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Overcoming trastuzumab resistance in HER2-positive breast cancer using combination therapy

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Abstract

Human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC) comprises around 20–30% of all BC subtypes and is correlated with poor prognosis. For many years, trastuzumab, a monoclonal antibody, has been used to inhibit the HER2 activity. Though, the main resistance to trastuzumab has challenged the use of this drug in the management of HER2-positive BC. Therefore, the determination of resistance mechanisms and the incorporation of new agents may lead to the development of a better blockade of the HER family receptor signaling. During the last few years, some therapeutic drugs have been developed for treating patients with trastuzumab-resistant HER2-positive BC that have more effective influences in the management of this condition. In this regard, the present study aimed at reviewing the mechanisms of trastuzumab resistance and the innovative therapies that have been investigated in trastuzumab-resistant HER2-positive BC subjects.

KEYWORDS

breast cancer, drug resistance, HER2 positive, trastuzumab

1 | INTRODUCTION

Breast cancer (BC), the most popular type of cancer in females, still remains the second important cause of cancer-associated deaths, in spite of significant improvements in its treatment (Derakhshani et al., 2019; Siegel, Ma, Zou, & Jemal, 2014). BC can be categorized as a heterogeneous disease according to the status of molecular profiles and microscopic appearances such as estrogen receptor (ER) expression and HER2 amplification (Slamon et al., 1987).

Human epidermal growth factor receptor 2 (HER2), a trans membrane tyrosine kinase receptor, a member of the EGF receptor (EGFR) family, is encoded by the *HER2* gene. This family includes EGFR/HER1, HER2, HER3, and HER4 that promote growth factor-related signaling, for example, PI3K-AKT and RAS-MAPK pathways (Yarden & Sliwkowski, 2001). ErbB receptors embody an extracellular region that includes four domains (i.e., I/L1, II/CR1, III/L2, and IV/CR2) ordered as repeating identical segments of a two united part, a cytosolic tyrosine kinase, and a single membrane-spanning region. After a ligand binds to the extracellular region makes cytosolic kinase dimerization and activation, which leads to auto-phosphorylation and starting of down-stream signaling events (Cho et al., 2003; Vahidian et al., 2019).

Typically, on the epithelial cell surfaces, HER2 has a low expression that is necessary for the normal progress in various tissues like ovary, breast, liver, lung, central nervous system, and kidney. In contrast, immunohistochemical analyses have revealed that HER2 is over-expressed in BC cells, which can extend to two million receptors in

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each cell. In metastatic breast cancer (MBC), the increased levels of HER2 are related to reduced disease-free survival (Press et al., 1997). At the cell membrane, the localization of HER2 is a heterogeneous and dynamic process regulated by differential rates of endocytosis and recycling (Bertelsen & Stang, 2014; Z Rezaei & Derakhshani, 2018).

Trastuzumab (or Herceptin), was developed as a recombinant humanized monoclonal antibody (mAb) targeting the domain four of the HER2. It was produced by Genentech Inc. (San Francisco, CA). Trastuzumab is among the highly successful targeted treatments for BC that selectively exerts its antitumor effects on HER2-amplified BC tissues with no impact on tumors with normal expression of HER2. When combined with other chemotherapy drugs, trastuzumab increases the prognosis of subjects with metastatic diseases and decreases the risk of cancer recurrence and death (Drebin, Link, Stern, Weinberg, & Greene, 1985; S. Paik, Kim, & Wolmark, 2008; Seidman et al., 2008; Vogel et al., 2002). Nowadays, choosing the patients who can undergo trastuzumab therapy is based on HER2-positivity using protein- and DNA-based assays (Drebin et al., 1985; S. Paik et al., 2008; Seidman et al., 2008; Vogel et al., 2002).

Despite the clinical efficacy of trastuzumab, acquired resistance to this antibody challenges the use of this drug. Hence, overcoming trastuzumab resistance can pave the way for effective therapeutic methods suggested for the therapy of HER2-positive BC resistant to trastuzumab.

2 | MECHANISMS OF TRASTUZUMAB ACTION

There is more than one antitumor mechanism for trastuzumab. The antibody's direct action is based on receptor downregulation and intracellular signalings, such as downstream protumorigenic cell signaling and HER2 shedding inhibition. Indirect action that has been recommended as a mechanism of action for trastuzumab is the activation of an immune response via antibody-dependent cellular cytotoxicity (ADCC; Clynes, Towers, Presta, & Ravetch, 2000). Trastuzumab has a wide range of antimalignancy effects, especially on HER2-upregulated tumor cells. In this regard, the present review aimed at studying the molecular mechanisms of trastuzumab actions against cancer cells.

2.1 | Inhibition of HER2 ectodomain shedding

HER2 undergoes proteolytic cleavage in its extracellular domain (EDC) in HER2-overexpressing tumor cells which results in a truncated type of HER2 that is a type of receptor without the extracellular binding part for trastuzumab. This shortened membrane-bound fragment of HER2 retains downstream kinase activity and causes trastuzumab resistance (Hudziak et al., 1989). Christianson et al. (1998) in their study showed that the truncated receptor is also present in 24 human BC specimens in vivo. Also, Fornier et al. (2005) mentioned that it might establish a convenient approach for monitoring the patients that taking trastuzumab-based medication. Another study by Isola, Holli, Oksa, Teramoto, and Kallioniemi (1994) displayed that the serum ECD of HER2 released into the circulation and can be raised in the serum of women with MBC. In fact, the phosphorylated truncated receptor has been distinguished in approximately half of the human BC serum samples. A reduced HER2-ECD in serum is considered as a predictor of host response due to its association with improved progression-free survival (Molina et al., 2001).

2.2 | Internalization and degradation of HER2 protein

In BC cells that highly expressed HER2, trastuzumab leads to downregulation and degradation of HER2. It is proposed that trastuzumab begins internalization and degradation of HER2 by encouraging the function of tyrosine kinase-ubiquitin ligase c-Cbl. It is also suggested that the connection of trastuzumab to HER2 results in c-Cbl recruitment to the Tyr1112 site where c-Cbl ubiquitinates HER2 and induces its cleavage (Klapper, Waterman, Sela, & Yarden, 2000). Baldassarre et al. clarified the roles of Endo II (endocytic adapter protein endophilin A2) in HER2-positive cancer cells. They displayed that silencing Endo II can decrease the responses to trastuzumab by causing defects in HER2 internalization (Figure 1).

