

Targeted therapy in breast cancer: what's new?

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Summary

Breast cancer is the most commonly diagnosed malignancy and one of the major causes of death among women. Breast cancer is also one of the most investigated diseases but whose biological features are still not well understood, several effective treating strategies having been explored in dealing with different types of advanced breast cancer, such as endocrine therapy and molecular targeted therapy. Trastuzumab is the first approved targeted anti-cancer agent to show an attractive response rate and outcomes in treating HER-2 positive metastatic breast cancer patients. However, primary or acquired trastuzumab resistance usually occurs some time into the use of trastuzumab and leads to treatment resistance or tumour progression. The promising results with trastuzumab targeted therapy encouraged further investigations in this area exploring several novel targeted agents aiming to overcome the resistance drawback of trastuzumab. In this review we discuss the major newly developed targeted agents in breast cancer treatment, including the novel anti-HER-2 monoclonal antibody pertuzumab or ertumaxomab, small molecular tyrosine inhibitor lapatinib, selective PARP1 inhibitor olaparib, mTOR inhibitor rapamycin analogues, and sheddase inhibitors. Many of these novel targeted drugs or molecules showed additional or complementary effects to trastuzumab therapy that need further and wider investigation.

Key words: breast cancer; targeted therapy; HER-2; BRCA1; BRCA2

Introduction

Breast cancer as a heterogeneous disease presents a wide range of pathological characteristics and clinical features. Breast cancer is the most commonly diagnosed cancer type and one of the major causes of death among women worldwide [1]. Breast cancer mortality has been falling steadily since the 1990s, as a result of the successful promotion of earlier breast cancer screening techniques and the improvement of therapeutic strategies. For instance, over half of breast cancer cases are in hormone receptor positive patients, most of whom show a high response rate to endocrine therapy. However, primary and secondary resist-

ance to hormone therapy decreases its efficacy in advanced breast cancers and finally leads to tumour progression.

Tumorigenesis is a multistep process involving several sequential or overlapping abnormal alternations in cellular physiology, briefly categorised as self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), unlimited replicative potential, sustained angiogenesis, and tissue invasion [2]. The emerging studies reveal the molecular and genetic mechanisms of neoplastic transformation, including local progression and remote metastasis leading scientists into individualised therapeutic perspectives such as taking single or several aberrant molecules or mutated genes as key initiators or promoters in cancer progress in order to develop precise targeted therapy. These perspectives theoretically offer a more effective and less detrimental way of dealing with malignant diseases. Based on several specific biomolecular features of breast cancer, such as breast cancer susceptibility gene type 1, 2 (BRCA1/BRCA2) mutations [3], human epidermal growth factor receptor-2 (HER-2) overexpression [4], and vascular endothelial growth factor (VEGF) receptor activation [5], some targeted agents aiming at these genetic or molecular alterations have been developed to expand the beneficiary groups and augment treatment efficacy. In a more comprehensive perspective, several novel endocrine therapies targeting endocrine receptors, such as G-protein coupled receptor 30 (GPR30), should be included in targeted therapy. However, endocrine therapy is not the main focus of this review, in which several newly established or under-evaluating targeted therapies besides hormone receptor related therapy will be discussed.

Novel anti-HER-2 therapy

Trastuzumab and mechanisms of trastuzumab resistance

Trastuzumab (HerceptinTM, Genetech/Roche, South San Francisco, CA) is the first approved anti-HER-2 targeted agent to show a highly promising response rate in the treatment of HER-2 positive metastatic breast cancer patients [6], as well as in combination with or after standard adjuvant chemotherapy of HER-2 positive breast cancer patients [7].

Despite the fact that trastuzumab-based treatment strategy has established a milestone in the therapy of HER-2 positive breast cancer with attractive clinical benefits, either as a single agent or in combination settings, one of the major drawbacks of the trastuzumab-containing regimen is trastuzumab resistance, even in highly selected HER-2 overexpressed patients. In fact, only about 30% of HER-2 positive metastatic breast cancer patients respond to trastuzumab and approximately 70% of patients with overexpressed HER-2 receptor may have primary resistance to trastuzumab [8]. Additionally, the majority of those patients who achieve initial efficacy tend to develop secondary trastuzumab resistance within one or two years [9].

