molecularly for added prognostic value and therapeutic guidance.[20]

Biomarkers for molecular subtyping of BC

Up to 10 different subgroups of molecular BC have been proposed, though 5 main groups have substantial clinical relevance: (1) Luminal A: HR positive (HR+) (ER+ and/or PR+) and HER2 negative (HER2–); (2) Luminal B: HR+, and either HER2 positive (HER2+) or HER2–; (3) HER2-enriched BC: HR negative (HR–) and HER2+; (4) Triple-negative breast cancer (TNBC)/basal-like: HR– and HER2–; (5) Normal-like BC, which like luminal A, is HR+, HER2–, but its prognosis is slightly worse than luminal A.[21-24]

HR+ means that greater than 1% of tumor nuclei express ER and/or PR, as determined by IHC.[25] Two hypotheses explain the estrogen and the ER roles in BC. The first suggests that ER binding stimulates mammary cell proliferation, increasing cell division and DNA synthesis, thereby increasing the risk for replication errors and accumulation of mutations in processes of DNA repair, cell proliferation, and apoptosis.[26] The other hypothesis states that the metabolism of estrogen causes the formation of genotoxic by-products that directly damage DNA, causing mutations. HR+ cancer has the advantage of having a high response rate to hormonal therapy, including SERMs and AIs.[26]

Immunohistochemical staining can reveal HR+ cancer; however, a significant number of women present with resistance or develop resistance during endocrine-based therapies.[27] Estrogen receptor status and mutation, as well as the crosstalk between ER, HER2 signaling pathways, and growth factors, are common contributors to endocrine resistance.[28,29] Other mechanisms that explain these drug resistances include estrogen-independent growth, hypersensitivity to low estrogen concentrations, cyclin D1 overexpression, constitutive nuclear factor kappa B (NFκ-B) activation, upregulation of growth-factor-signaling pathways, and downregulation of ER-alpha expression.[27] The identification of resistance mechanisms leverages fruitful research on biomarkers of clinically significant and the development of new drug classes and targeted therapy.

Approximately 20 to 30% of patients with BC demonstrate overexpression of HER2,[30] a member of the ERBB family of receptor tyrosine kinases (RTK), which is involved in critical cellular functions, including cell growth and survival.[31] Human epidermal growth factor receptor 2 is an oncoprotein connected to the ERBB signaling pathways, mediating cell-cell interactions in organogenesis and adulthood. *ERBB2*, the gene that encodes HER2, may be amplified, leading to the overexpression of HER2 on the surface of BC cells known as HER2+. This overexpression of HER2 causes overactivation of the ERBB2 signaling pathway.[32]

The American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) updated the guideline recommendations for HER2 testing in BC and advocated the improvement of the accuracy of HER2 testing by IHC or in situ hybridization (ISH).[33] Human epidermal growth factor receptor 2+ criteria were defined as HER2 protein overexpression (IHC, microscopic field of vision > 10% of adjacent homogeneous tumor tissue cell region with complete and intense circumferential membrane staining) or gene amplification, ISH, average HER2 copy number ⩾6.0 signals/cell or average HER2 copy number ⩾4.0 signals/cell and HER2/chromosome enumeration probe 17 (CEP17) ratio ⩾2.0. If indeterminate results appear, a reflex test using an alternative assay (IHC or ISH) is required. If the test results do not conform to other histological tests, they should be repeated. The test results from laboratories should be highly consistent with the validated HER2 test, and the test should be carried out in the laboratories certified by CAP or other authorized institutions.[34]

The prognostic implication of HER2 status has also been discussed in the settings of ductal carcinoma in situ (DCIS).[35] Ductal carcinoma in situ represents 20 to 25% of all BC detected by population-based BC screening programs.[35] Although it is not invasive, DCIS has a higher proportion of HER2 amplification than the invasive disease, 34% and 13%, respectively.[35,36] The frequency of HER2 positivity in DCIS was found comparable to invasive BC, and HER2+ and DCIS were associated with poor prognosis features.[37,38]

Guided by selected biomarkers, these molecular subtypes are relevant for prognosis and planning therapeutic strategies. Patients with Luminal A BC have low levels of a biomarker that participates in controlling the rate at which cancer cells grow (Ki-67) and have the best prognosis. Compared with luminal A, the normal-like BC has low levels of Ki-67. Luminal B has high levels of Ki-67 and usually grows slightly faster than luminal A, and the patients' prognosis is slightly worse than luminal A. Human epidermal growth factor receptor 2-enriched cancers tend to grow faster than luminal BC (A and B) and can have a worse prognosis.[24,35,38]

Nowadays, decision-making is based on numerous factors, including tumor morphology and grade classification, tumor size, presence of lymph node metastases, and expression of ER, PR, and HER2 (a review on this topic was published by Fragomeni et al).[39]

The molecular subtype, defined by PAM50, which considers the expression profiles of 50 different genes, was proposed to add prognostic and predictive information gene signature to classify invasive BC into the 5 intrinsic subtypes (luminal A, luminal B, HER2-enriched, basal-like, and normal-like).[40] However, specific properties for the PAM50 subtypes reflect changes in the microenvironment instead of specific molecular changes in epithelial cells, highlighting the importance of the

tumor microenvironment for the progression and disease outcome.[41] Prat et al[42] proposed a surrogate immunohistochemical-based definition of luminal A tumors as HR+/HER2−/Ki-67 less than 14%, and PR+ more than 20%. PAM50 and determination of luminal A or luminal B are further used for the determination of prognosis. Patients with luminal A BC carry a higher 5-year OS rate of nearly 96% compared to a rate of about 86% in luminal B[43] in premenopausal women treated with tamoxifen versus tamoxifen followed by anastrozole, respectively. These molecular subtypes can also be used to assess recurrence rates, with luminal A having a 93.9% distant recurrence-free survival as opposed to 82.2% in luminal B.[44]

When considering the expression of HER2, cancers that are HER2+ have a better prognosis if they are HR+ as well. A study looking at routine clinical care of molecular subtypes of BC found that HR+ and HER2+ cancers had a 5-year OS of 92.5% while HER2+ tumors that were HR− had an OS of 85.6%.[45] This is an improvement from 20 years ago due to the development of anti-HER2 therapies, such as trastuzumab,[46] pertuzumab,[47] lapatinib,[48] neratinib,[49] and ado-trastuzumab emtansine, also known as trastuzumab emtansine (T-DM1).[50] However, resistance and adverse events (AEs) are frequently observed during HER2-directed therapy and are obstacles to the continuous administration of these agents.[51] Therefore, it is crucial to improve anti-HER2 strategies for patients who are intolerant of standard therapies, as well as determine the mechanisms of resistance (for a review, see Chen et al[52]).

Triple-negative BC is considered the most aggressive form of BC and the worst prognostically among the 4 main molecular subtypes.[9,53] Homologous recombination repair (HRR) pathway deficiency results in chromosomal instability, which characterizes TNBC,[54] germline BRCA1/2 mutations, and BRCA gene promoter methylation, and genetic mutations of the HRR pathway are considered the common causes for the deficiency in the HRR pathway.[55] Atchley et al[56] reported more than 80% of BC involving BRCA mutations being triple negative.[57] Studies have shown that TNBC has a higher risk of recurrence and worse prognosis after recurrence than HR+ cancers (median survival 12-18 months vs 50-60 months).[58] Although targeted therapies are becoming part of first-line treatments for several cancers, sequential chemotherapy remains the standard of care for TNBC due to the lack of receptor expression for targeting.[58] The OS for TNBC is lower than all non-TNBC, regardless of the staging of the disease.[59]

Testing for ER, PR, and HER2 status has been standard in the BC evaluation for some time, though other biomarkers are also emerging in research data.[60] The predictive value of different biomarkers is under investigations, such as estrogen-receptor1 (ESR1, a gene that encodes the ER), CDK4, mitogen-activated protein kinase kinase kinase 1 (MAP3K1).[61,62]

Considering advanced breast cancer (ABC), PIK3CA mutations have a strong predictive value for treatment with α-selective and β-sparing PI3K inhibitors, and its use has recently entered clinical practice.[61-63]

Pharmacological BC Therapies and Predictive Biomarkers

Chemotherapy

Chemotherapy is the typical treatment of most molecular types of BC. According to Herr et al,[64] nodal positive patients with luminal A BC have a limited benefit of adjuvant chemotherapy. Adjunct chemotherapy is an approach with estrogen-targeted therapy (SERM or AI) for luminal B breast tumors or in combination with trastuzumab in HER2+ BC.[65,66] In metastatic breast cancer (MBC), systemic chemotherapy is the first-line approach in HER2+ and TNBC and a possible addition in ER+ MBC.[67]

Chemotherapy is focused on taxane and anthracycline agents (Figure 3). A Cochrane review reported that a taxane-containing chemotherapy regimen has significantly improved disease-free survival (DFS) and OS when used as an adjuvant in early BC.[68] The current adjuvant regimen for early invasive breast carcinoma comprises doxorubicin or (epirubicin) and cyclophosphamide followed by paclitaxel in a dose-dense schedule of docetaxel, doxorubicin, and cyclophosphamide.[69,70] These chemotherapeutic drugs are equally effective and differ only in their toxicities.[69] Either of these regimens can be used as a monotherapy or in combination with endocrine therapy in HR+ BC. In ABC or MBC, chemotherapy is routinely used in a sequential method. This has been shown to have no difference in OS but a significant improvement in the quality of life.[71]

Platinum containing agents, particularly carboplatin, are also used in neoadjuvant treatment in HER2+ BC. Carboplatin has been shown to be effective when used in a regimen with trastuzumab and a taxane for HER2+ early stage of BC. Carboplatin, trastuzumab, and docetaxel used in the neoadjuvant setting were just as efficacious as doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab.[72] The main difference in these 2 regimens is that the carboplatin regimen has fewer acute toxic events and lower levels of cardiotoxicity. Additional information about mAbs is further presented.

