

Review Article

A perspective on anti-EGFR therapies targeting triple-negative breast cancer

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Abstract: Triple-negative breast cancer (TNBC), which lacks estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), accounts for about 15-20% of breast cancers and is the most aggressive breast cancer subtype. There are currently no effective therapies against metastatic TNBC. Compared with other breast cancer subtypes, EGFR is frequently overexpressed in TNBC and a potential therapeutic target for this disease. There are two types of EGFR inhibitors, small molecular tyrosine kinase inhibitor (TKI) and monoclonal antibody (mAb), for the treatment of cancers, such as non-small cell lung cancer and colorectal cancer. For breast cancer, however, the clinical trials of EGFR inhibitors have failed due to low response rates. Because a small portion of patients do demonstrate response to EGFR inhibitors, it may be necessary to stratify patients to enhance the efficacy of EGFR inhibitors in TNBC and to develop the effective combination therapy for this patient population. In this review, we describe some of the molecular mechanisms underlying EGFR inhibitor sensitivity and further discuss the possible therapeutic strategies to increase the efficacy of EGFR inhibitors in TNBC.

Keywords: TNBC, EGFR, EGFR inhibitors, drug resistance

Introduction

Breast cancer is the most common cancer type and the second leading cause of cancer-related deaths in women in the United States. The significant increase in the 5-year survival rate of breast cancer patients from 75 to 90% can be attributed to the advances in early detection and therapeutic approaches [1]. Although early-stage breast cancer is highly curable, the 5-year survival rate of patients with metastatic breast cancer is about 20%. Breast cancer is also a heterogeneous disease with multiple subtypes. About 15-20% of breast cancer patients are diagnosed with triple-negative breast cancer (TNBC), which is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2). TNBC is generally characterized by aggressive clinical course and poor prognosis compared with other breast cancer subtypes [2]. Moreover, due to the lack

of druggable targets, such as ER and HER2, chemotherapy is still a primary option for systemic treatment. While TNBC patients respond better to chemotherapy than do non-TNBC patients, TNBC patients who do not respond eventually develop the metastatic form of the disease, which is virtually incurable [3, 4]. Moreover, because the current treatment regimens are ineffective against a significant number of TNBC patients, new effective treatment strategies are urgently needed. In TNBC, the epidermal growth factor receptor (EGFR) is frequently overexpressed, and anti-EGFR therapies, including small molecule tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs), have been developed and are currently used for treatment of some cancers such as non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). Unfortunately, no EGFR therapies are currently approved for treatment of breast cancer. In this review, we briefly summa-

size the results from clinical studies of EGFR therapies and potential resistant mechanisms against anti-EGFR therapies in various cancer types and discuss the perspective on anti-EGFR therapies in TNBC.

Breast cancer subtypes

Immunohistochemistry (IHC) classification by ER, PR, and HER2 is currently used as the standard assessment of this disease in clinic. However, breast cancer is a heterogeneous disease that exhibits distinct clinical behavior, and recent advances in new technologies for gene and protein expression profiling further revealed the complex features of breast cancer. On the basis of gene expression profiling, breast cancer can be classified into five different subtypes that include basal-like, HER2-enriched, luminal A and B, and normal-like [5, 6]. These subtypes, referred to as intrinsic subtypes, well match IHC classification [7], and among them, basal-like breast cancer (BLBC) is the most aggressive subtype with a high histologic grade, and accounts for about 15% of invasive breast cancer. About 80% of TNBC overlaps with BLBC, both of which are highly aggressive and exhibit poor clinical outcomes. Furthermore, TNBC harbors substantial genetic heterogeneity, and is further divided into six subtypes, including basal-like 1 (BL1), BL2, immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (M), MSL (mesenchymal-stem-like), and luminal androgen receptor (LAR) [8]. These TNBC subtypes have been reported to differ in drug sensitivity and require different therapeutic approaches based on their characteristics.

TNBC and BLBC are also closely associated with hereditary breast and ovarian cancer syndrome caused by germline mutations in the *BRCA1/2* genes [9, 10]. *BRCA1* and *BRCA2* are involved in double-stranded DNA damage repair and play an essential role in DNA integrity. The incidence of breast cancer in individuals with *BRCA1/2* germline mutations is 20-30 times higher than those without the mutations, and 75% of breast cancer patients with *BRCA1* mutation turned out to have TNBC [11]. Recently, inhibitors against poly (ADP-ribose) polymerase (PARP) have been shown to induce synthetic lethality in *BRCA*-deficient ovarian and breast cancers. One PARP inhibitor, olaparib, is approved for the treatment of *BRCA* mutated

advanced ovarian cancer. Several other PARP inhibitors are being tested in clinical trials and are expected to receive approval for treatment for patients with TNBC and BLBC.