Several mechanisms have been postulated in an attempt to explain both intrinsic and acquired resistance to trastuzumab. Emerging evidence suggests that a variety of mechanisms which alter any step of the complex HER-2 signalling network may participate in the development of trastuzumab resistance [9, 10], including: a) cleavage of HER-2 extracellular domain to form the truncated HER-2 receptor and the overexpression of membrane associated mucin MUC4 to mask or block the trastuzumab binding site, which can interrupt the interaction between HER-2 receptor and this antibody; b) the loss of expression or function of tumour suppressor gene phosphatase and tensin homologue deleted on chromosome ten (PTEN) and the constitutive activating formula of PI3K mutant finally enhances the PI3K/Akt pathway and alters the intercellular HER-2 signalling network; c) the lost expression of a cyclin-dependent kinase (CDK) inhibitor p27 can induce the failure of trastuzumab-mediated cell cycle growth arrest; d) signal transduction through other EGFR family members (HGFR-1 for instance) could compensate the blocking signal of HER-2; e) bypass signalling through the non-EGFR family growth factor receptor insulin-like growth factor-1 receptor (IGF-1R) enables activation of the PI3K/Akt and MAPK downstream signal cascades without the participation of HER-2. Therefore, the further understanding of trastuzumab action and resistance mechanisms highlights the vital need for novel targeted drugs aiming at HER-2 overexpression.

Lapatinib and tyrosine kinase inhibitors (TKIs)

Lapatinib (TykerbTM, GlaxoSmithKline, Research Triangle Park, NC) is a dual, orally administered small molecule tyrosine kinase inhibitor (TKI) of both HER-2 and EGFR (HER-1), which can bind reversibly to the intercellular ATP-binding pocket within the catalytic domain of both receptors and inhibit receptor auto-phosphorylation. This action prevents receptor activation and leads to the blocking of subsequent downstream signal transmission, such as MAPK pathway and PI3K/Akt pathway cascades, which are responsible for regulation of gene transcription, cell proliferation and apoptosis [11, 12].

Several reasons support development of the clinical use of lapatinib beyond trastuzumab [13]. First of all, lapatinib, as mentioned before, has dual tyrosine kinase inhibitory activity that targets the intercellular domain of both EGFR and HER-2, whereas trastuzumab only binds the extracellular domain of HER-2. Thus, lapatinib may achieve more efficacy than trastuzumab in breast cancer patients with EGFR

and/or HER-2 overexpression. Secondly, lapatinib directly blocks the intercellular kinase receptors otherwise than by binding the extracellular domain as trastuzumab does. This mechanism can overcome the trastuzumab resistance form associated with the truncated HER-2 receptor, p95HER-2, and the lack of extracellular domain, but with the presence of tyrosine kinase activity [14]. Thirdly, although trastuzumab is effective in controlling the progression and invasion of metastatic breast cancer, it is insufficient to prevent the occurrence of brain metastases due to the molecular weight limitation for crossing the blood-brain barrier. Whereas lapatinib is a small molecular compound that may penetrate the blood-brain barrier easily and achieve the effective concentration level in the cerebrospinal fluid, it may therefore prevent the refractory effect of brain metastasis [15]. Furthermore, the cardiotoxicity related to HER-2 targeted therapy is one of the major side effects, some 11% of trastuzumab treated metastatic breast cancer patients developing cardiodyfunction. However, systemic analysis demonstrated that only 1.6% of lapatinib treated patients develop symptomatic or asymptomatic diminished cardiac function, which is a quite small proportion compared with the trastuzumab cohort [16].

In March 2007, lapatinib was approved by the FDA as the first small molecular inhibitor targeting HER-2 for the treatment of patients with advanced or metastatic breast cancer based on a phase III, randomised trial of lapatinib-capecitabine combined regimen in patients with progressive, HER-2 positive, locally advanced/metastatic breast cancer as compared with capecitabine treatment alone [17]. This trial showed that the addition of lapatinib to capecitabine was associated with a 51% reduction in the risk of disease progression with no significant increase in toxic effect and less central nervous system (CNS) metastasis occurrence as compared with capecitabine monotherapy. Several clinical trials are underway to evaluate the efficacy of lapatinib when combined with chemotherapy, endocrine therapy or trastuzumab, such as the Tykerb Evaluation After Chemotherapy (TEACH) trial, the global Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO) trial [18]. Recent published data demonstrated that lapatinib plus trastuzumab working as a dual blockade of HER-2 pathway might be a better solution than lapatinib or trastuzumab alone in neoadjuvant treatment for HER-2-positive primary breast cancer (NeoALTTO study), as well as in trastuzumab-refractory breast cancer [19]. Additionally, a recent published phase II study observed potential efficacy and good tolerance of lapatinib single agent treatment for relapsed or refractory HER-2 positive inflammatory breast cancer [20]. As the further study data become available, the treatment effect of lapatinib in different settings will be better understood.