Treatment for TNBC with a BRCA 1/2 germline mutation can be enhanced by adding a platinum-containing chemotherapeutic agent (Figure 3), such as carboplatin, whether used as adjuvant or neoadjuvant therapy. The addition of a platinum agent to the treatment results in double the objective response rate (68% in carboplatin vs 33% in docetaxel).[73]

Platinum-containing neoadjuvant chemotherapy was tested in TNBC to improve long-term outcomes. Despite resulting in varied results, it may be considered an option in TNBC patients.[74,75] Although the use of a platinum-containing chemotherapy agent can be expanded to all women with TNBC, it does not appear to have the same positive effects in patients without a BRCA 1/2 mutation or a mutation in another HRR gene when compared to those patients who do have a mutation.[64]

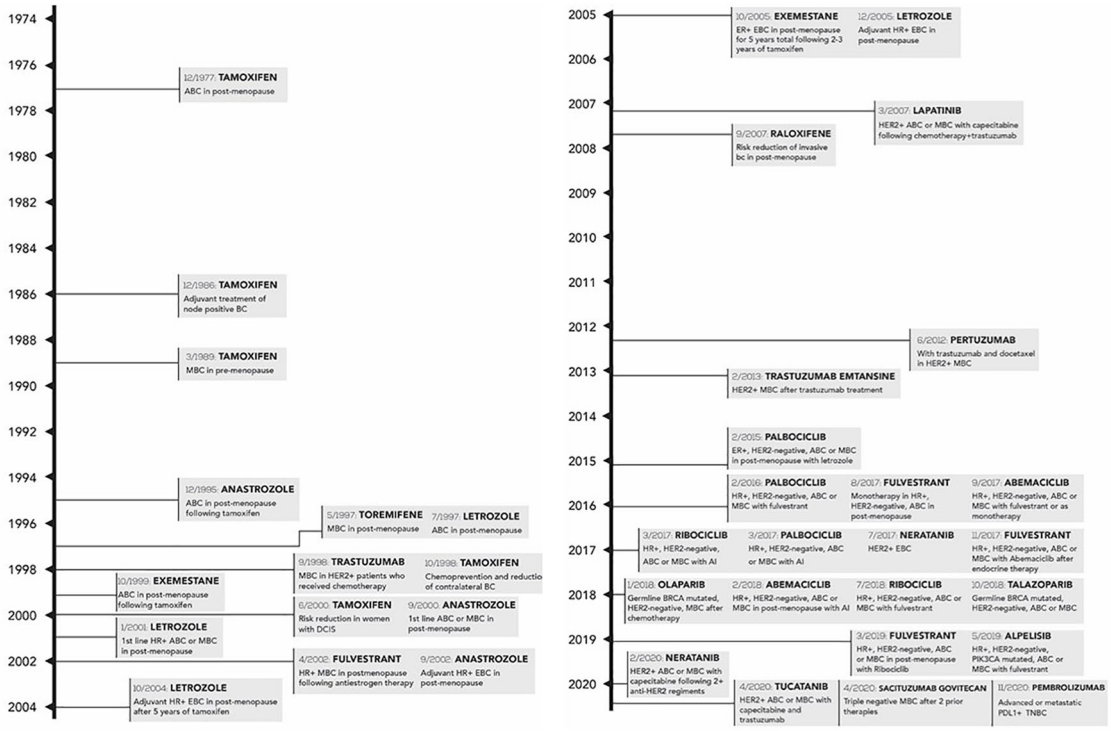

Figure 1. Timeline of drug approvals by the FDA (1974-2020) and the respective main clinical indications to treat breast cancers.
ABC indicates advanced breast cancer; AI, aromatase inhibitor; BC, breast cancer; BRCA, breast cancer gene; DCIS, ductal carcinoma in situ; EBC, early breast cancer; ER, estrogen receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MBC, metastatic breast cancer; PD-L1, programed cell death ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TNBC, triple-negative breast cancer.

Although chemotherapy remains the standard treatment of BC, particularly in early stages[76] and TNBC, the absolute benefits may be small and not worth the added risk of toxicity among women with a low baseline risk of recurrence. Likewise, chemotherapy is neither feasible nor likely to change OS[77] for patients with significant comorbidities[78] or advanced age. In this sense, chemotherapy has been supplemented by other drug classes, especially mAbs.

Hormone-based therapies

Selective ER modulators. Around 78% of breast tumors are ER+, which can block estrogen's action in the breast, a suitable therapy for most tumors.[79] One of the original pharmacological agents used to treat BC is tamoxifen, followed by toremifene (Figure 1).

SERMs act as partial ER agonists in some tissues while acting as ER antagonists in other tissues (Figure 3). The standard endocrine therapy for premenopausal women is tamoxifen. On the other hand, raloxifene and toremifene are SERMs of clinical significance in treating BC in postmenopausal women, acting as antagonists in breast tissue.[80,81] Their pharmacological differences are seen in other tissues. In all ER+ cancers, tamoxifen therapy is indicated as an adjuvant with a standard of treatment lasting at least 5 years. Regardless of menopausal status or age, the initial standard adjuvant therapy with tamoxifen for 5 years presented a reduction of BC recurrence and mortality by 30% and 33% after 15 years, respectively.[82] The ATLAS and aTTom trials compared 10 years of adjuvant tamoxifen to 5 years in women of any menopausal status or age. Both trials showed that 10 years of adjuvant tamoxifen had an absolute reduction of recurrence by 3.7% and mortality by 2.8% at 10 years compared to 5 years of treatment.[83,84] The SOFT and TEXT trials built upon tamoxifen and exemestane's therapeutic outcomes, showing that the addition of ovarian suppression to both resulted in significant increases in 8-year DFS and OS. The addition of ovarian suppression was also associated with increased osteoporosis, thrombosis, embolism, and musculoskeletal symptoms. Due to the increased adverse effects, the clinical usage of ovarian suppression is assessed in a case-by-case manner and is usually reserved for those considered to be at higher risk.[85,86] Adverse effects of tamoxifen and raloxifene result from their effects as agonists in tissues other than breasts, such as bones (raloxifene) and uterus (tamoxifen).[87-89] The clinically significant risks include increased rates of thromboembolic disease, cataracts, endometrial cancer, and stroke.[81] Despite potentially severe AEs, use of tamoxifen and raloxifene results in a decrease in all-cause mortality due to their reduction in BC mortality.[81,82]

Five-year treatment with tamoxifen decreased BCs' risk in the next 20 years from 12.3 to 7.8%. The risk of invasive BC within 20 years, specifically, was reduced from 8.3% with placebo to 4.9% with tamoxifen.[90] These results indicated that the number needed to treat (NNT) for 5 years of tamoxifen treatment was 22 to prevent one BC over the next 20 years. The most significant factor found to decrease tamoxifen's benefit in

BC prevention was the concurrent use of menopausal hormone therapy while taking tamoxifen.[90] Tamoxifen is also used for the prevention of BC in patients who are at increased risk, such as women above 60, or with a history of lobular carcinoma in situ, or a 5-year risk of BC of at least 1.66%.[91]

Initial results from the MORE trial showed that raloxifene reduced invasive BC risk by 76%. Since that time, the STAR trial updates have reigned in these numbers, comparing 5 years of tamoxifen versus raloxifene. The trial showed that after a median follow-up of 81 months, postmenopausal women treated with raloxifene resulted in a higher rate of invasive BC, about 24% higher than at the same time in the tamoxifen group.[91]

Despite a more significant BC risk reduction, tamoxifen comes with an increased risk of endometrial cancer, cataract, and thromboembolic events.[91]