2.3 | Inhibition of angiogenesis

Trastuzumab also reduces angiogenesis, which has shown to result in the inhibition of endothelial cell migration in vitro and a decrease in microvessel density in vivo. The expressions of proangiogenic factors were inhibited in trastuzumab-treated tumors, while these factors were enhanced in their respective controls (Klapper et al., 2000).

2.4 | Inhibition of DNA repair

The particular molecular mechanisms used by cells for the inhibition of DNA repair, which are activated by trastuzumab, remain unclear. Pietras et al. (1999) suggested that DNA adduct repair induced by cisplatin is partially inhibited by trastuzumab. Also, in vitro studies show that trastuzumab can block DNA synthesis after radiation (Baselga, Albanell, Molina, & Arribas, 2001). Julien et al. proposed that the inhibition of HER2 by trastuzumab markedly affects DNA interstrand crosslink repair (Table 1). Le et al. (2005) demonstrated that trastuzumab can induce DNA damage and downregulated PCNA. In the presence of a PARP inhibitor, low expression of PCNA may impair homologous recombination (HR) causing synthetic lethality (Le et al., 2005). García-Parra et al. (2014) displayed that poly (ADP-ribose) polymerase (PARP) blockers increased the antitumor effects of trastuzumab in vitro and in vivo in HER2 BC cancer cells.

2.5 | Antibody-dependent cellular cytotoxicity mediated by Fc

ADCC as an important extracellular mechanism of trastuzumab that is able to recruit major effector cells (e.g., NK cells that express

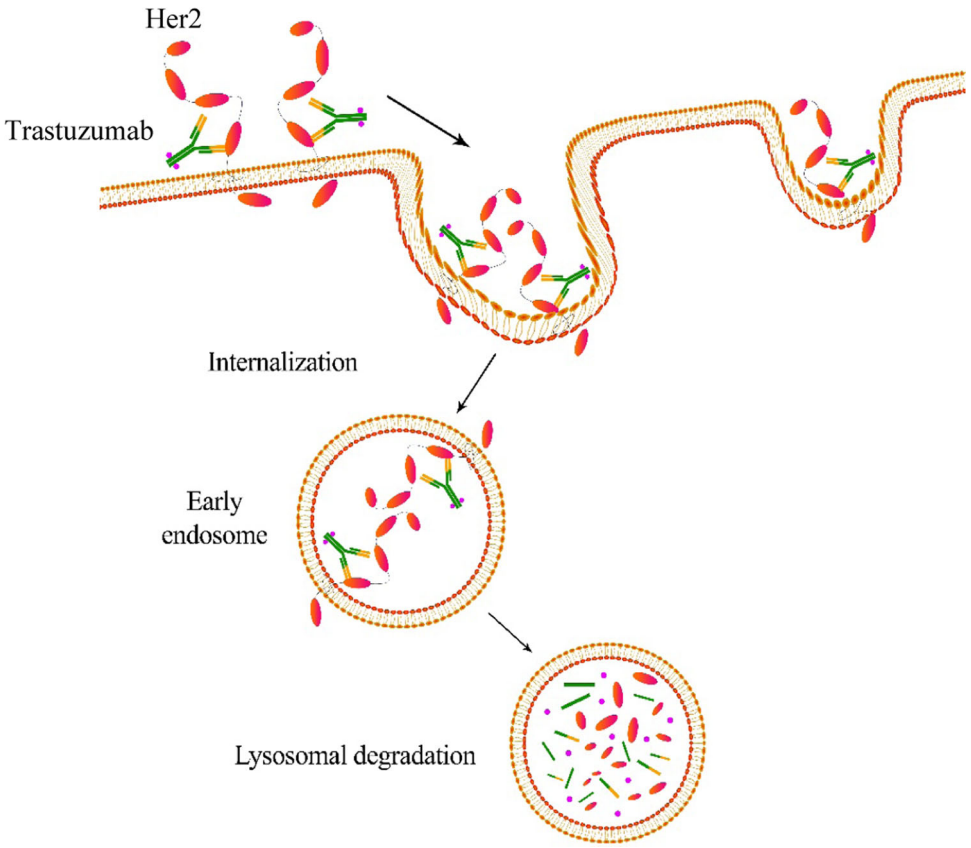

FIGURE 1 Internalization and degradation of HER2 proteins by trastuzumab. Trastuzumab is effective in downregulation and degradation of HER2 in HER2-overexpressing breast cancer cells. Trastuzumab begins internalization and degradation of HER2 by encouraging the activity of tyrosine kinase-ubiquitin ligase c-Cbl. It is also suggested that binding of trastuzumab to HER2 leads to recruitment of c-Cbl to the Tyr1112 site where c-Cbl ubiquitinates HER2 and induces its degradation. All the mentioned factors are presented in Figure 1 (see the text for additional details)

FcγRIIIA [CD16]) to overexpress HER2 tumor sites. Clynes et al. (2000) suggested that ADCC was enhanced in mice lacking FcγRIIB (inhibitory receptor), while it was decreased in mice deficient in the activating Fc receptors (FcγRIIIA). Thus, Fc-receptor-dependent mechanisms contribute mainly to the function of cytotoxic antibodies in tumors (Sliwkowski et al., 1999). Moreover, the ADCC activity was mainly enhanced in BC patients' serum in the presence of trastuzumab. Arnould et al. (2006) reported that enhancement in several natural killer cells (NK cells) and cytotoxic proteins such as granzyme B, were observed in tumor samples from women with

TABLE 1 Different mechanisms of trastuzumab in breast cancer therapy

Mechanism	Different functions	References
Blocking receptors	Receptor downregulation and reduce intracellular signaling	(Claret & Vu, 2012; Moasser, 2007)
Inhibition of HER2 ectodomain	Prevention of HER2 cleavage	(Christianson et al., 1998; Codony-Servat, Albanell, Lopez-Talavera, Arribas, & Baselga, 1999)
Inhibition of angiogenesis	Inhibition of endothelial cell migration	(Fornier et al., 2005; Lane, Motoyama, Beuvink, & Hynes, 2001)
Degradation of HER2 receptors	Trigger HER2, internalization, and degradation by ubiquitination	(Claret & Vu, 2012; Klapper et al., 2000)
The antibody-dependent cellular cytotoxicity	Able to recruit major effector cells	(Nagata et al., 2004; Sliwkowski et al., 1999)
Inhibition of DNA repair	Block the DNA synthesis after radiation and effect on DNA interstrand cross-link repair	(Baselga et al., 2001)
Effect on cell cycle	Inhibition of G1 phase cell cycle	(Filipits et al., 2018)
Effect on the PI3K pathway	Directly inhibits EGFR and ERBB2 activation	(Junttila et al., 2009; Zhang et al., 2011)

progressive BC after treatment with trastuzumab and docetaxel (Nagata et al., 2004). It is shown that trastuzumab-induced ADCC can be augmented by increasing NK cell activities, modulating the antibody itself, and inhibiting different proteins such as caspases, CD112R, and TIGIT, histone deacetylase (HDAC), and Adams (Nami, Maadi, & Wang, 2018). These findings proposed that ADCC acts as a mechanism of trastuzumab action, and clarifies an increase in response to trastuzumab in HER2 overexpressing cancer cells.