EGFR family members are the major targets for development of treatment strategies for metastatic breast cancer. Gefitinib (IressaTM, AstraZeneca Pharmaceuticals, London, UK) and erlotinib (TarcevaTM, Genentech/Roche, South San Francisco, CA) are both selective reversible small molecular tyrosine kinase inhibitors of EGFR (HER-1) and have won FDA approval for the treatment of head and neck cancer or lung carcinoma with mutated EGFR expression. However, the outcomes of clinical stud-

ies of both gefitinib and erlotinib in treating breast cancer were unimpressive [21]. On the other hand, Neratinib (HKI-272, Wyeth Corp, Madison, NJ), similar to lapatinib, is an oral, dual-activity but irreversible pan-inhibitor of EGFRs tyrosine kinases which targets EGFR, HER-2 and HER-4 [10]. The phase II trial showed benefit from neratinib in treating advanced HER-2 positive breast cancer. The objective response rate was 51% in trastuzumab naive patients and 26% in those who received prior treatment with trastuzumab [22].

Pertuzumab

Pertuzumab (OmnitargTM; Genentech/Roche, South San Francisco, CA) is a humanised monoclonal antibody that targets the extracellular domain of HER-2. However, the structure and function analysis demonstrated that pertuzumab binds to a quite distinct site of the HER-2 extracellular part from that of trastuzumab and is considered to serve as a HERs dimerisation inhibitor [23]. Receptor dimerisation can occur between two different EGFR family members (heterodimerisation) or between two symmetric EGFR receptors (homodimerisation). The intercellular tyrosine kinase of one receptor can only be phosphorylated and activated through dimerisation. This mechanism is quite important for HER-2/HER-3 dimers as the HER-3 receptor which lacked active tyrosine kinase domain and disabled to form the homodimers. Different investigations suggested that HER-2/HER-3 can act as an oncogenic unit, which initiates activation of the PI3K/Akt signal pathway to enhance tumour progression [24]. Pertuzumab is designed to bind to the junction part of HER-2 extracellular domain and thereby ultimately block the formation of HER-2 related homo- and hetero-dimerisation as well as their downstream signal transduction. The good tolerance and antitumour activity of pertuzumab has been identified in several completed phase I and phase II studies [25]. Pertuzumab showed a compensating anti-HER-2 efficacy after use in trastuzumab refractory metastatic breast cancer patients, which meant that it could partially reverse trastuzumab resistance [26]. A recently published phase II trial reported a 24.2% objective response rate and 50% clinical benefit rate in the regimen consisting of pertuzumab plus trastuzumab in advanced breast cancer patients with HER-2 overexpression and patients whose disease progresses aftertrastuzumab-based therapy. The promising results strongly demonstrate a synergistic efficacy of the two combined antibodies [27]. Two randomised phase III studies with pertuzumab that can increase knowledge of it are currently ongoing. The CLinical Evaluation of PErtuzumab and TRAstuzumab, sponsored by a Genentech (CLEOPATRA) study aims to compare the efficacy and safety of docetaxel plus trastuzumab with or without the combination of pertuzumab in previously untreated breast cancer patients. The neoadjuvant treatment with herceptin and pertuzumab, sponsored by a Hoffmann-LaRoche (NEOSPHERE) study recruits patients with locally advanced, inflammatory, or early-stage HER-2 positive breast cancer to evaluate the complete pathological response rate of the combination of trastuzumab, pertuzumab, and docetaxel. The first clinical data shows that pertuzumab and trastuzumab plus docetaxel given in a neoadjuvant setting

prior to surgery significantly improved the pathological complete response rate as compared with trastuzumab plus docetaxel (San Antonio Breast Cancer Symposium, SABCs 2010).

Trastuzumab-DM1

Trastuzumab-DM1 (T-DM1; Genentech/Roche, South San Francisco, CA) is a novel chemistry-driven conjugated HER-2 monoclonal antibody in which the trastuzumab is conjugated with a fungal toxin DM1 (maytansine) [28]. The purpose of developing this compound is to overcome trastuzumab resistance and boost the efficacy of this targeted strategy. Maytansine is an antimicrotubule agent that inhibits the assembly of cellular microtubules. In vitro studies showed that the cytotoxicity of maytansine is over 1000 times that of any other chemotherapeutic agent [29]. In trastuzumab-DM1, trastuzumab mainly works as a carrier that delivers DM1 to the tumour cells labelled with HER-2. Trastuzumab-DM1's mechanism of action is independent of functional HER-2 signalling, but only requires a high expression level of HER-2 on the cellular surface. Therefore, trastuzumab-DM1 can successfully overcome several trastuzumab resistance mechanisms related to HER-2 downstream signalling, such as PI3K mutation and PTEN downregulation. In a phase II study, trastuzumab-DM1 was administered to patients with metastatic, HER-2 positive and trastuzumab-refractory breast cancers. With a median follow-up of 9.5 months, the objective response rate was 25% and the clinical benefit rate was 34.8% [30]. In fact, trastuzumab-DM1 represents the true exploration of combined targeted and chemotherapy. This formulation, conjugating trastuzumab with potential cytotoxic agents, offers an attractive resolution to trastuzumab resistance.