Ultimately, studies have been inconclusive on the effects of prophylactic use of tamoxifen and raloxifene on both BC-specific survival and OS, leading to a minimal use of these agents in chemoprevention in clinical practice.[90,92]

Aromatase inhibitors. Anastrozole, exemestane, and letrozole are AIs. They inhibit the enzyme aromatase that converts androgens into estrogens in target cells (Figure 3). AIs were developed after tamoxifen and are becoming an essential part of the therapeutic plan for many ER+ cancers (Figure 1). AIs are used in pre or postmenopausal women. However, treatment efficacy is influenced by different variables. Resistance to AI therapy is common, occurring in more than 20% of patients with early-stage disease, and is inevitable in patients with metastatic disease. The development and maintenance of AI resistance involve mechanisms dependent on interactions with cell types within the tumor microenvironment, such as fibroblasts, immune cells, adipose cells, and mesenchymal stem cells (for a review, see Ma et al[93]). Obesity is the most substantial variable embodying probable endocrine resistance[78,94] in premenopausal patients, and it is associated with an increased risk of BC recurrence.[95]

Along with SERMs, AIs are used as a long-term treatment to decrease mortality and recurrence long after diagnosis and primary therapeutic interventions. Postmenopausal women treated for 5 years with AIs presented a decrease in BC recurrence by 3.6% over 10 years and a mortality reduction by 2.1% over 10 years when compared to tamoxifen.[96] Extension of treatment to 10 years in postmenopausal women further increased DFS by 4% compared to the standard 5-year treatment.[97] The aforementioned SOFT and TEXT trials also looked at exemestane. They found that the addition of ovarian suppression to exemestane improved DFS and OS,[85,86] which is of particular importance when using AIs in premenopausal women. AIs block peripheral conversion of androgens to estrogens, and in premenopausal women, most of their estrogen is formed in the ovaries; therefore, when using AIs in this population, it is necessary to use ovarian suppression to increase the efficacy.[98] Like tamoxifen, exemestane and anastrozole reduced the risk of BC after menopause by up to 65%.[99] Despite the results showing the efficacy of exemestane and anastrozole in chemoprevention of BC, they are rarely used for this purpose in clinical practice. Their low utilization in clinical practice may be due to lack of awareness on the part of primary-care physicians of the clinical trials showing the efficacy of exemestane and anastrozole as methods of chemoprevention for BC and concerns over adverse effects, including loss of bone mineral density and intensification of menopausal symptoms, mainly hot flashes and fatigue.[100] Since 2013, AIs, particularly letrozole, have gained increased clinical significance in combination with other agents, including CDK 4/6 inhibitors, PI3K inhibitors, and HDAC inhibitors.[15,61,101,102] Details about these agents will be discussed in the next sections.

Selective ER degraders. The prevalence of ER+ BC, combined with the risk of recurrence after treatment with both AIs and SERMs, led to the development of the third class of drugs that acts through ER known as SERDs. This class currently contains an orphan drug, fulvestrant, a 17-β-estradiol analog,[103] which acts as a selective ER antagonist that also speeds the ER degradation and downregulation.[104] Due to its mechanism of action (Figure 3), fulvestrant is useful for treating HR+ cancer and is mainly reserved for local ABC or MBC.

Fulvestrant is currently approved only for use in postmenopausal women. In head-to-head trials, fulvestrant resulted in an increased OS and PFS over anastrozole.[105,106] Fulvestrant monotherapy is approved for postmenopausal women with HR+ MBC following antiendocrine therapy, or those with HR+, HER2– ABC not previously treated with endocrine therapy (Figure 1).[107] However, fulvestrant is most often used as an adjuvant instead of monotherapy.[108,109] In combination with palbociclib, fulvestrant resulted in an absolute prolongation of OS of 6.9 months among patients with HR+, HER2– ABC who had disease progression after previous endocrine therapy.[106]

The most common adverse effects for fulvestrant are arthralgia, hot flush, fatigue, nausea, and back pain.[106] Although infrequent, cardiac failure, and arrhythmias are the most severe adverse effects leading patients to discontinue the therapy.[110,111]

Targeted therapy and immunotherapy

Cyclin-dependent kinases 4/6 inhibitors. A combination of acquired and new resistance to hormonal based treatments has led to the search for newer agents to aid in treating HR+ tumors. Food and Drug Administration-approved CDK 4/6 inhibitors include palbociclib, abemaciclib, and ribociclib.[62] CDK4/6 interact with the protein cyclin D1 (CCND1) to allow for progression through the G1 checkpoint and into the S phase of the cell cycle. CDK4/6 inhibition prevents DNA replication by arresting progression from the G1 to S phase (Figure 3).[112]

Mainly due to ABC, current guidelines include CDK4/6 inhibitor combined with endocrine therapy for the treatment of premenopausal/postmenopausal women with HR+/HER2– (Figure 1).[113] Palbociclib combined with letrozole was shown to increase PFS by 45% when compared to letrozole alone in HR+, HER2– BC[101] and significantly improved OS by 48% versus letrozole alone.[114]

Abemaciclib and ribociclib presented similar results as palbociclib in recent clinical trials. The MONARCH 2 trial compared the use of abemaciclib + fulvestrant versus placebo + fulvestrant in HR+, HER2– BC. Abemaciclib resulted in significant increase in both OS and PFS when compared to placebo.[115] The MONALEESA-7 trial, ribociclib + endocrine therapy (AI or tamoxifen + goserelin) was compared to placebo + endocrine therapy in HR+, HER2– BC. The results showed the addition of ribociclib significantly increased OS.[116]

Selected biomarkers are predictive of these pharmacological agents' efficacy. High CDK4 expression was associated with resistance to letrozole, indicated by a shorter PFS than low CDK4 expression; the addition of palbociclib mitigated this.[62] Higher expression levels of fibroblast growth factor receptor-2 (FGFR2), and ERBB2 RTK 3 were associated with longer PFS when treated with letrozole plus palbociclib.[62] Finally, low expression of CCNE1 was associated with greater PFS when treated with letrozole.[117] Despite these findings, testing of these biomarkers is not used routinely in clinical practice.[118,119]

Common AEs associated with all CDK4/6 inhibitors are neutropenia and leukopenia. Patients often report fatigue, nausea, and arthralgia. Serious AEs occurred at a rate of 19.6% with the use of palbociclib and 12.6% with letrozole alone.[27] Of note, the rate of neutropenia with abemaciclib was much lower than with palbociclib or ribociclib.[62,101,102]

Phosphoionositide-3 kinase inhibitors. Phosphoionositide-3 kinase is a critical enzyme for many cellular functions, including proliferation, apoptosis, and nutrient response.[120] Approximately 40% of HR+, HER2– BC patients presented with mutations in the *PIK3CA* gene, leading to constitutive activation of the alpha subunit (p110α) of PI3K.[121-123]

The novel PI3K inhibitors (Figure 3) used to treat BC are alpelisib, taselisib, and copansilib.[61,124-126] Of the 3, the only currently FDA approved is alpelisib in combination with fulvestrant for postmenopausal women or men, who have HR+, HER2–, PIK3CA mutated, ABC or MBC (Figure 1).[127-132] Alpelisib works as an α-specific PI3K inhibitor, by selectively inhibiting p110α, leading to interruption of PI3K signaling pathways.[128-132] The SOLAR-1 trial alpelisib + fulvestrant resulted in a PFS of 11 months versus 5.7 months in the placebo group. This effect was only seen in patients with PIK3CA mutated cancer, though, with the effect disappearing in those without the mutation.[124] Taselisib and copansilib, have shown promise, particularly in combination with other anticancer drugs, with all showing increased PFS in clinical trials involving HER2–, HR+ advanced, or MBC.[125,126]

Currently, the clinical benefit of PI3K inhibitors is only achieved in patients with *PIK3CA* mutations. Recently it was shown that double mutations might increase sensitivity even more than a single mutation. When comparing the response to a PI3K inhibitor taselisib, patients with multiple PIK3CA mutations had an overall response rate (ORR) of 30.2% compared to 8.7% in placebo. Patients with a single mutation only had an ORR of 18.1% compared to 10% in placebo, which was not a statistically significant difference.[133] The presence of *MAP3K1* mutation simultaneously with *PIK3CA* mutation is associated with the enhanced clinical benefit of PI3K inhibitors.[61] Like other potential biomarkers mentioned, this offers an avenue for further studies to determine the clinical impact of testing for inactivating *MAP3K1* mutations in combination with activating PIK3CA mutations.[61]

AEs associated with PI3K inhibitors are determined by the isoform they affect. Alpelisib is more specific to the α isoform of PI3K, and therefore, the most common adverse effects are hyperglycemia, diarrhea, and nausea. The most common grade 3 or above AEs witnessed with alpelisib + fulvestrant treatment is hyperglycemia, with rash, and diarrhea following.[124]