2.6 | The effect of trastuzumab on cell cycle

Trastuzumab arrests cell cycle during G1 phase by the inhibition of proliferation. The cell cycle is arrested by inhibition of proteins' expression that is related to the cleavage of the cyclin-dependent kinase (CDK) blocker p27kip1. On the other hand, assessing p27 expression in women with initial stage HER2-positive BC after treatment with trastuzumab. Filipits et al. (2018) explained that using a predefined cutoff, a low p27 expression can be associated to positive effects of trastuzumab (Table 1).

2.7 | The effect of trastuzumab on the PI3K pathway

The PI3K signaling pathway is one of the pivotal pathways with significant oncogenic features in different malignancies. BC tumorigenesis is assumed to be related to this pathway. Some studies have proposed that the complex of HER2/HER3/PI3K and PI3K-AKT signaling pathway have critical roles in cell proliferation in HER2-amplified cells (Klos et al., 2003). Trastuzumab can block EGFR signaling through binding to the extracellular subdomain IV of HER2 but, lapatinib reversibly interacts with the cytoplasmic ATP-binding domain of the kinase and inhibits phosphorylation of HER2. Reduced downstream signaling induces p27kip1, and then induces cell cycle arrest and apoptosis (Baselga et al., 2001; Sliwkowski et al., 1999). Trastuzumab also can promote dissociation of Src (a nonreceptor tyrosine kinase) from HER2, which reduces its activity. Consequently, Akt and mammalian target of rapamycin (mTOR) would be inhibited (Nagata et al., 2004; Figure 2).

3 | MECHANISMS OF TRASTUZUMAB RESISTANCE

The critical step for the improvement of new anti-HER2 strategies is recognition of the trastuzumab resistance mechanisms. Just a third of women with HER2-positive BC show a primary response to trastuzumab that indicates "initial" or "inherent" trastuzumab resistance in the patients (Nagata et al., 2004; Z. Rezaei, Sebzari, Kordi-Tamandani, & Dastjerdi, 2019). The trastuzumab resistance is unlikely to be the result of an intrinsic defect of HER2 degradation but it is probably related to impaired HER2 down-regulation due to its uncoupling from Cbl, causing another and impaired pattern of endocytosis. Manufacturing of TGFa presumably involved the uncoupling mechanism and the resulting changed trafficking of the receptor and could thus account for acquired or primary resistance to trastuzumab (Valabrega et al., 2005). Furthermore, About 70% of women with $HER2^+$ MBC who primarily responded to trastuzumab, showed progress within a year suggesting "secondary" or "acquired" resistance to trastuzumab (Valabrega et al., 2005).

Interruption of the interplay between the therapeutic agents and their target proteins is a potential mechanism for the resistance of the targeted antibodies. The molecular mechanisms for trastuzumab resistance are listed below (Klos et al., 2003; Pietras et al., 1999; Wen et al., 2006).

3.1 | Epitope masking

3.1.1 | Breast cancer stem cell markers $CD44^+$ and CD24

Overexpression of breast cancer stem cells (BCSC) markers including CD44 may cause to inhibition of the trastuzumab-binding site at the HER2-ECD (Martin-Castillo et al., 2013). It was shown that trastuzumab decreases the population of the ALDEFLUOR-positive BCSCs in trastuzumab-sensitive and HER2-amplified BC cell lines via blockade of PI3K/Akt signaling, but not in trastuzumab-resistant cell lines (Korkaya, Paulson, Iovino, & Wicha, 2008). Recent studies have shown that the $CD44^+/CD24^-$ phenotype can be utilized as a predictive factor of trastuzumab response and a prognostic factor for the clinical result in patients with $HER2^+$ primary BC (Seo et al., 2016; Figure 3A).

3.1.2 | Mucin-1 (MUC1)

The mucin-1 (MUC-1) an O-linked glycosylated polypeptide that undergoes autocleavage to generate two subunits (Kufe, 2013). MUC1-N is located extracellularly at the cell surface with the MUC1 C-terminal (MUC1-C) transmembrane subunit (10). MUC1-C acts as an oncoprotein through association with receptor tyrosine kinases (RTK), such as EGFR and HER2, by coupling to their signaling cascades (9). It has been shown that MUC1-C play a critical role in the activation of the HER2 pathway which indicates a potential approach to revoke trastuzumab resistance (Raina et al., 2014; Figure 3B). Particularly, cell-penetrating peptides like GO-203 that specifically bind to the MUC1-C are currently very effective therapy in preventing growth and making the death of BC cells (38).

3.1.3 | Mucin-4 (MUC4)

Membrane mucins such as Mucin-4 (MUC4) can decrease matrix–cell interactions and adhesion. MUC4 is generally expressed in the apical surface of mammary cells. In BC cells, MUC4 can also stimulate HER2 signaling through an EGF-like domain on its ASGP-2 subunit (Clynes et al., 2000). It is suggested that MUC4 acts as a ligand for HER2, causing induced phosphorylation on Tyr1248 residue of HER2 as a