Ertumaxomab

Ertumaxomab (RexomonTM; Fresenius Biotech, Hamburg, DE) is a trifunctional, bispecific antibody that targets both the HER-2 expressed on tumour cells and the CD3 antigen on T cells. Ertumaxomab conducts formation of a HER-2-ertumaxomab-CD3 complex leading to the aggregation and activation of T cells, macrophages, dendritic cells and natural killer cells to either initiate or metastatic tumour sites that subsequently cause the death of the tumour cells through phagocytosis [31]. In vitro experiments have indicated that ertumaxomab has the potential to kill many different HER-2 positive tumour cell lines. The acceptable phase I study results lead to the start of a phase II trial to evaluate the efficacy and safety of ertumaxomab administered to patients with HER-2 positive and progressive metastatic breast cancer after trastuzumab treatment. The results of the recently published phase II study (SABCs 2010), offer continuing support for further clinical development of ertumaxomab.

BRCA1, BRCA2 mutations and targeted therapy

BRCA1 and BRCA2 mutations were both first identified and cloned as the candidate of the breast cancer and ovarian cancer susceptibility gene through linkage analysis among selected high risk family members some 16 years ago [32,

33]. Approximately 5% to 10% of all breast cancers are accounted for by hereditary breast cancers, which are chiefly attributed to germ line mutations of either BRCA1 or BRCA2 genes; the BRCA1 gene alterations occur in 40% to 45% of hereditary breast cancer as well as 80% occurrence in both hereditary breast and ovarian cancers [34, 35]. Women who have a mutation in either of these genes have a high cumulative lifetime risk of developing breast and ovarian cancers. Recent meta-analysis studies demonstrated that BRCA1 mutation carriers bear a 57–65% lifetime risk of developing breast cancer and 39–40% probability of evolving ovarian cancer; whereas the BRCA2 mutation carriers have 45–49% lifetime probability of breast and an 11–18% risk of ovarian cancer [3, 36]. The overall prevalence of BRCA1 or BRCA2 mutations is estimated to be from 1 in 400 to 1 in 800, a ratio which is quite variable among races or ethnic groups [37, 38]. However, some ethnic subgroups have higher carrier prevalence due to founder mutations which result from geographical or cultural isolation during population development [39, 40]. The detailed revision of BRCA1 and BRCA2 mutations' distributions and their related clinical implications across race and ethnicity was summarised by Kurian AW in a very recent review [41].

The BRCA1 tumour suppressor gene is localised on the long arm of chromosome 17 at 17q21, which contains 24 exons that could be transcript into a 7.8kb mRNA encoding the breast cancer type 1 susceptibility protein. BRCA1 abnormalities result in a dysfunction of homologous recombination (HR) repair mechanisms, which predispose the carriers to exposure to DNA-damage events such as ionising radiation, interstrand cross-linking agents, and spontaneous chromosomal aberrations [42, 43]. BRCA1 also interacts with several intracellular factors to participate in the regulation of a wide range of cellular processes such as cell cycle checkpoint control, gene transcription regulation, DNA damage repair and apoptosis regulation [44, 45]. The location of BRCA2 tumour suppressor gene on the chromosome is quite different from the BRCA1 localisation. BRCA2 was identified on the long arm of chromosome 13 at 13q12.3, which could transcript a 10.4kb mRNA that encodes breast cancer susceptibility protein type 2 through 27 encoding exons [33]. Different studies have suggested that the major function of BRCA2 is direct regulation of the availability and activity of Rad51, which works as a key enzyme catalyser in the reaction of HR process [46, 47]. High levels of spontaneous chromosomal aberrations were reported in BRCA2 defective cells due to the error-prone homology-directed repair of DSBs caused by BRCA2 mutations [48].

Although BRCA1 and BRCA2 are located on two different chromosomes, they share a number of functional similarities that are known as “caretakers” of chromosomal stability maintenance [44]. Functional wild-type BRCA1 and BRCA2 genes play an important role in the repair of DNA double strand breaks (DSBs) resulting from different types of carcinogenesis. DNA DSBs are particularly detrimental to genomic integrity and stability. HR and non-homologous end joining (NHEJ) are two major DNA repair mechanisms. Cell line studies have demonstrated that the HR mechanism plays the prominent role during the DSBs re-

pair process which can precisely and completely reverse this kind of DNA injury, whereas NHEJ contributes to a template and inherently error-prone repair process [49, 50]. BRCA1 and BRCA2 act as tumour suppressor genes and play a central role in the process of the HR repair mechanism, which is an error-free cell response to the DSBs and thus prevents the occurrence of inheritable tumour evolving gene mutations [51]. In contrast to BRCA2, BRCA1 may also be involved in several additional DNA repair pathways besides HR, such as NHEJ in DSB repair [52] and nucleotide excision repair (NER) [53]. The BRCA1 and BRCA2 mutations, including frameshift deletions, insertions, and nonsense mutation, may lead to premature truncation of protein transcription with defective HR repair [54]. A defective HR mechanism results in DSBs shifting into a more error-prone repair pathway leading to the accumulation of spontaneous and damage-induced chromosomal aberrations and subsequently carcinogenesis [55, 56].