Many of these agents have been abandoned in development due to toxicity issues or lack of efficacy, such as buparlisib and pictilisib that have both had trials terminated.[134,135]

Tyrosine kinase inhibitors. Receptor tyrosine kinases are a family of tyrosine protein kinases. Receptor tyrosine kinases are transmembrane proteins with binding sites at their extracellular domains for polypeptide hormones and growth factors as ligands. Receptors of growth factors are members of the RTK family, such as the epidermal growth factor helping the regulation of cell growth and differentiation. The proto-oncogene HER2 encodes epidermal growth factor receptor (EGFR) with tyrosine kinase activity.[33,136]

HER2/neu is a transmembrane tyrosine kinase receptor that forms part of this ErbB family signaling network. Abnormal signaling by this network is present in BC.[137] The EGFR/HER1, a different ErbB receptor, is expressed or highly expressed in many tumors. Although this remains controversial,[138,139] overexpression of EGFR is linked to a more aggressive breast tumor phenotype, involving the increased potential for invasiveness and metastasis.[140-142]

TKIs play an essential role in the modulation of growth factor signaling. The mechanism of TKI is depicted in Figure 3. The TKIs targeting BC treatment are lapatinib, neratinib, tucatinib, pyrotinib, and afatanib. Among them, only lapatinib, tucatinib, and neratinib are approved by the FDA to treat BC (Figures 1 and 2).

Lapatinib (a prodrug metabolized by CYP3A4), combined with capecitabine, is used to treat patients with

HER2-overexpressing MBC who received an anthracycline, a taxane, and trastuzumab as prior therapy. Among the 3 TKIs approved, lapatinib is the only intracellular blocker acting on both HER2 (ErbB-2) and HER1 (ErbB-1), acting as a dual reversible inhibitor for receptors, thus blocking the downstream MAPK/Erk1/2 and PI3K/AKT pathways.[143,144]

The most common adverse effects during therapy with lapatinib plus capecitabine were gastrointestinal disorders (diarrhea, nausea, and vomiting) or dermatologic, such as palmar-plantar erythrodysesthesia and rash.[145]

Neratinib is an irreversible TKI of HER1, HER2, HER4, approved in combination with capecitabine for adult patients with advanced or metastatic HER2+ BC who have received 2 or more prior anti-HER2-based regimens in the metastatic setting.[146] Compared with lapatinib, neratinib has a more valid and consistent inhibitory effect in feasible resistance pathways.[49] Neratinib was shown to significantly improve the 2-year invasive DFS after trastuzumab-based adjuvant therapy in HER2+ BC. The most common adverse effects among patients using neratinib include diarrhea, and less frequently, neutropenia and dehydration were reported. These adverse effects were reversible and manageable with dose reduction, pause interruption, and proper supportive care.[147]

Tucatinib is approved in combination with trastuzumab and capecitabine for adult patients with advanced unresectable or metastatic HER2+ BC, including patients with brain metastases who have received one or more prior anti-HER2-based regimens in the metastatic setting.[148] Tucatinib increased PFS at 1 year to 33.1% compared to 12.3% in placebo and led to an increased OS at 2 years to 44.9% from 26.6% in placebo.[149] Tucatinib's common adverse effects are diarrhea, palmar-plantar erythrodysesthesia, nausea, fatigue, and vomiting.[146]

Pyrotinib and Afatinib are both TKIs that have shown promise in studies, with pyrotinib being approved in China.[150,151] Neither have been approved by the FDA, but they remain in numerous phases III and II trials (Figure 2).

ADP-ribose polymerase inhibitors. Cancer cells with deleterious mutations in BRCA1/2 are deficient in the repair mechanism for DNA double-strand breaks (DSB), leaving these tumors highly dependent on the repair pathway for single-strand DNA breaks (SSB).[10,152] DNA-SSB results directly from oxidative damage.[152] Poly ADP-ribose polymerase comprise a family of enzymes that modify targeted proteins by catalyzing the transfer of poly(ADP-ribose) (PAR) to these target proteins. Poly ADP-ribose polymerase acts as a damage recognition repair protein of an SSB and triggers the repair of SSBs in the DNA by base excision repair (BER).[153] Poly ADP-ribose polymerase detects SSBs, binds to DNA, and undergoes autopoly ADP-ribosylation, which acts as a signal for the recruitment of hundreds of downstream proteins that regulate DNA repair and eventually repair these SSBs.[154,155] Nevertheless, if these SSBs are not repaired, they finally progress to DBSs, which are cytotoxic. Poly ADP-ribose polymerase inhibition (Figure 3) is thought to decrease PAR levels, one of the earliest cellular responses to genotoxic stress.[156] In other words, targeted therapy with PARP inhibitors leads to SSBs accumulation and, consequently, DSB formation fostering DSB repair.[154-157]

In addition to catalytic inhibition, PARP inhibitors induce PARP trapping at sites of DNA damage. The capacity to trap PARP–DNA complexes varies among PARP inhibitors and is not correlated with PARP catalytic inhibition.[157-159] The synthetic lethality approach combines a PARP inhibitor and a BRCA mutation in a condition where a deficiency in one gene does not lead to cell death. Still, a combination of 2 or more deficiencies do.[158,160] Poly ADP-ribose polymerase inhibitors target PARP enzymes, mainly PARP1 and PARP2.[161]

Regarding PARP inhibitor treatment of TNBC, TP53, ATM, PALB2, and RAD51C might be prognostic biomarkers or predictive indicators for treatment response and could also provide targets for novel treatment strategies.[157]

To date, there are 4 PARP inhibitors approved by the FDA: olaparib, rucaparib, niraparib, and talazoparib.[162-164] Olaparib and talazoparib are the PARP inhibitors currently FDA approved as targeted therapies for treating MBC caused by a BRCA mutation (Figure 1).

Olaparib has been approved for the treatment of patients with advanced ovarian cancer as of 2014. The OlympiAD phase III trial then evaluated the efficacy and safety of olaparib in patients with metastatic HER2– BC that could be either HR+ or HR–.[165] The results indicated olaparib increased PFS by 2.8 months and response rate by 31.1% compared to placebo.[166-169]

Talazoparib (Figure 1) is a potent PARP inhibitor. It provides 100 times greater catalytic inhibition and PARP-trapping potential than other PARP inhibitors, indicating the trapping PARP on DNA may be more effective in inducing cancer-cell death than enzymatic inhibition alone.[170]After the phase III EMBRACA trial, talazoparib showed superior PFS by 3 months and increased ORR by 35.4% compared to standard chemotherapy.[10,171] The most common adverse effects observed with PARP inhibitors are anemia, fatigue, nausea, vomiting, and neutropenia.

Histone deacetylase inhibitors. Resistance to antiestrogens in ER+ tumors can either present initially or develop during treatment. One potential mechanism of antiestrogen resistance is through the alteration of genome sequences, resulting in gene silencing.[172] This has created an additional avenue for dealing with resistance beyond targeting new pathways, instead by altering the epigenetics of tumor cells. Acetylation of histones increases the transcriptional activity of DNA, and, in the face of antiestrogen resistance, HDAC has become a target for pharmacological intervention.[173,174] Histone deacetylase inhibitors block the enzyme, resulting in hyperacetylation of histones, relaxation of the chromatin, allowing for higher transcription of the DNA (Figure 3).