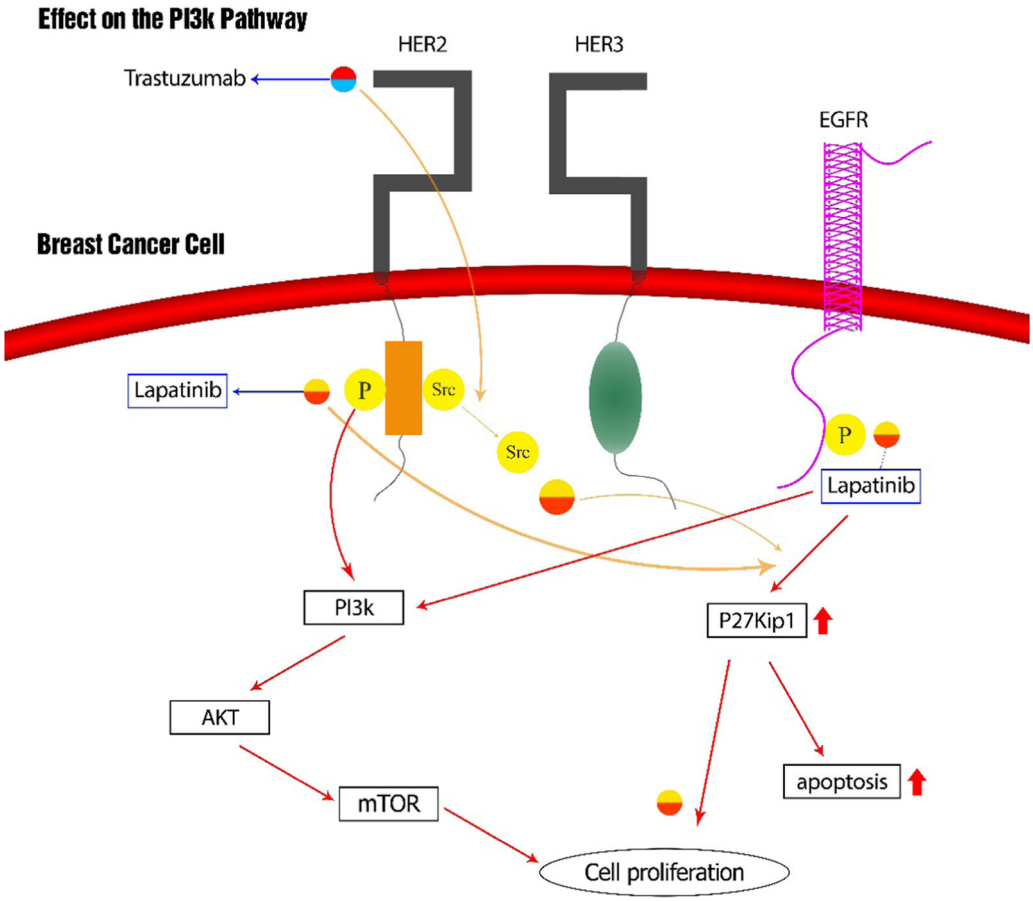

FIGURE 2 The effect of trastuzumab on the PI3K signaling pathway. In numerous diseases, the PI3K pathway is one of the essential signaling pathways with significant oncogenic features. In general, tumorigenesis of breast cancer is assumed to be associated with the PI3K pathway. HER2/HER3/PI3 K and PI3K-AKT signaling pathway complex have critical roles in cell proliferation in HER2-amplified cells. Trastuzumab and lapatinib can block this signaling, either by inhibiting the activity of EGFR and ERBB2 kinases directly or through ERBB2 binding at the cell surface. Trastuzumab can promote dissociation of Src (a nonreceptor tyrosine kinase) from HER2, which reduces its activity. Consequently, Akt and mammalian target of rapamycin (mTOR) would be inhibited

main phosphorylation target. Therefore, it contributes to the transforming function of HER2 oncoprotein. MUC4 does not affect the expression level of HER2 receptor completely (Nagy et al., 2005; Pai et al., 2016). Also, MUC4 has been described to hide the trastuzumab-binding sites of HER2 by decreasing its binding in vitro in JIMT-1 (a de novo trastuzumab-resistant BC cell line; 18). However, regulation of MUC4 expression through endogenous modulators and its effect on trastuzumab resistance in vivo are still unclear (Figure 3C).

3.2 | Activation of the IGF-IR pathway

Trastuzumab resistance is related to the enhanced signaling of the insulin-like growth factor-I receptor (IGF-IR). In BC cells, increased expression of IGF-IR could decrease growth arrest of HER2-overexpression mediated by trastuzumab, which mediated trastuzumab resistance (Saisana, Griffin, & May, 2016; Tóth et al., 2016). Previously, Nahta, Yu, Hung, Hortobagyi, and Esteva (2006) showed that IGF-1R and HER2 form a unique receptor complex and exhibit crosstalk in acquired resistance models. Moreover, it was shown that IGFIR interacts with HER2 physically and phosphorylates it in trastuzumab-resistant cells (de Groot et al., 2016). Also, the resistance can be reversed by inhibition of IGF-1R signaling. Jerome et al. (2006) and Xin et al. (2015) displayed that recombinant IGF-binding protein-3 (IGFBP3) or progressive heat-promoted expression of the dominant-negative IGF-1R 486/STOP by inhibition of IGF-1R signaling can significantly sensitize cells to trastuzumab (Figure 3D). Sanabria-Figueroa et al. (2015) found that the main biologic effect caused by IGF-1R was an invasion, which was mediated by both Forkhead box protein M1 (FoxM1) and Src-focal adhesion kinase (FAK) signaling. They strongly proposed that combinations targeting therapy of IGF-1R and HER2 can suppress the aggressive potential in cancer cells that are resistant to trastuzumab via mechanisms that related to the Src and FoxM1 (Sanabria-Figueroa et al., 2015).

3.3 | β2- adrenergic receptor signaling

β2-adrenergic receptor (β2-AR), is the main mediator of catecholamine-induced impacts on the malignant characteristics of the tumor cells (Armaiz-Pena et al., 2013). Recent research indicated that the