Several management strategies have been suggested for reduction of the cancer risk in individuals who bear the BRCA1 or BRCA2 mutations. In a recent study, the breast cancer incidence in 483 BRCA1 or BRCA2 mutation carriers was measured. Two of 105 (1.9%) women who underwent bilateral prophylactic mastectomy and 184 of 378 (48.7%) women who did not receive the surgery were diagnosed with breast cancer, which suggested an over 90% reduction of cancer risk for the bilateral prophylactic mastectomy group [57]. Bilateral prophylactic oophorectomy also proved to be sufficient to reduce the breast cancer risk among mutation carriers [58]. Tamoxifen, a selective oestrogen receptor regulator, was tested in a randomised trial which concluded on the risk reduction potential among women with inherited BRCA1 or BRCA2 mutations, especially carriers of BRCA2 mutation [59].

On the other hand, increasing knowledge of the roles of BRCA1, BRCA2 genes in DNA repair and the relations between their mutations and cancer predisposition suggests the therapeutic potential of strategies targeting the BRCA1 and BRCA2 defects. The principle of targeting tumour cells with HR deficiency followed by BRCA abnormalities is to induce DNA damage which could be repaired in normal circumstances depending on the BRCA function of the HR pathway, but they must be highly selective for BRCA-deficient cells and relatively harmless for normal cells [60]. This approach can be described with a therapeutic concept based on targeting of abnormal BRCA1 or BRCA2 via inhibition of DNA repair mechanisms for the single-strand breaks (SSBs), these mechanisms depending on poly-ADP-ribose polymerase-1 (PARP-1) activity [61, 62]. PARP-1 plays a major role in response to extensive DNA damage in SSB repair, especially for tumour cells with BRCA dysfunction and deficient HR pathways. Inhibition of PARP-1 converts SSBs to DSBs due to lack of HR repair mechanisms followed by BRCA mutation leading to accumulation of unrepaired DSBs and forming complex lethal chromosomal alternations. The first human phase I study reported that a single agent therapy with olaparib (AZD2281; AstraZeneca, London, UK), an oral selective inhibitor of PARP1, has promising antitumour activity in advanced solid tumors with either BRCA1 or BRCA2 mutations. A very

recent multi-centre proof-of-concept phase II trial further demonstrated a positive result with olaparib single agent therapy in women with both advanced breast cancer and BRCA1 or BRCA2 mutations [64]. This study showed an objective response rate of 41% in a cohort assigned 400 mg twice daily versus 22% in a cohort assigned 100 mg twice daily, with no unacceptable toxicities observed. Other than olaparib, several novel PARP inhibitors have also proved to be beneficial on the basis of either preclinical studies or phase I investigations, such as BSI-201, AG014699, and ABT-888 [65]. Iniparib (BSI-201, BiPar Sciences, Inc), another PARP inhibitor similar to olaparib, has been reported in a phase I study to improve the clinical benefits and survival of patients with metastatic triple-negative breast cancer, which shares clinical and pathological features with hereditary BRCA1-related breast cancer [66].

PI3K/Akt/mTOR pathway as a therapeutic target

PI3K/Akt/mTOR pathway and breast cancer

The intercellular signal pathway involving phosphatidylinositol 3-kinase (PI3K), protein kinase B/PKB (Akt) and mammalian target of rapamycin (mTOR), regulates several cellular functions, such as cell growth, survival and proliferation, which are essential for tumorigenesis and progression [67]. Tyrosine kinases contain trans-membrane growth factor receptors such as insulin-like growth factor 1 receptor (IGF-1R), fibroblast growth factor receptors family (FGFRs) and EGFR family, and are the upstream of the PI3K/Akt/mTOR pathway. Extracellular signals activate membrane receptors mentioned before, and the PI3K and Akt can be activated consequently by phosphorylation cascades, and eventually activate their downstream substrates to regulate the cell cycle progression, cell survival or apoptosis. mTOR, a serine/threonine kinase, is one of the best studied downstream kinases of Akt and a master regulator of protein translation, which can phosphorylate and activate the eukaryotic translation initiation factor eIF4E-binding proteins (4E-BP1) and the 70kD ribosomal protein S6 kinase (p70S6K). Additionally, mTOR can induce a positive feedback effect to phosphorylate Akt and enhance the signal transduction of this pathway [68].

