Current Breast Cancer Treatment Strategies and Resistance Mechanisms

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Abstract

Breast cancer, characterized by diverse subtypes and intricate molecular landscapes, poses persistent challenges in treatment. A nuanced understanding of current therapies and resistance mechanisms is essential. Subdivision based on hormonal receptors and HER2 expression yields four distinct subtypes with varying prognosis and treatments. Despite initial success in clinics, concerns arise due to patients developing resistance mechanisms over time. This review comprehensively explores evolving breast cancer treatment strategies, focusing on molecular landscapes and challenges associated with targeted therapies. It covers endocrine therapy, receptor roles, and emerging resistance strategies. Approved inhibitors' mechanisms and the importance of comprehending signaling pathway interplay are discussed. The review connects the themes by examining resistance strategies against inhibitors and advocating for combination therapies. Current and evolving treatment strategies are scrutinized, encompassing established and investigational modalities. The review aims to provide valuable insights for researchers, clinicians, and policymakers to advance therapeutic strategies and enhance patient outcomes.

Keywords

Introduction

Breast cancer develops when some breast cells begin to grow uncontrollably. Treatments for breast cancer include surgery, radiation therapy, chemotherapy, hormonal therapy, targeted therapy, and immunotherapy [1]. Most breast cancers are hormone receptor positive, meaning that they depend on hormones to grow and spread. Hormone therapy works by blocking the cancer cells from receiving the natural hormones that they crave. Targeted therapy uses specifically designed drugs such as monoclonal antibodies that act on specific tumor antigens (neoantigens), or those found in the tumor microenvironment [2]. Typically, when it comes to targeted therapy and biological therapy, the treatment is based on the molecular subtype of breast cancer that is in turn based on the genes the cancer cells express, which control how cells behave [3]. Herein, we will cover the current treatments and possible mechanisms of resistance against such options in the context of the most common breast cancer molecular subtypes, namely being luminal breast cancer, HER2-enriched breast cancer, and triple negative or basal-like breast cancer.

Luminal Breast Cancer Treatment Options

Luminal breast tumors, so-called estrogen receptor (ER) - positive (ER+) tumors represent around two-thirds of all breast cancers. This subtype is further divided into luminal A, luminal B, and B-like breast cancers. Hormonal therapy is well thought-out as an indispensable part of the management of patients with ER+ breast cancer [4].

Hormonal Therapy of Luminal Breast Cancer Subtype: Special Focus on ER Modulation-based Therapy

Endocrinal therapies that either disrupt the production of estrogens or hamper estrogen-mediated signaling pathways have become an important part of the management of hormone-dependent breast cancer [5]. Existing drugs for adjuvant

endocrine therapy can be separated into three classes: selective estrogen receptor modulators (SERMs), aromatase inhibitors (AIs), and selective estrogen receptor down regulators (SERDs) [5]. The most used class of estrogen receptor modulators is Tamoxifen (TAM), in both pre- and post-menopausal women. The antitumor attributes of TAM are reflected to be a result of its anti-estrogenic action, mediated by completive inhibition of estrogen binding to ER, where TAM belongs to SERMs. Prior to entering breast cancer cell, TAM is metabolized in the liver into 2 active metabolites, endoxifen and 4-hydroxytamoxifen (4-OHT) [5]. When these metabolites enter the cell, they bind to the cytosolic ERs, thereby blocking the binding of estrogen. TAM-bound ERs dimerize, translocate to the nucleus, and bind to the estrogen response element (ERE) in the promoter region of estrogen-induced genes. The ER-TAM complex, however, does not recruit the essential coactivators; as a result, TAM inhibits the expression of estrogen-induced genes, including growth factors and angiogenic factors secreted by the tumor, which may stimulate growth via autocrine or paracrine mechanisms [5]. As a result, the G1 phase of the cell cycle is stalled and cell proliferation is slowed. Because of the altered balance between cell proliferation and ongoing cell loss, tumors may regress [5].

Figure 1: Diagrammatic illustration of how fulvestrant works. AF1 and AF2 are known as activation functions 1 and 2, respectively. ER stands for estrogen receptor, and ERE is an acronym for estrogen receptor response element. F represents fulvestrant, and RNA POL II is short for ribonucleic acid polymerase II [6].

TAM has a partial ER-agonistic effect versus fulvestrant, which has an almost entirely antagonistic effect. Furthermore, TAM only affects the ER's AF2 domain, whereas fulvestrant affects

both activating function 1 (AF1) and activating function 2 (AF2) [7] (Figure 1).

There exist other options for hormonal therapy of ER-positive breast cancer such as Toremifene, used to treat metastatic breast cancer. It is structurally and pharmacologically similar to TAM, differing only by a single chlorine atom [7]. The major difference between the two compounds is in the preclinical activity. Fulvestrant, a SERD, is used to treat progressed breast cancer in postmenopausal women. When fulvestrant binds to estrogen receptor monomers it hinders receptor dimerization, AF1 and AF2 are thus left inactive, translocation of receptor to the nucleus is abridged, and degradation of the estrogen receptor is augmented [7]. While the medicines indicated above work by preventing hormones from binding to cancer cells, other medications work by preventing the body from producing estrogen after menopause. This comprises AIs, a class of medicines that lessen the quantity of estrogen in the body, depriving breast cancer cells of the hormones they need to grow. AIs are only utilized in women who have experienced menopause [7]. They cannot be used unless the body is in natural menopause or menopause triggered by medications or the elimination of the ovaries. Mechanism-based AIs are steroidal inhibitors that imitate the substrate and are transformed by the fat tissue enzyme aromatase to a reactive intermediate, causing the inactivation of aromatase. These different types of endocrine therapies have been used effectively to cause a momentous decline in cancer recurrence and death [7].

Resistance Strategies of Endocrine Therapy

Although existing endocrine therapies for women with ER+ breast cancer have resulted in significant improvements in outcomes, not all patients with ER+ tumors respond to endocrine treatment (de novo or primary resistance). Besides, ER+ patients, who at first, respond may later become non-responsive to the therapy (acquired resistance) [8]. Acknowledging the ultimate origins of treatment resistance has therefore been the interest of several studies to tackle this paramount clinical dilemma. The intricate crosstalk, both genomic and non-genomic, between

estrogen receptors and growth factors was well thought out to be a critical issue contributing to endocrine resistance (Figure 2). ERs refer to a family of nuclear transcriptional regulators that engage in an essential role in the progress of breast cancer [8]. ERs are classified into two isoforms: ER-α and ER-β. Because the role of ER-β in endocrine resistance is still debated, and because ER-α expression is higher in breast tumors than ER-β, it is the target of therapeutic intervention. Here, we will limit our discussion to ' ER-α ' which will be referred to as 'ER' in the following sections. Nuclear ER and membrane ER both act through genomic (nuclear) and non-genomic (membrane) pathways [8]. In general, one of the main features of the apparatus of ER-mediated antiestrogen treatment resistance is the loss of ER expression [9]. The fact is that nearly all primary ER+ patients will develop endocrine resistance, implying that some distorted pathways may affect ER expression and functions. For instance, ER loss has been linked to unusual methylation of CpG islands in the ER gene's 5′ regulatory regions. This in turn could result in transcriptional inactivation of the ER gene and lead to hormone resistance in various human breast cancers. Furthermore, 50% of patients with ER+ breast tumors express PR [9]. The increased resistance of ER+ breast tumors to SERMs could be because estrogen has a higher affinity to ER compared to SERMs. Numerous clinical studies have exposed that ER+/PR+ tumors are quicker to respond to SERMs therapy than ER+/PR- tumors. Indeed, two trials concerning the function of progesterone receptor (PR) in response to AIs revealed an improved response to endocrine therapy in PR+ tumors than PR- tumors [9] Multiple studies described that many growth factors of breast cancer could ultimately reduce PR levels through the PI3K/Akt/mTOR pathway and downregulate ER expression level and action. Sian Tovey et al. found that PR and HER2 status could help predict early decline in ER+ tamoxifen-treated breast cancer patients [9]. Furthermore, the status of expression of both HER-1 (EGFR) and HER-2 was drastically elevated in the ER+/PR- patients than in that of ER+/PR+ patients, despite recent clinical recommendations that such elevated levels of HER-1 and HER-2 were associated with TAM resistance [10]. It is noteworthy that treating HER2-positive breast cancer patients with TAM has resulted in weak outcomes.