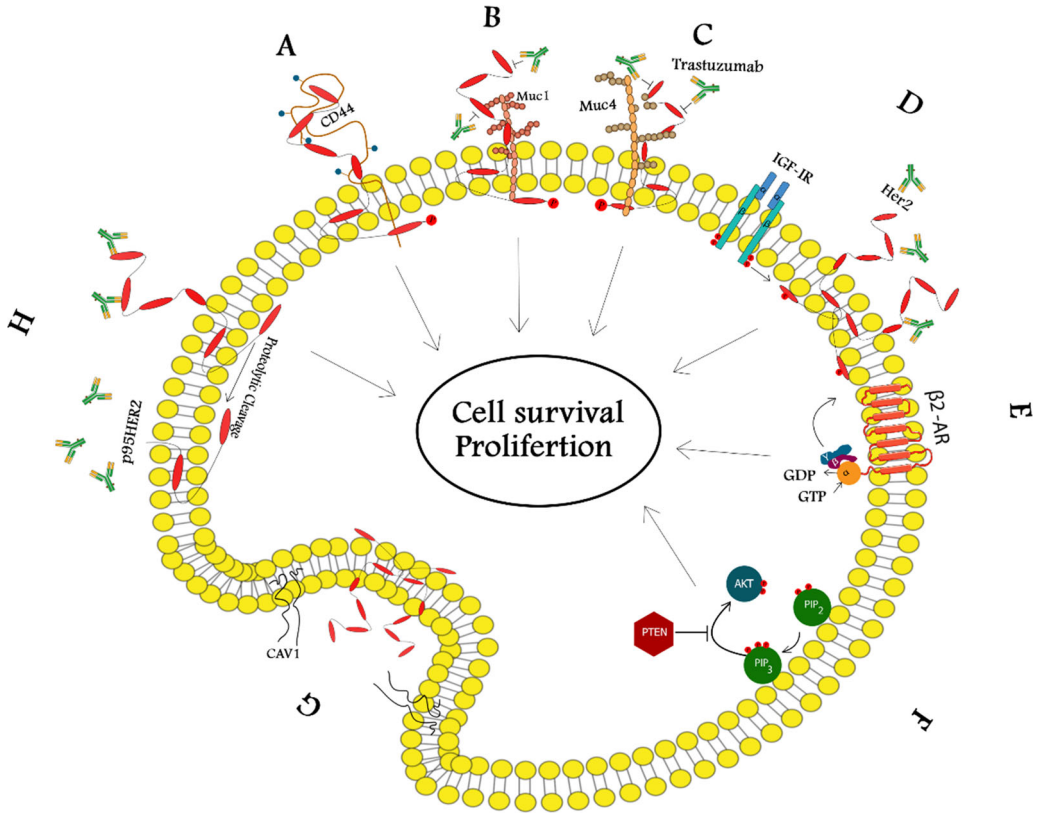

FIGURE 3 The molecular mechanism of trastuzumab resistance. In epitope masking, cell surface molecules like CD44, MUC1, and MUC4 (A, B, and C respectively) hide the epitope to which trastuzumab binds and also inhibit the recognition and binding of HER2 by trastuzumab; (D) and (E): Receptor crosstalk, where cell surface receptor kinase such as IGF-IR and β2-AR stimulate the activation of HER2 signaling; (F), PTEN blocks the PI3K signaling cascade. Loss of PTEN can activate cell survival processes; (G), caveolae can mediate endocytosis and remove the HER2 from cell surface which inhibits trastuzumab binding; (H), lack substantial parts of the extracellular domain of HER2 which termed p95HER2, can naturally evade antibody binding due to the absence of the extracellular domain (See text for details)

expression of β2-AR reversely correlates with trastuzumab response in patients with HER^+ BC. The data from this study indicated that β2-AR can be implemented for the forecast of the therapeutic outcome of trastuzumab-based therapy in BC (Liu et al., 2016; Figure 3E).

3.4 | Defects in the PTEN-PI3K/AKT pathway

There are many possible mechanisms leading to trastuzumab resistance, among which the most prevalent one in BC is the activation of the PI3K/Akt pathway. The main abnormality limiting the activation of the PI3K/Akt pathway is the loss of PTEN function or gain-of-function mutations in PI3KCA. Berns et al. (2007) proposed an advanced method for identifying patients with poor efficacy response to trastuzumab when combining low PTEN expression and mutations present in oncogenic PIK3CA (Figure 3F).

They also mentioned a relationship between PIK3CA hotspot mutations and reduced progression time after treatment. Another study showed a comprehensive functional RNA interference indicating PTEN as the central mediator of trastuzumab sensitivity in HER2-overexpressing BC cell lines. In addition, PTEN knockdown and transfection of PI3KCA mutant cell line can return their insensitivity to trastuzumab. In this regard, PTEN loss and PIK3CA mutations correlated with the inferior time of progression and survival in a study conducted on 256 women with trastuzumab-treated $HER2^+$ MBC (Berns et al., 2007; Razis et al., 2011).

3.5 | Caveolae

The availability and stability of cell surface HER2 is the main predictor of Trastuzumab influence. Intrinsic defects in the endocytic machinery are responsible for HER2 downregulation which is associated with unresponsiveness to trastuzumab therapy (Z. Rezaei, Kordi-Tamandani, & Dastjerdi, 2018; Valabrega et al., 2005). Caveolae-mediated endocytosis is suggested to be involved in trastuzumab and T–DM1 resistance (Chung et al., 2018; Figure 3G). In a recent study, accumulation of HER2 in response to pharmacological reduction of CAV1 was demonstrated using preclinical models of xenograft and organotypic cultures from freshly harvested human tumors. The finding that CAV1 controls the cellular distribution of HER2 can have a direct therapeutic indication for antibody-mediated targeting of HER2 and may shed light on the usage of these antibodies for therapeutic purposes and

molecular imaging of HER2$^+$ tumors. It was revealed that the availability of HER2 protein at the cell surface can be tuned by temporal reduction of cholesterol through lovastatin in a way that could serve to boost trastuzumab binding and therapy against HER2$^+$ tumors. This study demonstrated the potential advantage of using statins to develop the therapeutic outcomes of trastuzumab and HER2-targeted molecular imaging in the clinics (Pereira et al., 2018).

3.6 | Expression of p95HER2

Truncated p95HER2 fragments generated with constitutive kinase activity, revealed resistance to trastuzumab due to lack of trastuzumab-binding epitopes. Two kinds of mechanisms have been suggested to form the p95HER2 receptor (a) by proteolytic degradation of the HER2-ECD and (b) alternative TIS (translation-initiation sites) of the HER2 protein. HER2-ECD can be generated into cell culture or circulating media in serum in vivo. Enhanced levels of HER2-ECD in serum are related to poor prognosis in progressive BC patients (Colomer et al., 2000; Hayes et al., 2001; Leitzel et al., 1995; Y. Lin & Clinton, 1991; Pupa et al., 1993; Ruiz et al., 2018; J. Sperinde et al., 2016, 2018; Todeschini et al., 2011; Yamauchi et al., 1997; Zabrecky, Lam, McKenzie, & Carney, 1991; Figure 3h). In the mammary glands of transgenic mice, expression of p95HER2 receptors displayed more aggressive breast tumors compared with that determined by the full-length receptor (Louie & Sevigny, 2017). BC patients received trastuzumab who show overexpression of p95HER2, have a greater incidence of lung tumor invasion compared with patients expressing the full-length receptor (M. Scaltriti et al., 2007). Kallergi et al. (2015) investigated the expression of p95HER2 receptors on circulating tumor cells (CTCs) in both early and advanced BC women. In the MBC, their incidence enhanced and their presence was due to poor survival.