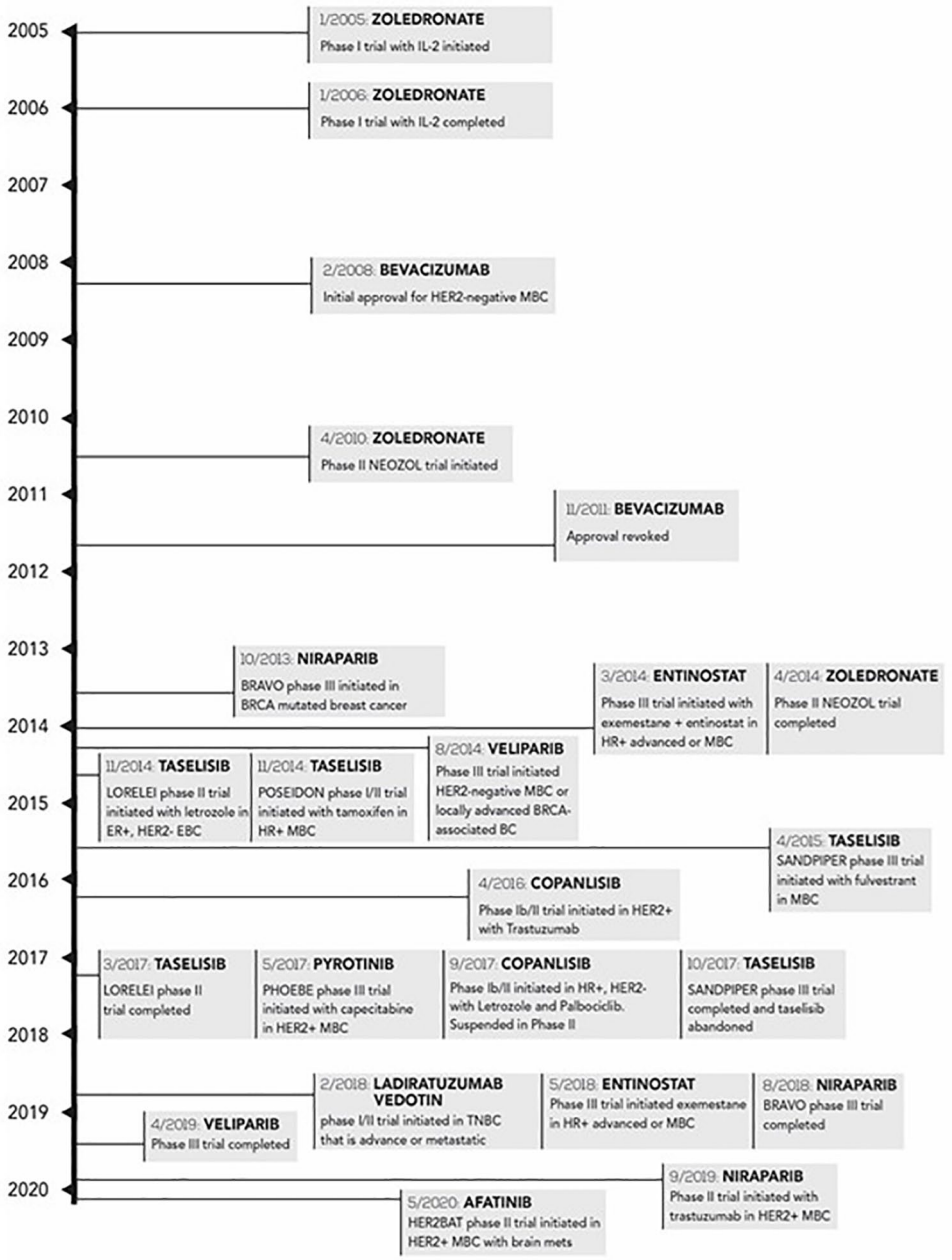

Figure 2. Timeline (2007-2020) of drugs under clinical trial toward the treatment of breast cancers and those that had their trials not completed.
BC, breast cancer; BRCA, breast cancer gene; EBC, early breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HER2BAT, a study of HER2+ breast cancer patients with active brain metastases treated with afatinib & T-DM1 vs. T-DM1 alone; HR, hormone receptor; IL-2, interleukin-2; LORELEI, study of neoadjuvant letrozole + taselisib versus letrozole + placebo in post-menopausal women with breast cancer; BRAVO, trial of niraparib versus physician's choice in HER2 negative, germline BRCA mutation-positive breast cancer patients; MBC, metastatic breast cancer; PHOEBE, pyrotinib plus capecitabine versus lapatinib plus capecitabine in patients with HER2+ metastatic breast cancer; POSEIDON, safety, efficacy and circulating tumor DNA response of the beta isoform-sparing PI3K inhibitor taselisib (GDC-0032) combined with tamoxifen in hormone receptor-positive metastatic breast cancer patients; SANDPIPER, a study of taselisib + fulvestrant versus placebo + fulvestrant in participants with advanced or metastatic breast cancer who have disease recurrence or progression during or after aromatase inhibitor therapy; TNBC, triple-negative breast cancer.

Preclinical studies determined that HDAC inhibitors increased transcription of the ER gene, restoring ER+ status and AI responsiveness to cell lines that were previously resistant.[175,176] Currently, no HDAC inhibitors are approved by the FDA to treat BC, though entinostat is presently in 2 phase III trials (Figure 2).[177]

The most common adverse effects triggered by entinostat use are fatigue, nausea, neutropenia, and peripheral edema. The most frequently observed grade 3 or higher AEs were neutropenia and fatigue.[15]

Monoclonal antibodies. Breast cancer with HER2 overamplification (HER2+) was historically more challenging to treat than the corresponding HER2–, resulting in a worse OS and prognosis.[178] This was before the development of mAbs, which have since improved the prognosis of HER2+ cancers, though they remain worse prognostically.[179,180] Overamplification of HER2 over-activates signaling pathways, causing proliferation, motility, and survivability of the tumor cells, which makes difficult the treatment. Human epidermal growth factor receptor 2 tyrosine kinase pathway

targets ER and promotes human BC cells to grow in a hormone-independent way.[181]

This scenario has been changed by introducing mAb, trastuzumab, and pertuzumab, targeting the HER2 receptor as a new strategy to treat HER2+ disease.[182]

Trastuzumab binds to extracellular domains of HER2[183] activating antibody-dependent cellular cytotoxicity, blocks HER2 signaling (mostly PI3K/Akt pathway), and finally interrupts HER2 angiogenesis.[184-187] Trastuzumab also blocks the proteolytic cleavage of the extracellular domain of HER2, therefore preventing the formation of a truncated, membrane-bound, constitutively active form of HER2 (known as p95HER2) (Figure 3).[184-187] Pertuzumab binds extracellular domains of HER2 and inhibits the dimerization of HER2[188] with other proteins of the HER family and therefore inhibits activation of the signaling pathway involved in promoting cell growth and opposing apoptosis (Figure 3).[189]

A third mAb, bevacizumab, is an anti-vascular endothelial growth factor (VEGF) targeting HER2– MBC. It is approved in Europe[190] and Australia[191] to treat BC. In the United States and Canada, bevacizumab is approved against other cancers, but not BC.[192] Clinical trials had previously suggested that bevacizumab combined with taxanes seemed to be a highly effective first-line treatment for MBC.[193,194] According to the FDA, based on 4 clinical trials (Figure 2), this mAb did not improve survival enough to outweigh the risks of increased blood pressure, internal bleeding, chest pain, and pulmonary embolism.[191]

Many patients with HER2+ tumors, especially those with MBC, demonstrate primary *de novo* or intrinsic resistance to trastuzumab as a monotherapy[195-197] Truncation of the HER2 molecule itself[198] represents the most prominent mechanism of resistance of HER2+ BC against trastuzumab.[199] In the same way, most patients who initially respond to trastuzumab demonstrate disease progression within 1 year of treatment initiation. On the other hand, trastuzumab improves prognosis when used in combinations. It delays the disease progression and increases the OS when used as an adjuvant to traditional chemotherapeutic regimens with doxorubicin, cyclophosphamide, and paclitaxel.[66,200,201] Trastuzumab administered concurrently with paclitaxel after doxorubicin, and cyclophosphamide is the most frequently used adjuvant schedule to treat HER2+ BC.[200] The joint analysis of North Central Cancer Treatment Group NCCTG N9831 (combination chemotherapy with or without trastuzumab in treating women with HER2 overexpressing BC) and the National Surgical Adjuvant Breast and Bowel Project NSABP B-31 (doxorubicin and cyclophosphamide plus paclitaxel with or without trastuzumab in treating women with node-positive BC that overexpresses HER2) reported that adding trastuzumab to paclitaxel after doxorubicin and cyclophosphamide in early-stage HER2+ BC leads to a substantial reduction in cancer recurrence.[200] Trastuzumab concurrently with paclitaxel increased 5 years DFS rates to 84.5%, and paclitaxel plus sequential trastuzumab increased 5 years DFS rates 80.1%.[201]

The NeoSphere trial showed the efficacy of mAb agents extends into the neoadjuvant setting as well, indicating that the combination of trastuzumab and pertuzumab with docetaxel had a 16.8% improvement.[202] The same group[203] previously reported a sustained benefit in event-free survival (EFS) from trastuzumab-containing neoadjuvant therapy followed by adjuvant trastuzumab in patients with locally advanced or inflammatory BC and provided a positive insight into the association between complete pathological remission and long-term outcomes in HER2+ disease.[203]

The addition of pertuzumab to the standard treatment of chemotherapy in combination with trastuzumab increased in 3 years DFS from 93.2 to 94.1% in patients with node-positive or high-risk node-negative HER2+ BC.[204]

The addition of carboplatin in a dose-equivalent, taxane (paclitaxel)-containing regimen plus trastuzumab had a significant advantage with respect to PFS and ORR over paclitaxel plus trastuzumab in women with HER-2-overexpressing MBC.[205]