A more extensive understanding of the function of HER2 in endocrine resistance sheds light on the crosstalk among HER2 and ER signaling tracks. It is notable that TAM, through membrane ER, is competent in activating HER2, which in turn causes ER and A1B1 (an essential ER co-activator) phosphorylation. Benz and colleagues discovered that transfecting HER2 in MCF-7 cells, which are hormone-dependent breast cancer cell lines, can result in TAM resistance [10]. Another in vitro study found that MCF-7 developed resistant clones to TAM; these clones were found to have increased levels of phosphorylated EGFR and HER2. Estrogen and TAM seem to turn on EGFR and HER2 signaling pathways through on-genomic mechanisms in HER2 overexpressing tumors. It should be noted that some downstream kinases, such as AKT, can phosphorylate ER and activate A1B1, resulting in a crosstalk between the nuclear TAM-ER complex and its co-activators that promote cell survival and proliferation [10]. Interestingly, the role of miRNAs in encouraging endocrine resistance is represented by, but not restricted to, their participation in controlling ERα. MiR-221 and miR-222, for example, are overexpressed in TAM-resistant and even ER-negative breast cancer cell lines and tumors. It is important to note that the 3′UTR of ERα is a straight target of miR-221/222 lessening ERα protein expression [11]. An experiment attempting to transiently overexpress miR-221/222 in TAM-sensitive MCF-7 cells led to TAM resistance whilst downregulation of miR-221/222 in ERα negative/TAM-resistant MDA-MB-468 cells brought back ERα expression and cells became susceptible to TAM-induced cell cycle arrest and apoptosis. However, miR-873 has been revealed to be reduced in TAM-resistant MCF-7 compared to TAM-sensitive and in breast tumors compared to usual tissues [11]. It is a target of cyclin-dependent kinase 3 (CDK3) for downregulation, where CDK3-mediated ERα phosphorylation enhances ERα function [11]. Lately, miR-519a was demonstrated as a new onco-miRNA via enhancing cell viability and cell cycle succession. MiR-519a level was elevated in TAM- resistant MCF-7 cells as compared with TAM-sensitive MCF-7 cells. Upregulated levels of miR-519a in primary breast tumors were linked with abridged disease-free survival in ERα+ breast cancer patients and thus

miR-519a was recommended as a possible contributor to TAM resistance. This is supported by the fact that miR-519a knockdown in TAM-resistant MCF-7 cells made the cells susceptible to TAM growth inhibition. In contrast, overexpressing miR-519a in TAM-sensitive MCF-7 cells desensitized the cells to TAM by averting growth inhibition while encouraging caspase action and apoptosis [11]. It is noteworthy that tumor suppressor genes (TSGs) involved in PI3K signaling p21, RB1, and PTEN, were reported to be actual targets of miR-519a, even though the function of such targets in causing TAM-resistance has not been discovered yet [12]. Thereby, it is critical to gain a more complete understanding of the underlying resistance mechanisms and elucidate targets for therapeutic intervention and by combining endocrine therapy with various molecularly targeted agents and signal transduction inhibitors, some success has been achieved in overcoming and modulating endocrine resistance in Hormone-positive breast cancer. Established strategies include selective ER modulators, anti-HER2 agents, mTOR inhibitors and inhibitors of PI3K are not at present a treatment alternative for women with ER+ breast cancer outside the milieu of clinical trials [12].
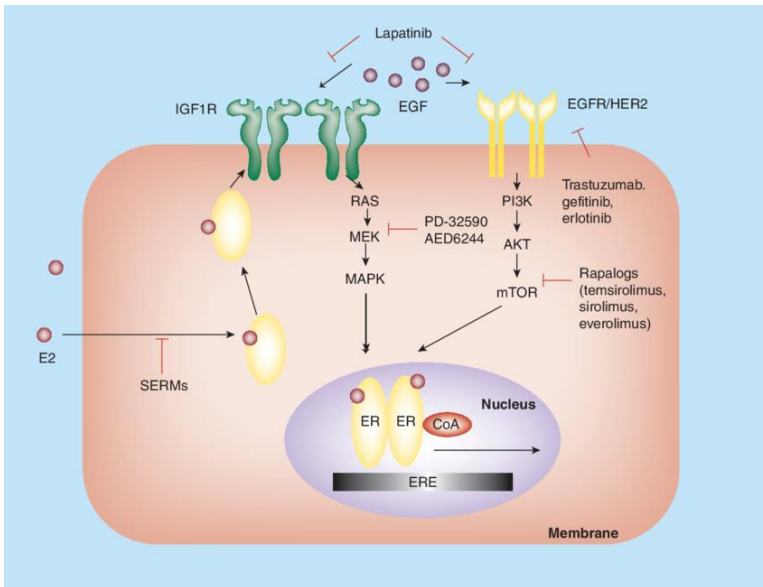
Figure 2: Molecular changes in endocrine-resistant breast cancer. This scientific diagram represents cellular signaling pathways involving IGF1R, EGF, and EGFR/HER2 receptors. It illustrates the roles and interactions of various molecules and inhibitors within these pathways, including Lapatinib, Trastuzumab, PD-32590, and AED6244. The diagram also depicts the internal cellular components like RAS, MEK, MAPK, and the AKT pathway leading to mTOR. The nucleus with ER, CoA indicating a complex formation at ERE is also shown [13].

It is demonstrated that resistance to TAM could be due to the activation of mER leading to the increase in HER2 expression levels, consequently, elevating HER2-mediated signaling pathways including PI3K/AKT/mTOR pathway. Additionally, the change in expression levels of certain miRNAs upon endocrine resistance implies a role of RNA interference in this mechanism which requires further investigation [12].

HER2-Enriched Breast Cancer Treatment Options

HER2-enriched breast cancer is ER-, PR-, and HER2+. HER2-enriched cancers are likely to develop quicker than luminal

cancers and can have poorer prognosis but are typically effectively treated with therapies targeting HER2 [14]. Its attributes are due to HER2-mediated activation of oncogenic pathways that force the different cancer cell traits, such as the Mitogen Activated Protein Kinase (MAPK) and the PI3K/AKT/mTOR cascades [14]. At present, specific monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) are the two HER2 targeting approaches that have effectually boosted the prognosis of patients with HER2+ breast cancer. Anti-HER2 therapies (also referred to as HER2 inhibitors or HER2-targeted therapies) are a set of medicines used to treat all stages of HER2+ breast cancer and HER2-low breast cancers. In addition, small molecule TKIs are an alternative for patients with early phase or progressive HER2 + breast cancer [14].

Anti-HER2 Mechanisms of Approved HER2 Inhibitors

HER2 is a transmembrane tyrosine kinase receptor and has a unique feature that differentiates it from the other members of the family in the absence of a known ligand. HER2 is overexpressed in 25 to 30% of human breast cancers and has been determined to be an adverse prognostic factor [10,15]. Since the level of HER2 in human cancer cells is with membrane overexpression than in normal adult tissue, they are potentially more sensitive to the toxicity of HER2 sensitive drugs. HER2 overexpression is typically found in both the primary tumor and at the metastatic sites which provides the rational for the effectiveness of anti-HER2 at all disease sites [10,15]. Research thus focused heavily on HER2 inhibitors as anticancer agents. Trastuzumab is the first of such agents which was registered for use in patients with HER2 overexpressing breast cancer. Trastuzumab is a recombinant monoclonal antibody (mAb) directed against the extracellular domain of the tyrosine kinase receptor, HER2. It is known to bind to domain 4 [10,15]. It has shown clinical activity in HER2 overexpressing breast cancers and is currently approved in patients in both metastatic and adjuvant settings. Although still subjects of discussion, different mechanisms of action have been attributed to its anti-HER2 activity: (i) antibody-dependent cell mediated cytotoxicity, (ii)