The aberrant activation of PI3K/Akt/mTOR pathway has been observed in various types of human malignancies including breast cancer. In addition, the high activation level of PI3K/Akt/mTOR pathway has been linked to resistance to conventional cancer therapy, resistance to endocrine therapy, and association with poor prognosis of advanced stage, lower histological grade as well as propensity to promote metastasis [69]. The somatic mutations of the PIK3CA gene, which encodes the p110 α catalytic subunit of PI3K, occur in approximately 18–40% of clinical cases and play an important role in activating PI3K/Akt/mTOR pathway to stimulate cell growth in breast cancer [70]. Although the amplification of Akt genes has rarely been observed in breast cancer, the phosphorylated/activate isoform of Akt has been correlated to drug resistance and poor prognosis [71]. More importantly, the dysfunction of tumour suppressor PTEN (phosphatase and tensin homo-

logue deleted on chromosome ten), which acts as a negative regulator of PI3K through its phosphatase activity, is another frequent genetic alternation observed in breast cancer. Gene mutation, deletion or promoter hypermethylation of PTEN gene can lead to loss of PTEN suppressor function and consequently activation of PI3K/Akt/mTOR pathway. It has been shown that PTEN dysfunction is correlated to lymph node metastasis and treatment failure in breast cancer patients [72].

PI3K/Akt/mTOR pathway inhibitors

Considering the crucial role the PI3K/Akt/mTOR pathway plays in tumour progression and drug resistance among breast cancer patients, developing specific drugs targeting and blocking related signal components within this pathway is believed to be a potential treatment strategy. Several inhibitors targeting the p110 catalytic subunit of PI3K, such as wortmannin and LY294002, have been tested in preclinical studies. Although the antitumour activity of these PI3K inhibitors has been observed, their poor solubility, instability and high toxicity have limited their clinical application [67]. Perifosine is an oral synthetic inhibitor which can prevent Akt recruitment to the membrane and block activation of downstream effectors. Preclinical models suggested that perifosine could inhibit cell growth in breast cancers through apoptosis mechanisms [73]. Clinical studies showed good tolerance of perifosine administered either as a single agent or in combination with other cytotoxic drugs; however, the objective response rates are disappointing and insufficient.

The mTOR inhibitors are the most highly developed targeted drugs as compared to all other PI3K/Akt/mTOR pathway inhibitors. Rapamycin, as the mTOR inhibitor first discovered, has been shown to have anti-fungal activity and an immunosuppressive effect which has been widely used as an immunosuppressant in organ transplants [74]. Along with the mTOR inhibitory activity, rapamycin has also been observed to be the proliferation inhibitor of cancer cells, but the poor solubility and instability of rapamycin made it unsuitable for *in vivo* use [74]. Temsirolimus (ToriselTM/CCI-779; Wyeth, Madison, NJ) is an ester derivative of rapamycin which was explicitly designed as an anti-cancer drug and was approved by the FDA in 2007 for the intravenous treatment of metastatic renal cell carcinoma, while everolimus (CerticanTM/RAD001; Novartis AG, Basel, Switzerland) is another oral hydroxyethyl ether derivative analogue of rapamycin initially developed as an immunosuppressant for renal and heart transplant patients. Both temsirolimus and everolimus share a similar anti-cancer effect and the mTOR inhibiting mechanism by binding to the FK506-binding protein (FKBP-12) [75]. Preclinical *in vitro* or *in vivo* data has demonstrated that both rapamycin analogues were capable of inhibiting the proliferation of multiple breast cancer cell lines which were ER-positive and with the overexpressed HER-2 or the loss of PTEN function, either administered alone or in combination with chemotherapeutic agents, endocrinal drugs, other targeted substances or radiotherapy [69]. The phase I and phase II clinical studies evaluating the efficacy and toxicity of the rapamycin analogous monotherapy in patients with local-regional advanced or metastatic breast cancers

showed promising anti-tumour activity and a generally tolerable safety profile. On the other hand, multiple clinical trials recruit mTOR inhibitors in combination with chemotherapy, radiation, endocrine therapy or other targeted therapies, in order to enhance treatment sensitivities, overcome resistance mechanisms, or reduce adverse effects [75]. The preclinical *in vivo* data also supported a variety of phase II and phase III randomised, controlled clinical trials to determine the efficacy and safety of combining aromatase inhibitors and mTOR inhibitors in hormone receptor positive breast cancer. The first randomised phase I study of everolimus has been reported in SABCs 2010, which shows improved 6-month benefit combined with tamoxifen as compared with tamoxifen alone in hormone receptor positive/HER-2 negative metastatic breast cancer patients. This promising result will encourage further studies on mTOR inhibitors in the treatment of breast cancer. The first large phase III study comparing results of temsirolimus in combination with letrozole-based treatment and letrozole alone in postmenopausal women with advanced or metastatic breast cancer was terminated prematurely because of high grade 3 toxicities but no significant clinical benefit in this combination protocol over letrozole alone [18]. However, another phase III trial is ongoing in postmenopausal metastatic breast cancer patients who are treated with first-line therapy in combination with temsirolimus plus letrozole or letrozole alone, and the primary endpoint of this study is to determine overall progression-free survival [69].