Tryphaena conducted a randomized study to assess the cardiotoxicity of the addition of pertuzumab plus trastuzumab with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2+ early BC. The results demonstrated that the addition of pertuzumab does increase rates of cardiotoxicity, but the overall incidence was low.[206]

The cardiac safety profile of a neoadjuvant doxorubicin-based regimen followed by paclitaxel, trastuzumab, and pertuzumab with completion of 1 year of adjuvant trastuzumab-based therapy was confirmed by Yu et al.[207]

The most significant AEs related to mAb agents are cardiac. The risk of a primary and overall risk of cardiac events is slightly higher with the concurrent use of pertuzumab and trastuzumab than trastuzumab alone (0.7% pertuzumab vs 0.3% with trastuzumab alone).[204] Apart from cardiac toxicity, the most common severe adverse effects associated with those mAb are diarrhea, neutropenia, and anemia.[204]

Antibody-drug conjugates. Monoclonal antibodies are also components of a subclass of drugs used to treat BC, the ADCs. They are composed of a targeted mAb linked to an antineoplastic agent.[208] Currently, this class contains the following drugs approved by the FDA for BC treatment: trastuzumab emtansine (T-DM1)[209] and trastuzumab-deruxtecan[29,210] and sacituzumab govitecan-hziy.[209] Trastuzumab emtansine is composed of trastuzumab linked to a fungal derivative that is a microtubule inhibitor.[7] Trastuzumab emtansine is indicated for HER2+ MBC in both men and women following results of the EMILIA phase III trial showing an increased PFS with T-DM1 when compared to lapatinib plus capecitabine.[50] The most frequent AEs seen with T-DM1 administration was thrombocytopenia, followed by elevated transaminases.[50]

The efficacy of T-DM1 for treating advanced HER2+ BC was further tested in the MARIANNE trial, which compared T-DM1 use to taxane plus trastuzumab. The findings showed

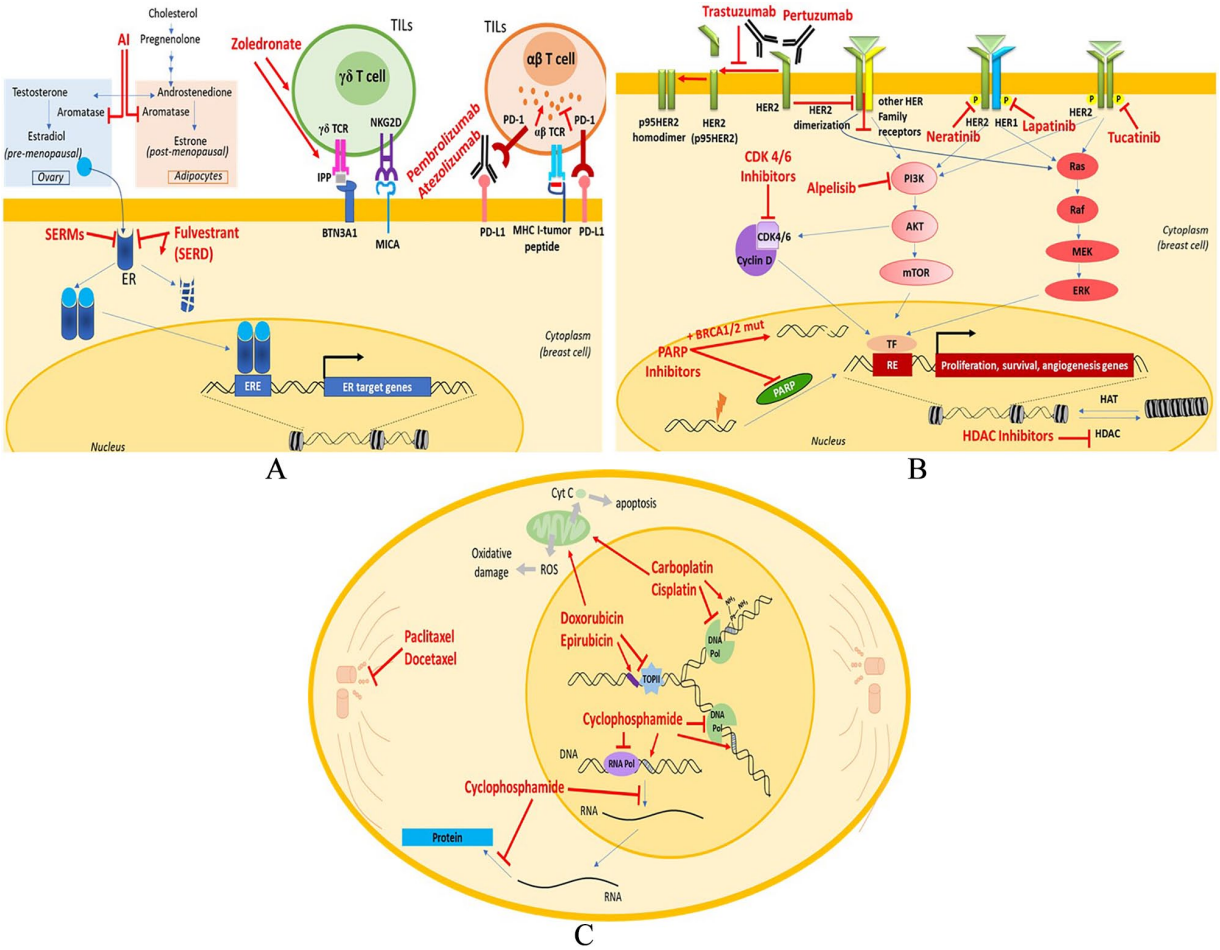

Figure 3. Schematic diagram depicting sites of action of prominent drugs affecting principal pathways associated with breast cancer: (A) aromatase is the enzyme that catalyzes the conversion of androgens to estrogens. AIs inhibit the enzyme, therefore reducing the levels of estrogen which is needed for ER+ cancer cells growth. SERMs (ER antagonism) act as estrogen receptor antagonists in breast tissue. SERD (fulvestrant) is an ER antagonist leading to degradation and downregulation of ER. Zoledronate is a direct γδ *T* cell stimulator and also leads to accumulation of IPP in cancer cells leading to stimulation and activation of γδ *T* cell via γδ TCR recognition of phosphoantigens presented by butyrophilin 3A1 (BTN3A1) on the BC target cells and/or interaction of MICA with NKG2D. The immune response of γδ *T* cells can be via stimulatory and regulatory effects on other components of the immune system (secretion of cytokines or direct antigen presentation) and via direct cytotoxicity (through perforin-granzymes). Monoclonal antibodies: (B) PARP inhibitors lead to PARP inhibition and PARP trapping at sites of DNA damage. PARP acts as damage recognition repair protein for initiation of base excision repair of DNA SSB. PARP inhibition causes inability to repair and accumulation of SSB, leading to DSBs, which in cells with BRCA1/2 mutation cannot be fixed and accumulate ultimately triggering cell death. CDK4/6 inhibitors prevent DNA replication by arresting progression from the G1 to the S phase of the cell cycle. mAbs inhibit activation of the signaling pathway of HER involved in promoting cell growth and opposing apoptosis. Pertuzumab inhibits HER2 dimerization; trastuzumab prevents cleavage of the extracellular domain of the HER2 receptor, which leads to the formation of a truncated form of HER2 (p95HER2). p95HER2, which contains tyrosine kinase activity, can form constitutively active stable homodimers. PI3K inhibitor (alpelisib) selectively inhibits the p110α catalytic subunit of PI3K interrupting both AKT-dependent and AKT-independent PI3K signaling pathways. HDAC inhibitors block the enzyme, resulting in hyperacetylation of histones, relaxation of the chromatin, and allowing for higher transcription of the DNA. Tyrosine kinase inhibitors: tucatinib, lapatinib, neratinib are homologous of the adenosine triphosphate (ATP) that act by competing for the ATP-binding domain of protein kinases preventing phosphorylation and subsequent activation of the signal transduction pathways, leading to apoptosis and decreasing cellular proliferation. Moreover, TKIs target other kinase receptors due to the homology that they share with the EGFR family in the catalytic domain. (C) Anthracyclines: doxorubicin and epirubicin inhibit cancer through multiple pathways: to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. Doxorubicin inhibits the enzyme topoisomerase II (TopII), causing DNA damage and induction of apoptosis—also cause ROS-mediated oxidative damage to DNA, further limiting DNA synthesis. Alkylating agents: cyclophosphamide (a nitrogen mustard compound), carboplatin and cisplatin (platinum-containing compounds) develop cytotoxic effects mainly due to substitution of alkyl groups for hydrogen atoms on DNA, resulting in the formation of cross-links within the DNA chain and thereby resulting in misreading of the DNA code and the inhibition of DNA, RNA, and protein synthesis and the triggering apoptosis in rapidly proliferating tumor cells. Taxanes: paclitaxel and docetaxel stabilize microtubules act mainly by binding to beta-tubulin, enhancing its proliferation and stabilizing its conformation. Doing so inhibits the proper assembly of microtubules into the mitotic spindle, arresting the cell cycling during G2/M. conferring enhanced survival.