3.7 | The effect of trastuzumab on cell cycle

Binding of trastuzumab to HER2 with a high affinity can induce cytostatic effect, that is related to G1 arrest and decrease of apoptosis and cell proliferation as a result of the increase in the level of the p27kip1 (Baselga et al., 2001). The CDK can be inhibited by P27^{Kip1} that blocks cyclin E/CDK2 complexes and induces cell cycle arrest. P27^{Kip1} could be phosphorylated by Akt and then marked for proteasomal destruction. It has been demonstrated that increased levels of p27kip1 in trastuzumab-treated cell lines causes a reduction in CDK2 activity (M. Scaltriti et al., 2011). PPM1H is related to The PP2C family that consists of metal-dependent Ser/Thr phosphatases. S. Tuen et al. explored that PPM1H knockdown might have an important effect on total levels or phosphorylation of HER family members and downstream MAPK, PI3K, or cell-cycle regulatory signaling pathways. They suggested a model through which PPM1H can dephosphorylate the T187 site on p27 and prevent its ubiquitylation and degradation. Stabilized p27 could inhibit the cell cycle. Therefore, PPM1H may be related to poor patient outcome in BC.

4 | THERAPEUTIC STRATEGIES

4.1 | Anti-HER2 antibodies

4.1.1 | Lapatinib

Lapatinib is a double EGFR/ErbB2 tyrosine kinase inhibitor (blocking both HER1 and HER2) that links HER1 and HER2 kinase domains, especially to the adenosine triphosphate (ATP) binding site. It also prevents the activation of phosphorylation and receptors actions. The activation of downstream pathways like MAPK and PI3K/Akt is blocked in BC cells leading to a decreased growth and proliferation of the cancer cells. Using this data, clinical studies have tested the impacts of lapatinib in combination with trastuzumab on treating advanced HER2$^+$ BC that does not respond to trastuzumab (Blackwell et al., 2010; Konecny et al., 2006). The hypothesis behind this combinatorial strategy is the distinct action mechanisms in how these drugs work. In fact, trastuzumab is a monoclonal antibody, which prevents HER2 receptor from dimerizing, and lapatinib is a tyrosine kinase blocker, which inhibits nascent signaling through HER dimers. The other advantage is that unlike trastuzumab, lapatinib is a small molecule; thus it is much more appropriate to enter to the central nervous system (CNS) and has the potential to better control of MBC (Arteaga et al., 2012).

Also, it can promote trastuzumab dependent ADCC due to the accumulation of HER2 on the cell surface. The dual efficacy of HER2 inhibition with trastuzumab and lapatinib was studied in the Phase III study of EGF104900. In this study, patients with HER2$^+$ MBC who indicated progression during trastuzumab therapy were randomly selected to take lapatinib in monotherapy or lapatinib in combination with trastuzumab. The combination was led to more satisfying outcomes both in progression-free survival (PFS) and in OS (Blackwell et al., 2012).

4.1.2 | Neratinib

Neratinib is a dual tyrosine kinase inhibitor of HER1 that is used for the management of different stages of HER2$^+$ BC. Neratinib is an irreversible tyrosine kinase inhibitor, unlike the reversible nature of lapatinib, this drug can covalently bind to the ATP-binding site of the receptor kinase. Cell-cycle arrest and antiproliferative impacts relate to the reduction in signaling in the HER2 model systems after therapy with neratinib (Burstein et al., 2010; Rabindran et al., 2004).

This drug was examined in research with HER2$^+$ patients who were previously exposed to trastuzumab or have never been treated with anti-HER2 in the past. In Phase II studies, neratinib showed a well-tolerated capacity and significant clinical activity. The 16-week survival rates were 59% for patients with prior trastuzumab therapy and 78% for patients with no prior trastuzumab therapy. It has also been shown that trastuzumab is more effective and has a longer inhibitory impact than lapatinib. It could also bypass potential pathways of resistance (Rabindran et al., 2004; Sharma & Jayanth, 2010).

4.1.3 | Afatinib

Afatinib, a small molecule drug, is an ErbB family blocker and tyrosine kinase inhibitor (TKI; Canonici et al., 2018; Sequist et al., 2013; Tsang & Finn, 2012). Afatinib has the potential to irreversibly block all homo- and heterodimers ErbB family receptor related to cancer which characterized it as a candidate for modulating sensitivity in trastuzumab-resistant BC. In fact, afatinib has displayed clinical activity in progressive solid tumors and trastuzumab resistance $HER2^+$ BC (N. U. Lin et al., 2012). Furthermore, dual inhibition of HER2 with the combination of trastuzumab and afatinib which is a TKI, alone or in combination with chemotherapy, is a treatment approach for metastatic and trastuzumab-resistant (Kümler, Tuxen, & Nielsen, 2014).

4.1.4 | Pertuzumab

Pertuzumab is another monoclonal antibody, which attempts to block ligand-induced HER2/HER3 heterodimerization (Gradishar, 2012). This drug has a temperate antitumor clinical activity and also, it seems to be a very proper synergistic drug combined with trastuzumab. The diverse mechanisms of action of trastuzumab and pertuzumab act complementary to prepare a more perfect inhibition in signal transduction of HER2. This combined treatment has strongly increased antitumor function in xenograft models with a reduction in the HER protein levels (Scheuer et al., 2009). Cortés et al. (2009) showed that the combination of pertuzumab and trastuzumab could reverse trastuzumab resistance. The patients who received pertuzumab monotherapy after trastuzumab had clinical benefit rate (CBR) and the objective response rates of 3.4% and 10.3%, respectively. However, in the patients who take the combination after progression on trastuzumab, these rates were 17.6% and 41.2%, respectively (Cortés et al., 2009).

4.1.5 | Poziotinib

Poziotinib is a new, oral pan-HER kinase blocking agent, which has revealed effective antitumor actions via suppression of HER family tyrosine kinases activity. This drug has shown significant clinical benefits in heavily-treated $HER2^+$ MBCs. Biomarker studies analyzing pre- and on-treatment biopsies are required to support further significant clinical outcomes of poziotinib in $HER2^+$ MBC (Han et al., 2017; Koga et al., 2018).