prevention of HER2 truncated membrane bound fragment following HER2 overexpression and (iii) HER2 receptor downregulation [16]. The binding of trastuzumab to HER2 receptor with high affinity and specificity prevents the formation of HER2-HER2 homodimers and HER2-HER3 heterodimers. This subsequently inhibits HER2-mediated signal transduction pathways, hence it is thought to be the main mechanism of action of trastuzumab [16]. Additionally, the binding of trastuzumab to HER2 on cancer cell membranes is documented by Fcɣ receptors expressed by cells of the innate immune system, including natural killer (NK) cells, antigen-presenting cells (APCs) as well as effector immune cells; this results in the clearance of T-bound cancer cells either through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Pertuzumab is a novel fully humanized mAB that binds to domain 2, a portion of the extracellular domain essential for dimerization [16]. While trastuzumab is known to bind to domain 4. This binding of pertuzumab efficiently sterically blocks ligand-induced homodimerization and importantly HER2-HER3 heterodimerization that is known to activate downstream survival signaling pathways such as PI3K/AKT, whereas trastuzumab has only a minor effect in the presence of a ligand [17]. Pertuzumab was approved in mid-2012 for use in combination with trastuzumab and Docetaxel to treat patients with metastatic or locally recurrent unresectable HER2 positive breast cancer who have not received previous anti-HER2 therapy or chemotherapy for their metastatic disease. To increase the potency of antibody directed therapy, the specificity of the antigen binding site has been combined with a wide variety of effector agents including toxins [17]. This led to the development of antibody-drug conjugate (ADC) Ado-trastuzumab emtansine (T-DM1). T-DM1 is comprised of the anti-HER2 antibody trastuzumab bound to the potent antimicrotubule cytotoxic agent maytansine (DM1) by a thioether linker. T-DM1 uses trastuzumab to specifically localize the highly active chemotherapy to HER2 positive tumor cells. Trastuzumab and DM1 are degraded by the lysosome leading to cell cycle arrest and apoptosis [17]. It was initially approved in 2013 for metastatic patients. In 2020, trastuzumab deruxtecan (T-DXd) was the second approved ADC for patients who had

received at least 2 lines of anti-HER2-based therapy in the metastatic setting [17]. Like T-DM1, it is made of a mAb backbone of trastuzumab, its cytotoxic payload, obtained from exatecan, is a powerful topoisomerase I inhibitor rather than a microtubule inhibitor [18]. Moreover, T-Dxd comprises a cleavable linker where cathepsins, lysosomal enzymes upregulated in numerous cancer cells, are thought to act on [18]. The payload is membrane permeable and, thus is able to perform the bystander effect, supposedly enabling action even in tumors with varied or low expression of HER2, a property not noticed with T-DM1. All these attributes could clarify the anticancer activity of T-DXd in tumors that are intractable to T-DM1 [18].
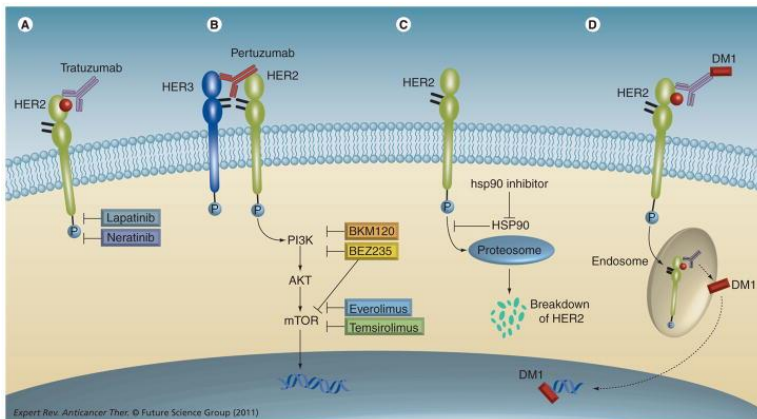

Figure 3: Novel therapeutic agents' points of intervention for HER2-enhanced breast cancer. (A) Lapatinib, a dual inhibitor of EGF receptor (EGFR)/HER2 tyrosine kinase, is sanctioned for use in patients resistant to trastuzumab. Neratinib, on the other hand, is an irreversible inhibitor of the tyrosine kinase of EGFR/HER2. (B) Pertuzumab, a monoclonal antibody for HER2, attaches to a unique epitope on HER2, distinct from the binding site of trastuzumab, and inhibits ligand-induced heterodimerization with HER3. The PI3K–AKT–mTOR pathway, when dysregulated, can lead to resistance to trastuzumab, and therapies targeting the direct inhibition of PI3K, AKT, and mTOR are under development. © Inhibitors of HSP90 facilitate the degradation of HER2 by impeding the function of HSP90, a chaperone protein that safeguards HER2 from proteasomal degradation. (D) TDM-1, a conjugate of the antibody–drug of trastuzumab and maytansine, enables the selective delivery of a potent microtubule inhibitor into cells overexpressing HER2 [18].

The HER2 extracellular domain has no known ligand and is activated by the formation of homo or heterodimers. These dimers lead to the phosphorylation of tyrosine kinase residues in the cytoplasmic domain which function as docking sites for proteins that activate the PI3K and MAPK signaling pathways downstream leading to cell cycle progression and proliferation. Both trastuzumab and pertuzumab work by binding to the extracellular region of HER2 at domains 4 and 2, respectively. Trastuzumab binding to HER2 on breast cancer cells enhances their clearance by innate immune cells [18] (Figure 3).
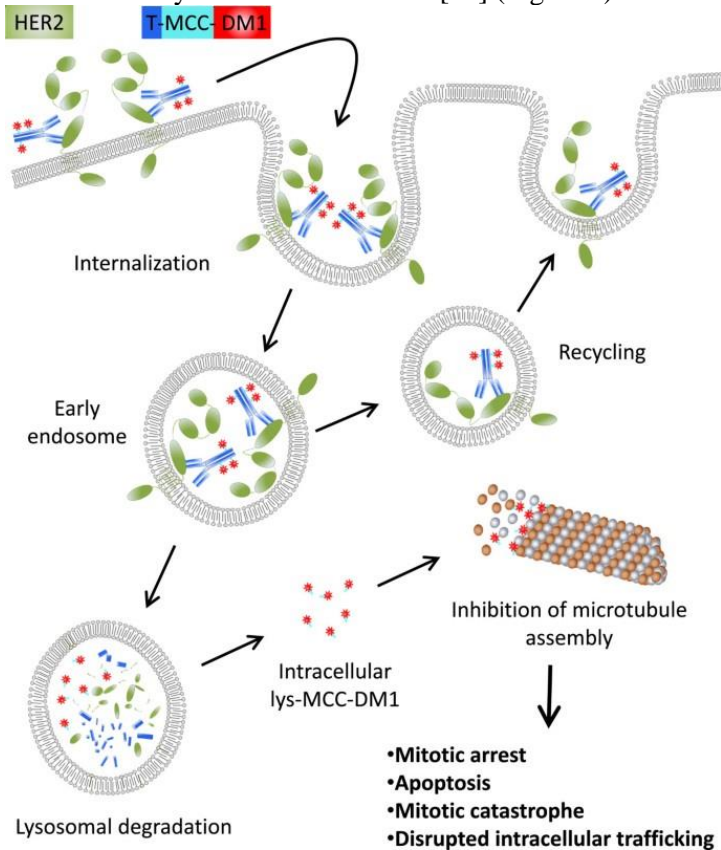

Figure 4: The internal movement and processing of trastuzumab emtansine (T-DM1) within a cell. The T-DM1 compound binds to the human epidermal growth factor receptor-2 (HER2) located on the plasma membrane, leading to the formation of a HER2-T-DM1 complex that enters the cell through receptor-mediated endocytosis. This process results in the formation of early endosomes

from the internalized endocytic vesicles. The contents of these early endosomes can either be recycled back to the cell membrane or the endosome can mature into a lysosome. The DM1 component is released following the proteolytic degradation of the antibody portion of T-DM1 in the lysosomes. The intracellular lysine (lys)-MCC-DM1 then inhibits the assembly of microtubules, leading to mitotic arrest, apoptosis, mitotic catastrophe, and disruption of intracellular trafficking. MCC refers to a non-reducible thioether linker [19].