AI indicates aromatase inhibitor; AKT, serine/threonine-protein kinase 1 (also known as PKB); ATP, adenosine triphosphate; BC, breast cancer; BRCA, breast cancer gene; CDK, cyclin-dependent kinase; DSB, double-strand breaks; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERE, estrogen-responsive element; ERK, extracellular signal-regulated kinase; HAT, histone acetyl transferase; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; IPP, isopentenyl pyrophosphate; MHC, major histocompatibility complex; MICA, MHC Class I polypeptide-related sequence type A; mTOR, mechanistic target Of rapamycin; PARP, poly ADP-ribose polymerase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; RE, responsive element; ROS, reactive oxygen species; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator; SSB, single-strand break; TF, transcription factor; TIL, tumor-infiltrating lymphocytes; TKI, tyrosine kinase inhibitor; γδ TCR, gamma delta *T* cell receptor.

that the PFS with T-DM1 was equivalent to taxane plus trastuzumab, but T-DM1 appeared more tolerable due to a lower proportion of grade 3+ AEs.[211] The TH3RESA trial looked at T-DM1 versus physician's choice treatment in patients with HER2+ ABC, which had progressed after treatment with 2 or more HER2-directed regimens. Trastuzumab emtansine demonstrated an absolute increase in OS of 6.9 months compared to physician's choice treatment.[212]

The KATHERINE trial expanded the indications of T-DM1 by comparing adjuvant usage of T-DM1 to trastuzumab in women with HER2-positive early BC who had undergone neoadjuvant chemotherapy + trastuzumab yet still had invasive breast disease. The results showed an increase in DFS from 77% in the control group to 88.3% with T-DM1. This has led to the expansion of the clinical indications for T-DM1 and the development of a new standard of care.[213]

Three-year results from KRISTINE[214,215] data showed a higher risk of an EFS associated with adjuvant T-DM1 plus pertuzumab and neoadjuvant, a similar risk of an invasive DFS, and less quality-of-life impairment in the neoadjuvant phase compared with neoadjuvant docetaxel, carboplatin, trastuzumab plus pertuzumab followed by adjuvant trastuzumab plus pertuzumab. Furthermore, relative to docetaxel, carboplatin, trastuzumab plus pertuzumab followed by adjuvant trastuzumab plus pertuzumab, the conventional systemic chemotherapy-sparing regimen of T-DM1 plus pertuzumab was associated with fewer serious AEs, grade 3 or greater AEs, and more AEs leading to treatment discontinuation. Overall, the observed worse EFS and similar invasive DFS associated with T-DM1 plus pertuzumab indicate the importance of selecting patients for conventional chemotherapy-sparing neoadjuvant regimen.[214,215] Data from KAITLIN may further outline the clinical utility of adjuvant T-DM1 plus pertuzumab in patients with HER2-positive early BC.[216]

Trastuzumab-deruxtecan is composed of the mAb linked to a topoisomerase I inhibitor, similar to irinotecan.[217] It is indicated in patients with HER2+ MBC that has previously been treated with T-DM1 following the results of the DESTINY trial showing an increase in response rate and OS.[210] There are other DESTINY breast trials testing the efficacy of trastuzumab-deruxtecan in different populations or compared to other regimens for treating HER2-positive BC.[218-220] Frequent AEs include decreased neutrophil count, anemia, and nausea. However, the most worrisome AE is the development of interstitial lung disease.[210]

ADCs have shown efficacy in treating HER2-negative cancers, with one being approved for treatment in April 2020: sacituzumab govitecan-hziy, an ADC composed of SN-38 an active metabolite of the topoisomerase I inhibitor irinotecan coupled to an anti-Trop-2 mAb. A trial in patients with metastatic TNBC showed an ORR of 33.3%, with a duration of response of 7.7 months.[221] Other ADCs are on the horizon, one of which is ladiratuzumab vedotin, which is a microtubule disrupting agent conjugated to a mAb against the zinc transporter LIV-1, which was originally identified as an estrogen-induced gene in the cell line ZR-75-1 of BC.[222] The presence of LIV-1 mRNA is associated with an ER + status and has been correlated with lymph node involvement of BC, suggesting a role for LIV-1 in metastasis.[223]

There are currently multiple phase I and II studies evaluating the safety and efficacy of ladiratuzumab vedotin in different BC populations.[224,225]

Bone modifying drugs. Denosumab, a human mAb that acts by neutralizing the receptor activator of nuclear factor κB ligand (RANKL)[226] and zoledronate are targeted therapies classified as bone modifying drugs. Zoledronate acts by binding to and accumulating in the bone, inhibiting bone resorption by osteoclasts. Denosumab, on the other hand, binds to the RANK receptor on osteoclasts and promotes osteoclast differentiation and activity without accumulating in the bone. They are indicated for the prevention of skeletal-related events in patients with BC[227,228] with bone metastases and the treatment of osteoporosis.[229-232]

The ASCO recommends adjuvant bisphosphonates to reduce bone recurrence and improve survival in postmenopausal patients with non-MBC.[233,234] According to the ASCO, the absolute benefit is more significant in patients who are at higher risks of recurrence, and almost all trials were conducted in patients who also received systemic therapy. Most studies evaluated zoledronic acid or clodronate, and data are extremely limited for other bisphosphonates. While denosumab was found to reduce fractures, long-term survival data are still required.[231]

St. Gallen consensus (2019)[234] considered the use of bisphosphonates in premenopausal patients on ovarian suppression with either tamoxifen or AIs. For postmenopausal patients, the panel strongly supported the use of bisphosphonates to improve DFS. And the panelists were clear in stating that denosumab twice a year should not be used as a substitute for bisphosphonate as suggested by the ABCSG-18 Trial,[235] despite a recent publication on the positive impact on DFS.[236]

Immune checkpoint inhibitors and gamma-delta T cell stimulators. The microenvironment of BC tumors is composed of fibroblasts, leukocyte lineage cells (including lymphocytes, macrophages and myeloid-derived stromal cells), and the extracellular matrix, all of which play an important role in BC development and progression.[237] Triple-negative breast cancer and HER2+ show the highest immunogenicity among the BC molecular subtypes.[238,239] We further discuss 2 emerging immunotherapies, ICI of the PD-L1/PD-1 receptor and gamma-delta (γδ) T cells.

Immune checkpoint inhibitors (ICI). Some BC cells express the immune checkpoint regulator PD-L1 to suppress anti-tumor immune responses, leading to a pro-tumor microenvironment. PD-L1 inhibitors relieve the anti-tumor immune responses' suppression, allowing the immune system to attack and kill the cancer cells.[240]

Programed death ligand 1, as a ligand of the PD-1 receptor, is an active biomarker that interacts with PD-1 receptor to suppress the immune system's response to cancer. Prgramed death 1 is a receptor that triggers inhibitory actions; it is quickly induced on naïve *T* cells to counteract T cell activation. PD-1 activation regulates *T* cell activation, tolerance, and exhaustion, and effector *T* cell responses.[241,242] Programed death ligand 1 expression can predict a favorable response to antibodies designed to unfetter anti-tumor activity.[243] This means that PD-L1 and its receptor PD-1 represent crucial therapeutic targets to treat cancer as immune checkpoints regulating host immunity.[244]

There are 2 monoclonal antibodies targeting PD-1 (nivolumab, pembrolizumab) and 3 targeting PD-L1 (atezolizumab, avelumab, and durvalumab) approved by the FDA. These drugs have been approved with different indications as monotherapy or as combination therapy with radiation, chemotherapeutics, or other ICI.[241] In this review, we approach pembrolizumab and atezolizumab, which are approved to treat BC.