4.1.6 | Pyrotinib

Pyrotinib is an oral, irreversible dual pan-ErbB receptor TKI acting against HER1, HER2, and HER4. The drug recently acquired conditional approval in China for utilizing in combination with capecitabine for the therapy of the patients with $HER2^+$ MBC (Ponde, Brandao, El-Hachem, Werbrouck, & Piccart, 2018; Zhu et al., 2016).

4.1.7 | Tucatinib

Tucatinib is an orally-active inhibitor of ErbB-2, which can reduce toxicity, and can improve dose-intensity and influence. Recent data indicate considerable advantages of using this drug, especially in patients with brain metastasis (Ponde et al., 2018; Zhu et al., 2016).

4.1.8 | PF-05280014

PF-05280014 is under investigation as a potential herceptin. Recent studies have reported that neoadjuvant treatment with PF-05280014 administered in a mixture with carboplatin and docetaxel showed non-inferiority in pharmacokinetics and comparability in immunogenicity, safety, and efficacy when compared with trastuzumab-EU in combination with carboplatin and docetaxel in patients with respectable $HER2^+$ BC (Lammers et al., 2018; J. Paik, 2018; M. D. Pegram et al., 2019).

4.1.9 | CT-P6 (Herzuma)

CT-P6 is a biosimilar to trastuzumab with same efficacy and pharmacokinetics and, equivalent immunogenicity profiles and safety (Jeong et al., 2018). In a very recent study, it has been shown that CT-P6 acts in a particularly similar manner to trastuzumab by binding to the HER2 receptor. Altogether, these results support further evidence of the similarity between trastuzumab and CT-P6 and indicate the possible usage of CT-P6 across trastuzumab indications (Jeong et al., 2018).

4.1.10 | Margetuximab (MGAH22)

Margetuximab is an antibody against HER2 binding with high affinity to the low- and high-affinity forms of CD16A, an Fc-receptor critical for ADCC in tumor cells. In a recent study, it was indicated that margetuximab was generally well tolerated and showed promising single antitumor activity (Oh et al., 2017; Table 2).

4.2 | Antibody–drug conjugates

4.2.1 | T–DM1

Antibody–drug conjugates (ADCs) are treatment approaches for the development of selectively deliver chemo-therapeutic drugs toward tumors whereas normal tissue toxicity limits. ADCs contain a tumor-targeting antibody and cytotoxic small molecules (payloads) attached through a chemical linker (Kim & Kim, 2015). T–DM1 consists of antibody trastuzumab, covalently attached by a thioether molecular bond to the antimicrotubule chemotherapy emtansine (DM1; Girish et al., 2012). Lapatanib plus capecitabine was compared in the EMILIA study with Trastuzumab emtansine (T–DM1), which showed an important variation in OS and PFS improvement in patients treated with the second regimen (Verma et al., 2012). T–DM1 improved overall survival compared with lapatinib plus capecitabine in $HER2^+$ MBC patients in the Phase III EMILIA study following the

TABLE 2 HER2 tyrosine kinase inhibitors

Type of molecules and antibodies	Mechanism of action	References
Lapatinib	Blocking the HER1 and HER2	(Blackwell et al., 2010; Konecny et al., 2006)
Neratinib	Blocking the HER1 and HER2	(Burstein et al., 2010; Rabindran et al., 2004)
Afatinib	Targeting the HER2 and HER4	(Canonici et al., 2018; Sequist et al., 2013; Tsang & Finn, 2012)
Pertuzumab	Blocking the heterodimerization of HER2 with HER3	(Gradishar, 2012; Scheuer et al., 2009)
Poziotinib	An oral pan-HER kinase inhibitor	(Han et al., 2017; Koga et al., 2018)
Pyrotinib	A dual inhibitor of both EGFR and HER2	(Ponde et al., 2018; Zhu et al., 2016)
Tucatinib	Selectively inhibits HER2	(Ponde et al., 2018; Zhu et al., 2016)
PF-05280014	Trastuzumab biosimilar	(Lammers et al., 2018; J. Paik, 2018; M. D. Pegram et al., 2019)
Margetuximab	Trastuzumab biosimilar	(Oh et al., 2017)
Herzuma (biosimilar of trastuzumab)	Binding to the HER2 receptor	(Jeong et al., 2018)

failure of first-line therapy with taxane and trastuzumab (Sung et al., 2018; Verma et al., 2012).

4.2.2 | SYD-0985

SYD-0985, an agent compound made up of trastuzumab conjugated with duocarmycin is tested in patients with advanced stomach and breast cancers (Ponde et al., 2018). In a recent study, it was proved that SYD985 is a new ADC with significant activity against epithelial ovarian carcinoma (EOC) and HER2/neu expression (Menderes et al., 2017).

4.2.3 | DS-8201a

DS-8201a, an agent combining an exatecan derivate and an anti-HER2 antibody, has also demonstrated Phase I results in a total of 130 patients suffering from advanced BC, with an ORR of 61.4% in $HER2^+$ patients and 31.6% in HER2-low patients (Ponde et al., 2018). In experimental studies, DS-8201a displayed a wider antitumor spectrum compared with T–DM1, especially against tumors with low HER2 expressing (Doi et al., 2017).

4.2.4 | TAK-522 (XMT-1522)

TAK-522 (XMT-1522) includes a novel, auristatin-based cytotoxic payload (Auristatin F-hydroxypropylamide, AF-HPA) and a novel human IgG1 anti-HER2 monoclonal antibody. Currently, TAK-522 is being tested in phase-1b trial patients with advanced breast, gastric, lung, and HER2 expressing cancers. In a very recent study, TAK-522 was indicated to be as a potential candidate for combination therapies with immune checkpoint modulators in patients suffering from poorly immunogenic HER2 expressing tumors (Khattar et al., 2018).

4.2.5 | MEDI4276

MEDI4276 is a HER2-targeting, bispecific antibody specifically targets and binds to two different epitopes on HER2 linked to a new anti-microtubule blocker called tubulysin. In $HER2^+$ tumor cells including T–DM1 resistant cells, MEDI4276 indicates increased cytolysis and cellular internalization. At higher doses, MEDI4276 has clinical activity with enhancing the toxicity (M. Pegram et al., 2018).