T-DM1 is a next-generation ADC that merges the anti-HER2 outcome of trastuzumab with the cytotoxicity of the anti-microtubule agent DM1. In order to target HER2+ breast cancer cells, T-DM1 must bind HER2 on the plasma membrane, in which the HER2-T-DM1 complex must be internalized via receptor-mediated endocytosis. DM1 is then freed into lysosomes due to proteolytic degradation of the antibody part of the complex, where the Lys-MCC-DM1 metabolite of DM1 acts as a microtubule inhibitor of assembly and inhibiting cell cycle progression through mitosis [19] (Figure 4).

Small TKIs Mechanisms of Approved TKIs against HER2-Positive Breast Cancer

Small TKI molecules have many benefits over monoclonal antibody therapies, such as the aptitude to target several family members concurrently, to act straight at the level of the intracellular signaling cascade, and to possibly cross the blood brain barrier (BBB). Two small molecule TKIs, lapatinib and neratinib, have been officially approved for HER2 breast cancer management [20]. Herein we will focus on the mechanism of action of these TKIs. TKI refers to certain oral small molecular drugs dynamic in encouraging apoptosis and hindering the proliferation of cancer cells. It competitively binds intracellular adenosine triphosphate (ATP) binding domains of the HER family owing to the homological organization of the ATP, inhibiting tyrosine kinase phosphorylation, and thereby blocking downstream signals [20]. In the context of brain metastasis cancer management, the effectiveness of monoclonal antibodies can be inadequate in crossing BBB, whilst small molecule TKIs, such as lapatinib, are considered to have permeability through the BBB. Both lapatinib and neratinib bind to inactive

conformation of HER family members, thereby limiting ligand-induced activation. Lapatinib reduces the phosphorylation of HER1 and HER2 to promote apoptosis [20]. However, Neratinib is an irreversible TKI of HER1, HER2, and HER 4. Neratinib was accepted by the FDA in 2017 as an unlimited adjuvant management for patients with early-stage HER2 overexpressing breast cancer following surgery and trastuzumab-based adjuvant management [20]. It acts by preventing phosphorylation of the ErbB family and downstream pathways comprising ERK and Akt, via its covalent combination with cysteine residues Cys-773 and Cys-805 of the ATP-binding domain of HER1, HER2, and HER4. Downstream signal transduction inhibition following neratinib treatment results in abridged cyclin D1 expression, thereby arresting the G1-S phase transition, ultimately leading to a reduction of cell proliferation. Furthermore, neratinib can also induce ubiquitylation and endocytic degradation and reduction in HER2 expression in a process involving HSP90 dissociation [21]. Besides, neratinib has been shown to hinder ATP-binding cassette transporter and subsequently overturn the multidrug resistance of cancer cells [21].

By reversibly and irreversibly inhibiting HER2 phosphorylation via Laptinib and neratinib respectively, HER2-mediated signaling pathways PI3K/AKT/mTOR and MAPK signaling cascades are hindered, thus reducing cell proliferation and cancer progression. Neratinib can also cause ubiquitylation, endocytic degradation, and a decrease in HER2 expression through a process involving HSP90 dissociation [21].

Resistance Strategies to Single HER2 Inhibitors

Regardless of the fact that in the last years, the introduction of mAbs, TKIs, and ADCs targeting HER2 notably improved patient prognosis in all disease stages, not all patients with limited-stage disease are cured and HER2+ metastatic breast cancer is still roughly considered a deadly disease. Primary or acquired resistance to anti-HER2 therapies is accountable for the majority of treatment failures. Lately, several resistance mechanisms have been recognized, such as ongoing activation of signaling pathways similar to or downstream of HER2, altered

binding of anti-HER2 agents to HER2, and abridged immune system activation. Even though trastuzumab noticeably enhanced the prognosis of HER2+ breast cancer patients, many patients still progress within 12 months from the start of trastuzumab treatment [22]. One way of resistance to trastuzumab could be due to its impaired binding to HER2 because of intratumor heterogeneity of HER2 expression which has been shown to be linked with abridged activity of Trastuzumab-based treatments [23]. This indicates the presence of some breast cancer clones with low expression levels of HER2 that may gradually become presiding throughout trastuzumab exposure. Additionally, some HER2 splicing variants can also affect the aptitude of trastuzumab to bind HER2. New studies involving breast cancer cell lines recognized a splicing variant of HER2 missing exon-16, which forms HER2 dimers in an SRC-dependent way and which has been shown to associate with in vitro resistance to trastuzumab [23]. Furthermore, the expression of specific molecules by the cancer cells themselves or even other cells within the surrounding microenvironment can affect trastuzumab binding to HER2 ectodomain could be a way of resistance to this treatment. One way of this is through the expression of membrane-associated mucin 4 (MUC4) which hides the trastuzumab binding site on HER2, thereby trastuzumab binding and inhibiting HER2 could be altered, however, this needs further evaluation [23]. Moreover, it is not surprising that mutations in genes encoding players in the PI3K/AKT/mTOR signaling cascade associated with the ongoing activation of such a pathway could account for the resistance to trastuzumab. Two activating mutations, namely E545K and H1047R, residing within PI3K catalytic subunit alpha, were linked with trastuzumab resistance in HER2+ breast cancers [23]. Comparable observations appeared in tumors expressing small levels of PTEN that are acknowledged to oppose PI3K-induced phosphorylation of inositide lipids. This could be further backed by the observation that the inhibition of PIK3CA or mTOR sensitizes cancer cell lines to trastuzumab [24]. Knowing that the tyrosine kinase SRC acts downstream of HER2, one possible mechanism of resistance to trastuzumab is the abnormal activation of SRC. Inhibition of SRC has been shown to re-establish sensitivity to trastuzumab both in vitro and in vivo [24].

Even though trastuzumab averts HER2 homodimerization and restrains its driven signaling, diverse RTKs, such as EGFR, HER1, and HER3, are capable of heterodimerizing with HER2, and trigger downstream signaling cascades in the same way as HER2 homodimers. This enables tumor cells to utilize a small number of HER2 molecules that are not trastuzumab-bound and thus reactivate the HER2 signaling cascade. It has also been demonstrated that the escape from ADCC could induce resistance to trastuzumab [24]. This is consistent with the observation that mice deficient in CD16A have altered ADCC-mediated lysis of cancer cells, and HER2+ve tumors developing in these animals are resistant to trastuzumab. Particularly, trastuzumab binding to the inhibitory receptor CD32B: FcγRIIB on myeloid cells averts ADCC. While recent data propose that T-DM1 could eliminate HER2+ tumor clones that are resistant to trastuzumab, resistance to T-DM1 limits the anticancer effectiveness of T-DM1 in the metastatic situation [24]. Resistance mechanisms to T-DM1 comprise besides altered binding of T-MD1 to HER2, an altered HER2-T-DM1 complex internalization, faulty lysosomal function that hinders DM1 release, and efflux pumps concerned in DM1 export [15,24]. In vitro and in vivo studies revealed that altered lysosomal acidification and degradation of the antibody part of T-DM1, or abridged export of lys-MCC-DM1 from the lysosome into the cytoplasm via SLC46A3 transporter, lead to an earned resistance to T-DM1 [15,24]. MDR1, a plasma membrane transporter, can promote T-DM1 resistance by inducing extracellular DM1 efflux, further backed up by the fact that MDR1 inhibitors might re-establish sensitivity to T-DM1. On the other hand, even though several tumors are initially resistant to lapatinib, HER2+ tumors obtain resistance following a median time of 6 months following lapatinib treatment [15,24]. Various HER2 amino acid substitution mutations have been shown to be linked with lapatinib resistance, with the HER2 L755S and T798I mutations accounting for the uppermost levels of resistance. However, neratinib has been shown to have an anticancer action in patients with metastatic breast cancer nurturing mutations in the HER2 tyrosine kinase domain separately from HER2 levels of expression [15,24]. Additionally, parallel signaling pathways to HER2 activation can be activated in a process involving elevated