Novel protocols regarding the mTOR inhibitors are strategies relating to combination with other targeted agents, such as EGFRs tyrosine kinase inhibitors, the anti-HER-2 monoclonal antibody trastuzumab, and anti-VEGF monoclonal antibody bevacizumab. Furthermore, the aberrant activation of the Ras/Raf/MEK/ERK pathway has been found in approximately 50% of breast cancers and has also been associated with poor prognosis and endocrine therapy resistance. Thus, the combination of targeting both the PI3K/Akt/mTOR and the Ras/Raf/MEK/ERK survival pathways might simultaneously suggest another strategy to overcome drug resistance and enhance response rates [76]. However, these combination regimens need further preclinical investigations to determine their clinical feasibility.

Antiangiogenesis and VEGF inhibitors

Angiogenesis is defined as the sprouting of new blood vessels from pre-existing vessels. One of the major hallmarks and prerequisites for most solid tumour growth is tumour-induced angiogenesis which is also responsible for the primary tumour invasion and distant cancer cell metastasis [2]. Vascular endothelial growth factor (VEGF) is a key mediator involved in the angiogenesis switch, which processes the development of a high density blood vessel network connecting the primary tumour to the host circulation, as well as a premature vascularisation characterised by high permeability status. The elevated expression of VEGF as an independent prognosis predictor has been observed in both early and late stage breast cancers and has been related to advanced stage of the diseases, poor prognosis, and decreased response to chemotherapy or endocrine therapy. The overexpression of VEGF is closely

linked to the loss of tumour suppressor p53 and the amplification of oncogene HER-2 [5].

Considering pathological angiogenesis as an essential step in tumorigenesis, the antiangiogenesis might provide a promising target for solid tumour treatment. Targeted therapies aiming at interrupting tumour neo-vascularisation can be categorised in two ways. Firstly, neutralisation of VEGF by the humanised recombinant monoclonal antibody bevacizumab (AcastinTM; Genentech/Roche, South San Francisco, CA), which recognises human VEGF-A, eliminates the ligands required for VEGF-R activation and inhibits the mitogenic and permeability-enhancing signal for neo-vascularisation; secondly, blockage of the downstream signal transduction cascade by small molecule tyrosine kinase inhibitors (TKIs), such as sorafenib (NexavarTM; Bayer HealthCare AG, DE) and sunitinib (SutentTM; Pfizer, New York, NY) [77].

Bevacizumab is the first approved anti-angiogenic agent for human cancers. Numerous clinical trials were conducted to test the efficacy of bevacizumab in metastatic breast cancers, especially in a form combining it with first-line chemotherapy. A recently published meta-analysis study summarised the available randomised trials using bevacizumab in addition to chemotherapy in metastatic breast cancer patients [78]. Their results concluded that regimens combining bevacizumab with chemotherapy provided substantial benefit for the treatment of metastatic breast cancer in terms of improving progression-free survival and objective response rate. However, there was no significant difference in overall survival. Sorafenib and sunitinib are both novel multitargeted TKIs that inhibit several proangiogenic tyrosine kinase receptors, including VEGFRs and PDGFRs [5]. Several phase I and II studies are ongoing currently to evaluate the safety and efficacy of these TKIs as combined with chemotherapy in treating metastatic breast cancers.

ADAMs and selective ADAMs inhibitors

The A Disintegrin And Metalloproteinase (ADAMs) comprise a family of multidomain transmembrane and secreted proteins and are involved in different biological functions, including fertilisation, adhesion, migration and proteolysis. Recent investigations implied that specific ADAMs are involved in tumour formation and progression, such as ADAM-9, ADAM-10 and ADAM-17 [79]. The primary substrates of ADAMs are the ectodomain of transmembrane proteins such as multiple precursor forms of growth factors, and the cleavage usually occurs at their juxtamembrane region. Specifically, two ADAMs, ADAM-10 and ADAM-17, have been shown to have the sheddase activity of releasing several ligands for EGFRs family receptors, such as epidermal growth factor (EGF) and transforming growth factor- α (TGF- α), which have been implicated as tumour-promoting factors. A number of reports have suggested that the abnormal overexpression of several ADAMs exists in multiple types of cancers and plays a role in cancer pathogenesis and progression [80], e.g. in lung cancer tissue overexpression of ADAM-28 correlated with the presence of lymph node involvement. And in breast cancer, ADAM-9 expression was significantly higher in node-positive than node-negative primary cancers. In ad-

dition, the ADAM-17 levels were shown to be an independent predictor of patient outcomes. However, the mechanisms of ADAMs overexpression in human malignancies remain to be investigated.