4.3 | PI3K/Akt/mTOR pathway inhibitors

mTOR is a serine-threonine protein kinase that modulates cellular metabolism through mRNA translation and protein synthesis. Activation of this pathway is involved in trastuzumab resistance. Also, some experiments have shown that targeting of mTOR might reverse trastuzumab resistance.

4.3.1 | Perifosine

Perifosine is an Akt inhibitor that undergoes clinical tests in the hematological and solid tumors of patients. Due to having excessive toxicity of Akt inhibitors in experimental models, another strategy for inhibition of PI3K/Akt signaling is utilized of small molecules that might inactivate the mTOR pathway. AP23573, RAD001, and CCI-779 were three mTOR inhibitors which evaluated for clinical test for the patients with BC and other solid tumors. According to the previous reports, lower PTEN-expression in breast tumors could lead to decreased response to trastuzumab. Also, drug discovery programs have begun to develop direct, more efficient and less toxic blockers of the Akt kinase family (C.-H. Lu et al., 2007; Ma et al., 2010; Pandolfi, 2004).

4.4 | IGF-1R inhibitors

As mentioned above, interactions and cross-signaling between IGF and HER2 receptor families causing alternative signaling

network activation and trastuzumab resistance (Nahta et al., 2006). By disrupting the IGF-1R/HER2 heterodimer, synergistic interactions with trastuzumab and inhibition of IGF1R downstream signaling, experimental models of trastuzumab-resistant HER2$^+$ BC have restored trastuzumab sensitivity (Tsang & Finn, 2012).

4.5 | Bispecific antibodies

Bispecific antibodies (BsAb) are monoclonal antibodies recognizing two distinct epitopes in tandem, either in different receptors or in the same receptor (Kontermann & Brinkmann, 2015). MCLA-128 recognize both HER2 and HER3, causing increased ADCC and ZW25 targets two distinct epitopes on the HER2-ECD (Yu et al., 2017).

4.6 | Blockade of PD-1/PD-L1 immune checkpoint

Programmed death 1 (PD-1) performs as a reverse modulator of the immune system. This coinhibitory receptor is highly expressed in tumor-infiltrating lymphocytes (TIL). PD-L1 (PD-1 ligand) is expressed by several cancers including BC. It is reported that targeting of PD-1/PD-L1 would inhibit PD-1/PD-L1dependent pathways in cancer. T cell infiltration could be used as predictive markers of the trastuzumab efficacy (Denkert et al., 2010). It has been shown that PD-1$^+$ TILs related to poor prognosis in HER2$^+$ BC (Muenst et al., 2013). Moreover, emerging data have proposed the correlation between the expression of PD-L1 on tumor cells and response to anti-PD-1 therapy (Brahmer et al., 2010; Topalian et al., 2012). Experimental studies indicated the synergistic effect of trastuzumab on anti-PD1 antibodies (J. Stagg et al., 2011). Other immune-based therapies, such as anti CD73, have also been investigated. Preclinical data recommended that anti CD73 can reduce tumor growth and metastases (J. Stagg et al., 2010).

4.7 | HSP90 inhibitors

Heat shock protein 90 (HSP90) is a molecular chaperone and has a critical role in the maturation and stabilization of many oncogenic signaling proteins, such as HER2/ERBB2 (Basso, Solit, Munster, & Rosen, 2002; Erlichman, Toft, Ames, & Goetz, 2003). Inhibition of HSP90 would degrade of HSP90 client proteins (Ehrlich et al., 2009). HSP90 inhibitors plus trastuzumab show antitumor function in HER2$^+$ BC. HER2 overexpression BC cell lines and xenograft models subjected to HSP90 inhibitors demonstrated the degradation of HER2, inhibition of PI3K signaling and growth suppression (X. Lu, Xiao, Wang, & Ruden, 2012). Phase I assessment of tanespimycin, an inhibitor of HSP90, plus trastuzumab showed a clinical advantage for trastuzumab resistance HER2$^+$ BC patients, with four minor and one partial response. In Phase II of the survey, tanespimycin plus trastuzumab exhibited a response rate of 24% and a clinical benefit rate (CBR) of 57% in the patients with HER2$^+$ MBC who had failed prior trastuzumab treatments. Now, second-generation of HSP90 inhibitors is in the development phase of the clinical trial (Chandarlapaty et al., 2010; Giacchetti et al., 2006; Miller et al., 2007; Modi et al., 2008; Solit & Chiosis, 2008). Other hsp90 inhibitors such as retaspimycin and AUY922, are now under assessment in the primary phase of clinical trials as single drugs or in combination with trastuzumab (Tsang & Finn, 2012).

4.8 | Network medicine based approaches

There is a growing insight that we have to enhance the complexity of our recommended therapies in case of complex mechanisms such as trastuzumab resistance from a gene-centered to a network-centered perspective (Pujol, Mosca, Farrés, & Aloy, 2010; Z. Rezaei et al., 2019). Network-centric therapeutic approaches mention targeting entire pathways instead of single proteins. The aim of these strategies would a combination of modulators and targets performing on many therapeutic points that can make synergistic effects operate by converging actions at particular pathway areas.

With the advances in high-throughput expression profiling technologies, trastuzumab resistance mechanism is being progressively dissected through analysis of BC omics data for prediction of response to treatment (Decker et al., 2018; Wang et al., 2016). For example, to recognize pathways linked to resistance, Creedon et al. characterized a novel set of epithelial-to-mesenchymal transition (EMT)-associated proteins linked to HER2-independent resistance using a systems biology approach. They proved that a subset of EMT-associated genes can be good candidates for prediction of BCs prognosis. Moreover, targeting the EMT-associated kinases effectively inhibited the proliferation of resistant cells. They concluded that inhibitors to these kinases can lead to an innovative approach for the management of HER2-independent resistance in different cancers.

5 | CONCLUSIONS

Recent surveys particularly suggest the effect of Trastuzumab on HER2-overexpressing BC. Nevertheless, the trastuzumab resistance clinical problem is growing progressively important. In this regard, the identification of the trastuzumab resistance molecular mechanisms is more essential than ever. Therefore, it is suggested to recognize the most appropriate HER2-targeted treatment combination toward the goal of improving overall survival for metastatic HER2-overexpressing BC patients.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

A. D. and Z. R. devised the main conceptual ideas. A. D., H. S., M. S., and A. M. wrote the initial draft of the manuscript. A. A., A. G., M. A. S., and F. V. prepared figures and tables. B. B and Z. R., K. H. reviewed and edited the manuscript. B. B. supervised the study.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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