expression of RTK ligands by tumor cells of nearby cells. For example, overexpression of neuregulin-1 (NRG1), the major HER3 ligand, turns on the EGFR-HER3-PI3K-PDK1 signaling cascade, thereby overcoming lapatinib-induced reversion of HER2/EGFR [15,24]. Likewise, the binding of HGF to MET as well leads to lapatinib resistance via PI3K/AKT/mTOR pathway reactivation throughout HER2 inhibition therapy. Interestingly, Lapatinib-induced inactivation of HER2 promotes adjusting PI3K/AKT elevation of HER3 expression [25], therefore encouraging HER2-HER3 heterodimerization. In the cases of trastuzumab and lapatinib, the enhanced activation of cyclin D1-CDK4/6 cascade seems to be linked to the resistance to these therapies as revealed in HER2+ breast cancer cell lines. This is consistent with the reversion of such resistance following pharmacological inhibition of CDK4/6 [25]. In terms of neratinib, Breslin et al. established that improved activity of the metabolism enzyme cytochrome P4503A4 results in neratinib resistance and cross-resistance to trastuzumab and lapatinib. Additionally, Seyhan et al. had acknowledged a set of genes linked to neratinib resistance by means of a genome-wide RNAi screen coupled with a lethal amount of neratinib, such as oncogenesis, transcription factors, protein ubiquitination, cell cycle, and genes recognized to cooperate with breast cancer-coupled genes [25]. The expression of RB1CC1, ERBB3, and FOXO3a has been shown to be elevated in HER2 TKI-sensitive breast cancer cell lines following management with lapatinib and neratinib. A study by Takeda et al demonstrated that the activation of YES1, being an SRC family member, has been shown to be upregulated in two neratinib resistant breast cancer cell lines [25]. This study showed that the knockdown of YES1 via siRNA made YES1 amplified cancer cell lines sensitive to neratinib. The authors revealed that YES1 interacts with and activates HER2 [25].

Numerous possible resistance mechanisms to anti-HER2 agents have been recognized. The majority of them engage genetic or epigenetic alterations causing either overexpression or ongoing activation of HER2/HER3/HER4 or other plasma membrane kinases (e.g. FGFR1) or downstream effectors. Regardless of the exact mechanism, reactivation of the PI3K/AKT/mTOR cascade

looks critical to promote and uphold resistance to anti-HER2 therapies. Regarding T-DM1 resistance, mechanisms including its internalization or lysosomal role might as well have an outstanding role [25]. Taken together, it is of use to assess the possibility of combining anti-HER2 mAbs/TKIs with the parallel players in the resistance to HER2 including PI3K inhibitor, AKT inhibitor, mTOR inhibitor, CDK4/6 inhibitor, and YES1 inhibitor among others.

Triple Negative Breast Cancer Treatment Strategies

Triple-negative breast cancer (TNBC) does not express ER and PR and doesn't overexpress HER2 (81). Since tumor cells do not have these proteins, hormone therapy and HER2 targeted therapy are not supportive; consequently chemotherapy (chemo) is the major systemic treatment alternative [26]. Nevertheless, TNBC regularly responds splendidly to chemotherapy and tends to relapse more often than other breast cancers. However, for women with TNBC who have a BRCA mutation and whose cancer no longer responds to ordinary breast cancer chemo drugs, targeted drugs called Poly ADP-ribose polymerases (PARP) inhibitors may be considered. Furthermore, in favor of advanced TNBC in which the cancer cells have the PD-L1 protein, the primary treatment may be immunotherapy besides chemo [26]. Also, antibody-drug conjugates (ADCs) like SG and T-DXd, as well as additional ADCs in later phases of research with alternative targets, will revolutionize the therapy landscape for BC and other cancer types. The PD-L1 protein is detected in around 1 out of 5 TNBCs [26].

Chemotherapy in the Context of TNBC

The goal of chemotherapy is to eliminate cancer cells in the original tumor and any sites of metastasis [26,27]. In addition to being a primary cancer treatment option in the case of TNBC, chemotherapy can also act as a secondary treatment before, during, and after other primary cancer treatments such as radiation therapy or surgical excision of a tumor. In most cases, several chemotherapy drugs could be administered to increase

their effectiveness [26,27]. This allows the body to recover and kills as many cancer cells as possible. Anthracycline/taxane-based chemotherapy is considered the standard of care for patients with TNBC, whether in the neoadjuvant or adjuvant setting. Both Paclitaxel and Docetaxel are anti-cancer chemotherapies. They are considered "plant alkaloids," "taxane" and "anti-microtubule agents. The main manne of paclitaxel's action is the hyper-stabilization of microtubules (a constituent of the cytoskeleton) made of repeating subunits of α- and β-tubulin vital for numerous cellular behaviors [26,27]. Paclitaxel binds to the N-terminal amino acids of the β-tubulin subunit and lowers the threshold concentration of purified tubulin subunits required for in vitro polymerization into microtubules while increasing the fraction of tubulin subunits that assemble [26,27]. Paclitaxel also interacts directly with microtubules, preventing depolymerization by cold and calcium [28]. As a result, cancer cells treated with the drug enter metaphase on bipolar spindles, and their growth is halted. The activation of the spindle assembly checkpoint prevents the progression of the cell cycle, specifically the separation of the chromosomes due to the presence of kinetochores that do not have a solid attachment to microtubules [28]. Cancer cells exposed to the drug exhibit decreased inner mitochondrial membrane potential, which causes the permeability transition pore channel to open and the release of cytochrome c and apoptosis-inducing factor. Apoptotic death is thereby carried out by activated effectory caspases. At the same time, docetaxel has been found to be twice as effective as paclitaxel in inhibiting microtubule depolymerization. Paclitaxel and docetaxel are both commonly used to treat a variety of tumors [28]. Other common chemo drugs used in TNBC include Anthracyclines. Its mode of action within cancer cells is based on growth arrest and programmed cell death by poisoning topoisomerase, a critical enzyme for unwinding DNA for replication and synthesis. One of the most promising new cytotoxic agents is gemcitabine, a pyrimidine nucleoside antimetabolite. The drug has been approved for the treatment of breast cancer and has shown activity in a variety of solid tumors. The most imperative mode of action of gemcitabine is DNA synthesis inhibition [28]. When gemcitabine triphosphate (dFdCTP) is incorporated into DNA, it is followed by a single

deoxynucleotide, preventing chain elongation. The chemo drugs mentioned above can be either used alone or in combination [29]. Unfortunately, chemotherapy drugs cannot tell the difference between fast growing normal cells and cancer cells; as a result, these drugs also damage or irritate some of the fast-growing normal cells such as those in the bone marrow, digestive system, and hair follicles. Death, irritation, or damage of these cells produces side-effects such as a weakened immune system, nausea, and hair loss [29].

BRCAness and PARP Inhibitors as a TNBC Treatment Option

A germline BRCA1 or BRCA2 mutation is present in 25% of patients with triple negative breast cancer [26,30]. BRCAness is defined as a set of traits in which BRCA1 dysfunction, caused by gene mutation, methylation, or deletion, results in a lack of DNA repair. Sometimes TNBCs seem to have BRCAness, and these tumors share clinicopathological features with BRCA1-mutated tumors. A better understanding of TNBC and the presence of BRCAness may have implications for both hereditary breast cancer screening and treatment of these tumors [26,30]. Tumors with BRCAness are thought to be extremely sensitive to chemotherapy. However, targeted drugs called poly (ADP-ribose) polymerase (PARP) inhibitors, like olaparib [Lynparza] or talazoparib [Talzenna] may be considered for women with TNBC who have a BRCA mutation and whose tumor no longer responds to common breast cancer chemo drugs. PARP1, which was discovered about 50 years ago, is involved in gene transcription, DNA repair, and cell death [26,30]. PARP inhibitor therapy is not currently approved by the FDA for patients with TNBC who do not have a germline BRCA mutation [31]. PARP is a major protein that is involved in DNA repair pathways, base excision repair (BER) mechanisms, homologous recombination (HR), and NEJ deficiency-based repair mechanisms. DNA damage repair deficiencies increase the likelihood of tumor formation. DNA DSB repair is inadequate in cancer cells affected by harmful mutations in the breast cancer susceptibility genes BRCA 1 and BRCA2 [31]. In fact, the homologous recombination repair (HRR) pathway relies heavily