Since the ADAMs play an important role in cancer initiation and progression, it is rational to hypothesise that therapies targeting and blocking their sheddase activity should display anti-tumour activity. In HER-2 positive breast cancers, the intracellular kinase activity of HER-2 can be amplified and over-activated through the cleavage of its extracellular domain to form the truncated HER-2 protein (p95HER-2) and to release multiple EGFR ligands, a process mediated by the sheddase ADAM10 and ADAM17 [18]. INCB7839 (Incyte Corporation, Wilmington, DE) is an orally bioavailable selective ADAM10 and ADAM17 inhibitor which can repress sheddase activity to prevent the formation of p95HER-2 protein in HER-2 positive breast cancers. First results from a Phase Ib trial demonstrated promising activity in a small cohort of HER-2 positive trastuzumab-refractory metastatic breast cancer patients with stabilisation of disease and a reduction in serum levels of cleaved HER-2 protein. Overall, the good tolerance and low toxicities supported INCB7839 into further phase II studies in combination with trastuzumab in treating trastuzumab-refractory metastatic breast cancers [81].

Heat shock protein 90 (HSP90) inhibitors

The heat shock protein 90 is an evolutionarily conserved molecular chaperone that participates in stabilising and activating multiple proteins, referred to as HSP90 clients, many of which are essential mediators for cell survival and signal transduction, including AKT, HER-2, EGFR, platelet-derived growth factor receptor (PDGFR) and tumour suppressor protein p53 [82]. Moreover, HSP90 has also been involved in diverse nuclear events, such as transcriptional regulation, chromatin remodelling and DNA mutation [83]. In cancer cells, HSP90 functions as a molecular chaperone which facilitates cell homeostasis and cancer cell survival by protecting a sequence of mutated or overexpressed oncoproteins from misfolding and degradation [84]. The increased expression level of HSPs is a common feature of human cancers, which might reflect a cytoprotective stress response to the hypoxic, acidotic and nutrient-deprived tumour-characteristic microenvironment. In breast cancers, overexpressed HSP90 was closely correlated with poor prognosis due to a low response rate to chemotherapy regimens as well as drug resistance. Based on the strong ties between HSP90 and carcinogenesis, several HSP90 inhibitors were developed as potential anti-cancer agents capable of interacting with the ATP-binding pocket of HSP90 to block its chaperoning function, thereby resulting in client protein degradation. Tanespimycin (TelatinibTM/17-AAG; Kosan Biosciences, Hayward, CA) is the first HSP90 inhibitor to enter clinical trials. Preclinical investigations suggested a therapeutic activity of tanespimycin for HER-positive breast cancer, as decreased HER-2 expression and inhibited breast cancer growth in both cell lines and animal models [18]. A phase II trial, which evaluated the efficacy of tanespimycin together with trastuzu-

mab regimen in HER-2 positive metastatic breast cancer patients, reported a response rate of 24% and an overall clinical benefit of 57% [83]. Further investigations should be conducted to evaluate tanespimycin or other HSP90 inhibitors in treating advanced breast cancer, and to test the feasibility of combining with other regimens, such as radiation therapy, conventional cytotoxic chemotherapy or other molecular targeted agents.

Conclusions

The administration strategies for advanced breast cancer patients have been steadily improved on the basis of the emerging investigation and understanding of the biological features of breast cancer. Trastuzumab, as the first developed clinically used targeted agent, has been approved for the treatment of HER-2 positive breast cancer and has achieved promising outcomes in selected patient groups, prolonging progression-free and overall survival time. However, in view of the inevitable drug resistance due to several bypass mechanisms, this targeted therapeutic strategy proves refractory or finally fails. In this review we try to highlight several recently investigated targeted agents aiming to carry breast cancer treatment beyond or compensate the treating mechanisms of trastuzumab. Many of these new drugs have shown a promising response rate in both preclinical experiments and clinical trials, either as single agent regimens or combined with other types of treatment. The promising results encourage further investigations into new drugs and greater understanding of their pharmaceutical mechanisms in optimal regimen models for targeted therapy of breast cancer, and to achieve the best outcomes in dealing with the present trastuzumab resistance challenge.

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