Based on the presence or absence of *T* cells and the expression of PD-L1 by cancer cells, a tumor can be categorized into 4 groups: (1) PD-L1 positive, *T* cell positive; (2) PD-L1 negative, *T* cell positive; (3) PD-L1 positive, *T* cell negative; and (4) PD-L1 negative, *T* cell negative.[245-247] Tumor responses with anti–PD1/L1 antibodies are not mediated by the antibody per se, but by tumor antigen-specific T cells that had been previously blocked by the PD1-PD-L1 interaction[248,249]

Many kinds of cancers are currently identified by IHC, which can detect the PD-L1 protein expression enhancing the response to anti-PD-L1-blockade. However, this method is not absolute regarding the use of PD-L1 as a predictor of responsiveness to the different anti-PD-L1 therapies.[250] The facts that some patients who test positive for PD-L1 may not respond to the treatment, and that some patients who test negative may still respond are potential caveats to predict therapeutic response through PD-L1[251,252] (reviewed by Ribas and Hu-Lieskovan[250]). Reasons for the concerns on the limitations of the predictive value of PD-L1 include the differences in the sensitivity/sensibility of the commercial assays available or artifacts related to IHC and tissue sampling. In addition, PD-L1 expression in the tumor microenvironment is highly complex, being upregulated by aberrant genetic alterations and is highly regulated at the transcriptional, posttr6anscriptional, and protein levels. Thus, PD-L1 IHC seems to be insufficient to fully understand the relevance of PD-L1 levels in the whole body and their dynamics to improve therapeutic outcomes.[251] Davis and Patel[253] evaluated PD-L1 as a predictive biomarker based on all ICI approved by the FDA from 2011 to 2019. They reported that PD-L1 was predictive in only 28.9% of cases and was either not predictive (53.3%) or not tested (17.8%) in the remaining cases.[253] A broad review on this topic has been published by Nimmagadda[241] and Cottrell and Taube.[243]

Nonetheless, fortunately, numerous IHC assays have been approved[254-256] to detect PD-L1 expression levels for specific drugs; each ICI has its own IHC assay specific to a distinct anti-PD-L1 antibody clone and a particular staining platform with specific tumor cell or immune cell thresholds.[243,257]

Although the different FDA-approved tests may detect the same biomarker, these tests' performance characteristics differ, meaning the tests may detect different patient populations, as they are based on the data generated in the course of the corresponding therapeutic trial. Thus, a test is uniquely paired with a specific drug, and FDA approval of this drug-device pair was shown to be safe and effective for the stated intended use, including the specific indication.[258,259]

The availability of multiple PD-L1 assays and the appropriate choice for immune checkpoint therapy has become increasingly significant in TNBC. Higher expression of PD-L1 is observed in TNBC, more than in other molecular subtypes of BC.[260] Moreover, in TNBC, PD-L1 is expressed mainly in tumor-infiltrating immune cells[259,261] making PD-L1 inhibition attractive immunotherapy for the treatment of TNBC.

Atezolizumab, a mAb against PD-L1, has shown mixed results in clinical trials. The Impassion-031 trial studied atezolizumab combined with nab-paclitaxel (a paclitaxel protein-bound) followed by doxorubicin and cyclophosphamide as a neoadjuvant treatment for patients with early-stage TNBC eligible for surgery. The trial showed a polymerase chain reaction (pCR) of 58% in the atezolizumab group and 41% in the placebo group.[262] Another trial, Impassion-130 looked at atezolizumab with nab-paclitaxel as a first-line treatment in untreated metastatic TNBC. Findings from this trial showed an increased PFS to 7.2 months with atezolizumab when compared to 5.5 months with placebo. Furthermore, Impassion130 showed that this increase in PFS was even greater in those patients who had PD-L1 positive tumors (7.5 months vs 5.0 months).[263] Final results from this trial showed an improvement in median PFS of 2.2 months with atezolizumab plus nab-paclitaxel for patients with tumors that express PD-L1 on immune cells that cover 1% or more of the tumor area (PD-L1 immune cell-positive patients)[264] while maintaining patient's health-related quality of life.[265] The results of these trials led to the approval of atezolizumab for early and metastatic TNBC with nab-paclitaxel. More recently, the Impassion-131 trial had less optimistic results when looking at atezolizumab in combination with paclitaxel for metastatic TNBC. The trial revealed that atezolizumab did not significantly improve PFS and that OS results at interim favored placebo over atezolizumab.[266] This led to the FDA releasing a statement that further studies are needed to confirm the efficacy of atezolizumab in treating BC, and that continued approval of atezolizumab with nab-paclitaxel could be contingent on these additional studies.[266]

Pembrolizumab, a mAb anti-PD-L1, was evaluated in 2 different phase III clinical trials to be used in conjunction with chemotherapy in the treatment of TNBC.[267,268] Keynote-522, a phase III trial, evaluated pembrolizumab plus chemotherapy

(paclitaxel/carboplatin followed by cyclophosphamide) compared with placebo plus chemotherapy as neoadjuvant therapy followed by adjuvant pembrolizumab or placebo in patients with early-stage TNBC.[267] This phase III trial showed a significantly higher percentage of patients with a pCR among those who received pembrolizumab plus neoadjuvant chemotherapy than those who received placebo plus chemotherapy regardless of PD-L1 status.[267] Pembrolizumab was also evaluated combined with chemotherapy (nab-paclitaxel; paclitaxel; or gemcitabine/carboplatin) as a first-line treatment for patients with locally recurrent inoperable or metastatic TNBC in the Keynote-355 phase III trial.[268] This study showed a statistically significant and clinically meaningful improvement in PFS when pembrolizumab was added to 3 different chemo regiments in TNBC patients whose tumors expressed PD-L1 (Combined Positive Score ⩾ 10).[268] Such results lead the FDA, in November 2020, to grant an accelerated approval of pembrolizumab in combination with chemotherapy (paclitaxel, nab-paclitaxel, or gemcitabine and carboplatin) for the treatment of patients with locally recurrent unresectable or TNBC whose tumors express PD-L1 (CPS ⩾ 10).[12]

Gamma-delta T (γδ T) cell stimulators: The γδ *T* cells are a subset of *T* lymphocytes characterized by *T* cell receptors (TCR) composed of γ and δ chains which can recognize a great variety of antigens including peptides, unprocessed proteins, sulfatides, and phospholipids without a requirement for major histocompatibility complex (MHC) antigen presentation.[13,14,269,270] Moreover, γδ *T* cells also express natural killer receptors such as NKG2D.[13,14] Different subtypes of γδ *T* cells display spectrums of phenotypic and functional characteristics ranging from innate to adaptive like features.[271,272]

γδ *T* cells are involved in the lymphoid stress-surveillance response by recognizing antigens that are upregulated in stressed and transformed cells but not expressed in healthy tissues, giving it an advantage as the first line of defense against transformed cells.[13,14,272,273] There are 2 major subtypes of γδ *T* lymphocytes in humans, the Vδ1$^+$ *T* cells, which are present in skin and intestine and the Vδ2$^+$ *T* cells which are present in the peripheral blood.[13,14,274] Vδ1$^+$ *T* cells represent the predominant γδ *T* cell subtype that has been found in healthy breast tissue[272] as well as tumor-infiltrating lymphocytes (TILs) for many cancers[14,275,276] and have also been implicated to be involved in BC.[13,274,277,278] A recent clinical study demonstrated PFS and OS correlated with Vδ1+ T cells representation for TNBC patients.[273] A subset of Vδ2$^+$ *T* cells expressing Vγ9Vδ2$^+$ TCRs is abundant in human peripheral blood, making it optimal for isolation and use in clinical trials. Vγ9Vδ2$^+$ *T* cells display antitumor immunity in response to phosphoantigens produced by cellular pathogens and overexpressed by cancers. Aminobisphosphonates (N-bis), such as zoledronate (zoledronic acid), also a phosphoantigen, is a promising drug in the treatment of cancer by targeting the mevalonate pathway (Figure 3).[13,14,279] As discussed above, zoledronate is the most potent and efficacious clinically approved N-bis, which are prescribed for osteoporosis.[280,281] and is also under clinical trial for BC as a γδ T cell stimulator (Figure 2), which is the newest potential class of drugs to treat ABC and MBC.[274]

Conclusion

Resistance to prior standard treatments is an important driving force for the discovery and development of new BC drugs and/or new strategies applying the existing drugs. Combined therapies comprising different classes of drugs is an approach successfully used as reasonable options to establish the best prognosis providing patients with the opportunity to obtain the best benefit while minimizing or abolishing recurrence, resistance, and toxic effects. Besides that, researches on cellular pathways have led to studies of new agents targeting or inhibiting tumorigeneses.

This rationale yielded the researches on biomarkers of clinical significance such as CDK4/6 expression and *PIK3CA* mutations. PIK3 inhibitors are the newest BC treatments approved by the FDA.

The forefront current drugs under clinical trials are mAb, ADC, HDAC inhibitors, PARP inhibitors, and ultimately the γδ *T* cell stimulators, but their role is unclear and still under investigation.

As research continues, new classes of drugs are emerging with increased benefits in BC treatment. The complexity of the cell cycle and its association with cancer biology provides a plethora of factors toward the development of more efficient agents as well as biomarkers with high predictive values.

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Author Contributions

J.S., R.P.R., and C.R. contributed equally. C.R. wrote the bulk of the manuscript and edited the final version. C.R. and R.P.R. conceived the project. C.R. coordinated manuscript production. C.R., J.S., and R.P.R. participated in literature searching. C.R., J.S., and R.P.R. wrote, edited, and approved the final version of the manuscript.

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