on both BRCA1 and BRCA2. BRCA 1 is a multifunctional enzyme with a direct role in HRR. In conjunction with CHK2, it is initially in charge of signal transduction; following that, ATM and ATR detect DNA double strand damage. It then works by establishing a structure that arranges the repair proteins at the DNA repair site. On the other hand, BRCA 2 brings in RAD51, a recombinase, at the DNA repair site [31]. Therefore, if additional occurrences that could hinder DNA damage repair take place, the damage could result in a gradual accumulation of DNA changes that could eventually result in apoptosis [32,33]. The first clinically approved synthetic lethality-exploiting drug, PARPib, has demonstrated promising activity in patients with BRCA-deficient tumors. It has been shown that PARPib primarily works by blocking the PARylation mechanism, which causes DNA damage to be trapped at the site of the damage, activating effector genes, and ultimately interrupting the replication fork by causing DSB damage with a cytotoxic effect [32,33]. Preclinical models thus demonstrated that DNA trapping on PARP may be a more potent means of inducing cell death than catalytic enzyme alone. Thus, the current inhibition of PARP enzymes results in the accumulation of unpaired damages in tumors harboring a defect in the HRR pathway, which ultimately results in tumor cell death. Contrarily, patients with BRCA 1 or 2 mutations may benefit clinically because healthy cells may be spared. Olaparib and talazopirib are the only two PARPibs that have currently been authorized for the treatment of patients with metastatic TNBC [32,33]. Olaparib is a small molecule that was initially thought to be an inhibitor of PARP-1 and PARP-2 but data revealed that it is also a potent inhibitor of PARP-3. On the other hand, talazoparib is a powerful PARP inhibitor with both a strong catalytic inhibition and a potential for trapping PARP [34,35].

PDL1 Inhibitors

Recent years have seen an increase in the relevance of research on PD-L1 expression in breast cancer, particularly in triple-negative breast cancer (TNBC). When compared to other breast cancer subtypes, TNBC has been reported to have higher rates of cell surface PD-L1 expression, and higher PD-L1 expression

implies a greater potential benefit from using PD-1/PD-L1 targeted immunotherapy in this population of patients [36,37]. It has been discovered that the PD-1 receptor of Treg cells enhances the de novo conversion of naïve CD4+ T cells to Treg cells in the presence of CD3 and TGF-, therefore attenuating immunological responses. Through blocking the mTOR-Akt signaling cascade, this conversion promotes the production of Treg and the immunological suppressive activity of CD4+ T cells [36,37]. As a result, PD-1 expression not only inhibits effector T-cell activity but also promotes the conversion of the population of immunosuppressive Treg cells. Although PD-1 has been extensively investigated in T-cells, its roles in B-cells for tumor immunosuppression have also come to light. However, PD-1 levels are negligible in pro-B cells, an early stage of the mature B cell, and rise with B cell development. It has been shown that PD-1 expression is heavily controlled during B cell differentiation [36,37]. PD-1 is a novel regulator of human B-cell activation. Additionally, PD-1 activated toll-like receptor 9 (TLR9) agonists can greatly improve B-cell maturation [38]. It has been demonstrated that inhibiting PD-1 activity on B cells improves antigen-specific antibody responses, proving that PD-1 suppresses B cell-mediated T-cell activation. Monoclonal antibodies (mAbs) are a kind of checkpoint inhibitor that inhibits the interaction of PD-1 and PD-L1 and thereby overcomes the drawbacks of traditional anticancer treatment [38]. mAbs can considerably reduce toxicity while shrinking solid tumors, suppressing advanced malignancies and metastasis, and improving overall patient survival. Interferon gamma (IFN)-induced increase of PD-L1 expression on tumor cell surfaces is one way of regulation. This is probably a way by which tumor cells avoid being destroyed by T lymphocytes that are specifically designed to fight tumors. Oncogenic signaling is a second pathway [38]. The FDA recently approved various anti-PD-1 and PD-L1 mAbs that target a variety of human malignancies. The clinical efficacy of anti-PD-1 and PD-L1 mAbs show promise in targeting PD-1 and PD-L1 immune checkpoints, consequently considerably improving patient conditions [39]. Atezolizumab (Tecentriq®), the first PD-1/PD-L1 immune checkpoint inhibitor for metastatic triple-negative breast cancer, was FDA approved in March 2019 as a treatment

for advanced TNBC. A monoclonal immunoglobulin-G1 (IgG1) antibody called atezolizumab is Fc-engineered, non-glycosylated, and humanized. It binds to PD-L1 and prevents it from interacting with PD-1 and B7.1 (CD80) receptors [39]. Tumor-infiltrating immune cells and/or tumor-associated tumor cells may both express PD-L1. Cytotoxic T-cell activity, T-cell proliferation, and cytokine production are suppressed when PD-L1 binds with PD-1 and B7.1 receptors on T-cells and antigen-presenting cells, preventing the anti-tumor immune response in the tumor microenvironment [39].

Other FDA Approved

TNBC patients who relapse soon after (neo) adjuvant treatment like chemotherapy have more severe conditions. Patients with TNBC who relapse within a year of following (neo) adjuvant chemotherapy have either primary resistance or early acquired resistance to cytotoxic chemotherapy. Shortened disease-free intervals in such cases are linked with a poor prognosis for successive lines of therapy [40]. Therefore, patients with TNBC chemotherapy resistance require improved therapies. Trop-2 is a protein that is over-expressed in more than 80% of TNBC. Sacituzumab govitecan (SG) is an antibody-drug conjugate (ADC) made of a humanized trophoblast cell-surface antigen-2 (Trop-2) antibody linked to an SN-38 payload, the active form of the metabolite topoisomerase 1 inhibitor Irinotecan (a chemotherapeutic medication), through a unique, hydrolyzable linker [40,41]. Breast cancer cells are immediately treated with chemotherapy when the antibody attaches to them. The high drug-to-antibody ratio of 7.6:1, the fact that internalization and enzymatic cleavage of SG by tumor cells are not necessary for SN-38 release from the antibody, and its bystander impact in tumor microenvironment make SG a unique Trop-2-directed ADC [42].

Breast Cancer Treatments Currently Evaluated in Clinical Trials

Intense research allowed a better understanding of the pathophysiology of breast cancer and led to the identification of

more effective, safe, and individualized novel drugs. Currently, many promising clinical trials targeting all the subtypes of breast cancer are in progress. This significant breakthrough changed the outlook of breast cancer therapy as it increased treatment options, reduced the risk of recurrence and progression, improved overall survival, and enhanced patient prognosis, especially for late-stage advanced breast cancer. Novel breast cancer therapies are numerous with diverse characteristics and different modes of action. Such therapies include PARP inhibitors, gene therapy, and immunotherapy [43].

PARP Inhibitors
Veliparib

Veliparib is a selective, oral inhibitor of PARP1 and PARP2. A phase 3 study showed that Veliparib enhanced the effect of platinum-based chemotherapy (Carboplatin/Paclitaxel) and it is considered a new treatment option for patients with HER2-negative, gBRCA-mutated metastatic or locally advanced breast cancer. It demonstrated promising anti-tumor activity with a tolerable safety profile as a single agent and in combination with carboplatin and paclitaxel in patients with BRCA mutation-associated breast cancer [44,45].

Recently, a phase II trial compared the outcomes in patients with different genomic characteristics treated with Cisplatin alone and in combination with Veliparib. 323 patients were classified into three groups: patients with a germline BRCA mutation, patients with a BRCA-like mutation in HR genes, and non-BRCA-like mutation. In addition, there was an unclassified group due to missing biomarker information. The studied endpoints were progression-free survival (PFS), objective response rate (ORR), overall survival (OS), and toxicity [44,45].

In the group of patients having a germline BRCA mutation, the results of PFS were not statistically significant. However, in the BRCA-like group, PFS with Veliparib treatment was enhanced compared to placebo (5.7 versus 4.3 months respectively). Besides in the same group OS (13.7 versus 12.1 months) and ORR (45% versus 35%). The patients in the non-BRCA-like

group and the unclassified group didn't benefit from Veliparib as the variation of PFS was not significant. Regarding toxicity, Grade 3/4 neutropenia (46% versus 19%) and anemia (23% versus 7%) occurred at a higher rate in the Veliparib arm compared to placebo. Consequently, the combination of Veliparib with Cisplatin was successful as it significantly improved PFS and OS for BRCA-like advanced TNBC. Biomarkers used in this study allowed the identification of a subgroup of BRCAwt TNBC that benefited from the addition of PARP inhibitors to cisplatin. This combination is promising and should be studied further in BRCA-like TNBC [44,45].

Rucaparib

Rucaparib is a PARP inhibitor that targets PARP1 and PARP2. It can also target PARP3 which is involved in chromosomal DNA double-strand break repair. Rucaparib is currently under a phase-II trial done on 78 patients with BRCA1/2-mutated advanced breast or ovarian cancers. This trial includes two cohorts with different treatment administration routes. The first cohort receives oral treatment while the second cohort receives intravenous treatment. In both cohorts, the first step of the study included a dose-escalation phase by which the best dose with the least side effects was determined. No objective response was observed in breast cancer patients of the two cohorts. However, 20% of patients in the oral cohort and 44% of patients in the intravenous cohort exhibited disease stabilization over 12 weeks. This means that the treatment can impede the spread of cancer. So, as a monotherapy, Rucaparib was well tolerated as the most adverse events were fatigue and nausea [46,47].

Another phase-II trial was done on patients with TNBC with residual disease after neoadjuvant therapy. These patients have a high risk of cancer recurrence. A total of 128 patients were recruited in the study, with 22% of them carrying a BRCA1/2 germline mutation. Rucaparib was administered in combination with cisplatin. This combination didn't impact the toxicity of cisplatin, and it didn't improve disease-free survival. Probably this is due to the dose of Rucaparib used in this study, as it was substantially less than the current phase II monotherapy dose,

and it may not have been sufficient to inhibit PARP activity. So, dose escalation may be required to check whether this combination is successful [48].

Immunotherapy

Various populations of immune cells are present in the breast stroma at different stages of development and maturation like post-natal development, puberty, and pregnancy. So, breast cancer is considered a moderately immunogenic cancer, with HER2-positive and TNBC subtypes, being the most immunogenic. Immune cells have a crucial role in the early detection and eradication of BC [49].

However, some breast cancer cells are less immunogenic as they have the capability to evade the immune system through different mechanisms. Tumor cells can reduce the expression of the major histocompatibility complex (MHC) that can decrease immune recognition and immune cell activation. This contributes to the development of low immunogenic tumor cells that can escape immune system surveillance. So, due to the importance of the immune system in breast cancer, immunotherapy emerged as a promising treatment option with fewer adverse reactions, strong specificity, and favorable clinical application. Immunotherapy boosts the immune system's ability to recognize, target, and eliminate cancer cells. The most important immunotherapies in breast cancer are immune checkpoint inhibitors, cytokine therapy, and cell-based immunotherapy (CAR-T cell therapy) [49].

Immune checkpoints negatively control immunity by induction of anergy or apoptosis of immune cells. The most important immune checkpoints are programmed cell death-1 (PD-1), programmed cell death ligand-1 (PD-L1), and cytotoxic T-lymphocyte antigen-4 (CTLA-4). Some tumors exploit the function of immune checkpoints to escape from immune surveillance. PD-1, a protein expressed on the surface of T lymphocytes, interacts with its ligand (PD-L1) expressed on tumor cells to inhibit T cells' proliferation and reduce their survival and cytotoxic abilities. CTLA-4, expressed on

regulatory T cells, weakens the immune response against tumor cells by inhibiting the interaction between T cells and antigen-presenting cells (APCs). It also inhibits the function of CD28, a protein that acts as a co-stimulator essential for T cell activation and survival [50,51]. The fact that immune checkpoints' function is triggered by ligand-receptor interactions makes it easy to develop inhibitors that can reverse the immunosuppressive state caused by such checkpoints. These inhibitors are mostly monoclonal antibodies that are currently in different stages of clinical trials; for example, anti-PD-1 mAbs (Nivolumab), anti-PDL-1 mAbs (Durvalumab, Avelumab), and anti-CTLA4 (Ipilimumab). Two mAbs are FDA-approved: Pembrolizumab against PD-1 for the treatment of patients with unresectable or metastatic solid tumors, and Atezolizumab in combination with nab-paclitaxel (a chemotherapy drug) against PD-L1 for the treatment of locally advanced/ metastatic TNBC [50,51].

Conclusion

To put it briefly, this comprehensive review has provided a detailed exploration of the current and evolving treatment strategies for breast cancer. It has elucidated the intricate molecular mechanisms underlying hormone receptor-positive (HR+), HER2-enriched, and triple-negative breast cancers (TNBC), shedding light on the challenges and therapeutic strategies associated with each subtype. The review has underscored the need for a personalized and multifaceted approach to breast cancer treatment, emphasizing the importance of understanding the dynamic interplay between various receptors, growth factors, and microRNAs, and the development of combination therapies to improve patient outcomes. The exploration of resistance strategies against single HER2 inhibitors and the advent of targeted therapies, notably PARP inhibitors and immunotherapy have broadened treatment options and introduced personalized dimensions to breast cancer management. The ongoing clinical trials and FDA-approved interventions present a tapestry of opportunities for enhanced patient outcomes. As the scientific community navigates this intricate landscape, collaborative efforts, rigorous research, and

an unwavering commitment to understanding breast cancer at its molecular core will be paramount for advancing the field and ultimately, enhancing the well-being of individuals impacted by this formidable ailment.

References

1. Yang K, Kim H, Choi DH, Park W, Noh JM, et al. Optimal radiotherapy for patients with internal mammary lymph node metastasis from breast cancer. Radiation Oncology. 2020; 15: 16.
2. Drăgănescu M, Carmocan C. Hormone Therapy in Breast Cancer. Chirurgia. 2017; 112: 413.
3. Kersh AE, Ng S, Chang YM, Sasaki M, Thomas SN, et al. Targeted Therapies: Immunologic Effects and Potential Applications Outside of Cancer. Journal of Clinical Pharmacology. 2018; 58: 7–24.
4. Pellegrino B, Hlavata Z, Migali C, De Silva P, Aiello M, et al. Luminal Breast Cancer: Risk of Recurrence and Tumor-Associated Immune Suppression. Mol Diagn Ther. 2021; 25: 409–424.
5. Ziyeh S, Wong L, Basho RK. Advances in Endocrine Therapy for Hormone Receptor-Positive Advanced Breast Cancer. Curr Oncol Rep. 2023; 25: 689–698.
6. Li J, Wang Z, Shao Z. Fulvestrant in the treatment of hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer: A review. Cancer Medicine. 2019; 8: 1943–1957.
7. Buzdar AU, Hortobagyi GN. Tamoxifen and toremifene in breast cancer: comparison of safety and efficacy. JCO. 1998; 16: 348–353.
8. Saatci O, Huynh-Dam KT, Sahin O. Endocrine resistance in breast cancer: from molecular mechanisms to therapeutic strategies. J Mol Med. 2021; 99: 1691–1710.
9. Ozyurt R, Ozpolat B. Molecular Mechanisms of Anti-Estrogen Therapy Resistance and Novel Targeted Therapies. Cancers. 2022; 14: 5206.
10. Rahem SM, Epsi NJ, Coffman FD, Mitrofanova A. Genome-wide analysis of therapeutic response uncovers molecular pathways governing tamoxifen resistance in ER+ breast

cancer. EBioMedicine. 2020; 61: 103047.

11. Muluhngwi P, Klinge CM. Roles for miRNAs in endocrine resistance in breast cancer. Endocrine-Related Cancer. 2015; 22: R279–300.

12. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Molecular Cancer. 2023; 22: 138.

13. Fan W, J C, fu P. Endocrine therapy resistance in breast cancer: current status, possible mechanisms and overcoming strategies. Future medicinal chemistry. 2015; 7: 1511–1519.

14. Kang I, Dong S, Lu J, Xia B. Recent Developments in HER2-Directed Therapy in Breast Cancer. Curr Breast Cancer Rep. 2019; 11: 311–325.

15. Thaper A, Tran J, Ali A. Current Updates in Management of HER2-Positive and HER2-Low Breast Cancer. Curr Breast Cancer Rep. 2023; 15: 135–141.

16. Xing F, Gao H, Chen G, Sun L, Sun J, et al. CMTM6 overexpression confers trastuzumab resistance in HER2-positive breast cancer. Molecular Cancer. 2023; 22: 6.

17. Omarini C, Piacentini F, Sperduti I, Cerma K, Barbolini M, et al. T-DM1 efficacy in trastuzumab-pertuzumab pre-treated HER2 positive metastatic breast cancer patients: a meta-analysis. BMC Cancer. 2022; 22: 623.

18. Gajria D, Chandarlapaty S. HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther. 2011; 11: 263–275.

19. Barok M, Joensuu H, Isola J. Trastuzumab emtansine: mechanisms of action and drug resistance. Breast Cancer Research. 2014; 16: 209.

20. Cunningham N, Shepherd S, Mohammed K, Lee KA, Allen M, et al. Neratinib in advanced HER2-positive breast cancer: experience from the Royal Marsden hospital. Breast Cancer Res Treat. 2022; 195: 333–340.

21. Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomized, double-blind, placebo-controlled, phase 3 trial. The Lancet Oncology. 2017; 18: 1688–1700.

22. Earl HM, Hiller L, Vallier AL, Loi S, McAdam K, et al. 6

versus 12 months of adjuvant trastuzumab for HER2-positive early breast cancer (PERSEPHONE): 4-year disease-free survival results of a randomized phase 3 non-inferiority trial. Lancet. 2019; 393: 2599–2612.

23. Wang ZH, Zheng ZQ, Jia SC, Liu SN, Xiao XF, et al. Trastuzumab resistance in HER2-positive breast cancer: Mechanisms, emerging biomarkers and targeting agents. Front Oncol. 2022; 12: 1006429.

24. Hunter FW, Barker HR, Lipert B, Rothé F, Gebhart G, et al. Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. Br J Cancer. 2020; 122: 603–612.

25. Takeda T, Yamamoto H, Suzawa K, Tomida S, Miyauchi S, et al. YES1 activation induces acquired resistance to neratinib in HER2-amplified breast and lung cancers. Cancer Sci. 2020; 111: 849–856.

26. Leon-Ferre RA, Goetz MP. Advances in systemic therapies for triple negative breast cancer. BMJ. 2023; 381: e071674.

27. Lafanechère L. The microtubule cytoskeleton: An old validated target for novel therapeutic drugs. Frontiers in Pharmacology [Internet]. 2022; 13. Available online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.969183

28. Zhang Z, Wang G, Li Y, Lei D, Xiang J, et al. Recent progress in DNA methyltransferase inhibitors as anticancer agents. Frontiers in Pharmacology [Internet]. 2022; 13. Available online at:
https://www.frontiersin.org/articles/10.3389/fphar.2022.1072651

29. Das M, Li J, Bao M, Huang L. Nano-delivery of Gemcitabine Derivative as a Therapeutic Strategy in a Desmoplastic KRAS Mutant Pancreatic Cancer. AAPS J. 2020; 22: 88.

30. Barchiesi G, Roberto M, Verrico M, Vici P, Tomao S, et al. Emerging Role of PARP Inhibitors in Metastatic Triple Negative Breast Cancer. Current Scenario and Future Perspectives. Frontiers in Oncology [Internet]. 2021; 11. Available online at:
https://www.frontiersin.org/articles/10.3389/fonc.2021.769280

31. Cortesi L, Rugo HS, Jackisch C. An Overview of PARP Inhibitors for the Treatment of Breast Cancer. Targ Oncol. 2021; 16: 255–282.

32. Kaur SD, Chellappan DK, Aljabali AA, Tambuwala M, Dua K, et al. Recent advances in cancer therapy using PARP inhibitors. Med Oncol. 2022; 39: 241.

33. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E. PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. Frontiers in Cell and Developmental Biology [Internet]. 2020; 8. Available online at: https://www.frontiersin.org/articles/10.3389/fcell.2020.5646 01

34. Boussios S, Abson C, Moschetta M, Rassy E, Karathanasi A, et al. Poly (ADP-Ribose) Polymerase Inhibitors: Talazoparib in Ovarian Cancer and Beyond. Drugs R D. 2020; 20: 55–73.

35. Bruin MAC, Sonke GS, Beijnen JH, Huitema ADR. Pharmacokinetics and Pharmacodynamics of PARP Inhibitors in Oncology. Clin Pharmacokinet. 2022; 61: 1649–1675.

36. Oner G, Önder S, Karatay H, Ak N, Tükenmez M, et al. Clinical impact of PD-L1 expression in triple-negative breast cancer patients with residual tumor burden after neoadjuvant chemotherapy. World Journal of Surgical Oncology. 2021; 19: 264.

37. Dong Y, Han Y, Huang Y, Jiang S, Huang Z, et al. PD-L1 Is Expressed and Promotes the Expansion of Regulatory T Cells in Acute Myeloid Leukemia. Frontiers in Immunology [Internet]. 2020; 11. Available online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.017 10

38. Thibult ML, Mamessier E, Gertner-Dardenne J, Pastor S, Just-Landi S, et al. PD-1 is a novel regulator of human B-cell activation. International Immunology. 2013; 25: 129–137.

39. Twomey JD, Zhang B. Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics. AAPS J. 2021; 23: 39.

40. Yin L, Duan JJ, Bian XW, Yu S cang. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Research. 2020; 22: 61.

41. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, et al.

Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. N Engl J Med. 2021; 384: 1529–1541.

42. Syed YY. Sacituzumab Govitecan: First Approval. Drugs. 2020; 80: 1019–1025.

43. Advances in Breast Cancer Research - NCI [Internet]. 2019. Available online at: https://www.cancer.gov/types/breast/research

44. Diéras V, Han HS, Kaufman B, Wildiers H, Friedlander M, et al. Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): a randomized, double-blind, placebo-controlled, phase 3 trial. The Lancet Oncology. 2020; 21: 1269–1282.

45. Rodler E, Sharma P, Barlow WE, Gralow JR, Puhalla SL, et al. Cisplatin with veliparib or placebo in metastatic triple-negative breast cancer and BRCA mutation-associated breast cancer (S1416): a randomized, double-blind, placebo-controlled, phase 2 trial. The Lancet Oncology. 2023; 24: 162–174.

46. Curtin N. Phase 2 multicentre trial investigating intermittent and continuous dosing schedules of the poly(ADP-ribose) polymerase inhibitor rucaparib in germline BRCA mutation carriers with advanced ovarian and breast cancer. British Journal of Cancer [Internet]. 2016. Available online at: https://www.academia.edu/94587776/Phase_2_multicentre_trial_investigating_intermittent_and_continuous_dosing_schedules_of_the_poly_ADP_ribose_polymerase_inhibitor_rucaparib_in_germline_BRCA_mutation_carriers_with_advanced_ovarian_and_breast_cancer

47. Michela P, Tedaldi G, Sirico M, Virga A, Ulivi P, et al. Moving Beyond PARP Inhibition: Current State and Future Perspectives in Breast Cancer. International Journal of Molecular Sciences. 2021; 22: 7884.

48. Kalra M, Tong Y, Jones DR, Walsh T, Danso MA, et al. Cisplatin +/- rucaparib after preoperative chemotherapy in patients with triple-negative or BRCA mutated breast cancer. NPJ Breast Cancer. 2021; 7: 29.

49. Li Y, Miao W, He D, Wang S, Lou J, et al. Recent Progress on Immunotherapy for Breast Cancer: Tumor Microenvironment, Nanotechnology and More. Frontiers in Bioengineering and Biotechnology [Internet]. 2021; 9.

Available online at:
https://www.frontiersin.org/articles/10.3389/fbioe.2021.6803
15

50. Yi H, Li Y, Tan Y, Fu S, Tang F, et al. Immune Checkpoint Inhibition for Triple-Negative Breast Cancer: Current Landscape and Future Perspectives. Frontiers in Oncology [Internet]. 2021; 11. Available online at:
https://www.frontiersin.org/articles/10.3389/fonc.2021.6481
39

51. Zhang W, Kong X, Ai B, Wang Z, Wang X, et al. Research Progresses in Immunological Checkpoint Inhibitors for Breast Cancer Immunotherapy. Frontiers in Oncology [Internet]. 2021; 11. Available online at:
https://www.frontiersin.org/articles/10.3389/fonc.2021.5826
64