EGFR in human cancers

EGFR is a receptor tyrosine kinase (RTK) that belongs to the ErbB family, and a transmembrane protein comprising an extracellular ligand binding domain, transmembrane domain, and cytoplasmic tyrosine kinase domain [12-14]. When a ligand binds to the extracellular region of EGFR, the receptor forms a dimer, turning on its kinase activity, followed by autophosphorylation at multiple tyrosine residues in the intracellular region to recruit various substrates. The receptor activation promotes cell proliferation, motility, and survival via activation of various downstream signaling pathways, such as Ras-Raf-MEK-ERK, PI3K-AKT-mTOR, and Src-STAT3 [15]. Ligand-activated EGFR molecules are then ubiquitinated, internalized, and isolated in endosomes. There are two major pathways of internalized EGFR, lysosome-mediated degradation pathway, which transports EGFR to the lysosomes for degradation, and receptor recycling pathway, which sorts EGFR to cell surface again [14, 15]. EGFR is also known to translocate into the nucleus, where it is involved in transcriptional regulation, DNA replication, and DNA repair [16]. The *EGFR* gene is frequently mutated or overexpressed lung, colon, head and neck, brain, pancreatic, and breast cancers and promotes tumor progression and drug resistance in these cancers [17-20]. Therefore, EGFR is an attractive drug target, and the inhibitors of EGFR, including TKIs and mAbs, have been developed and some are currently used in the clinic.

Overexpression of EGFR in cancer is partly due to gene amplification [21], but the underlying mechanisms are not yet fully understood. It has been reported that EGFR degradation through endocytosis is critical for upregulation of EGFR protein in some types of cancer cells, including breast cancer cells [22, 23]. Moreover, inhibition of *BRCA1* has been shown to induce upregulation of EGFR mRNA and protein in breast and ovarian cancer cells [24, 25] although the molecular mechanisms are still uncertain. Because TNBC is closely associated with *BRCA*-mutant breast cancer, the *BRCA1*-mediated

Anti-EGFR therapies in TNBC

Table 1. EGFR protein expression, gene mutations, and copy number in TNBC

Year	Authors	Country	Patient Number	Methods	Results
2007	Rakha et al. [29]	UK	282	IHC (Novocastra)	Positive: 37%
2008	Tan et al. [30]	China	31	IHC (Zymed)	Positive: 52%
2008	Toyama et al. [44]	Japan	110	IHC (PhamaDx) qPCR (TaqMan)	Positive: 31% No activating mutations
2009	Viale et al. [28]	Italy	284	IHC (Zymed)	Positive: 13%
2010	Gumuskaya et al. [35]	Turkey	62	IHC (Zymed, Novocastra) FISH (Abbot)	Positive: 61% (Zymed), 78% (Novocastra) Gene amplification: 2%
2011	Jacot et al. [41]	France	229	Direct sequencing	No activating mutations
2011	Shao et al. [38]	China	59	FISH (GP medical)	Gene amplification: 12%
2011	Teng et al. [42]	China	70	Direct sequencing	Exon 19 deletions and L858R mutation: 11%
2012	Choi et al. [27]	Korea	122	IHC (Novocastra)	Positive: 13%
2012	Liu et al. [33]	China	287	IHC (Dako)	Positive: 36%
2012	Meseure et al. [32]	France	18	IHC (PharmDx)	Positive: 72%
2012	Grob et al. [36]	Germany	65	FISH (Abbott) Direct sequencing	Gene amplification: 2% No activating mutations
2012	Martin et al. [31]	Switzerland	38	IHC (PharmDx) FISH (Abbot) Direct sequencing	Positive: 76% Gene amplification: 24% No activating mutations
2012	Lv et al. [43]	China	13	qPCR (TaqMan)	L858R mutation: 7.7%
2012	Santarpia et al. [39]	USA	267※	Sequenom technology	L858R mutation: 2.6%
2014	Nakajima et al. [45]	Japan	84	IHC (Ventana) DISH (Ventana) PCR (DNAFORM)	Positive: 33% No gene amplifications No activating mutations
2014	Park et al. [34]	Korea	151	IHC (PharmDx) FISH (Abbot) Direct sequencing	High EGFR copy number: 64% Gene amplification: 2% High polysomy: 31% L858R mutation: 2%
2014	Tilch et al. [40]	Australia	107	OncoCarta Assay Panel	No activating mutations
2015	Cao et al. [46]	China	50	ARMS assay	No activating mutations
2015	Bemanian et al. [48]	Norway	17	Direct sequencing	T790M mutation: 12%

Abbreviations: TNBC, triple-negative breast cancer; IHC, Immunohistochemistry; FISH, fluorescence in situ hybridization; DISH, dual-color in situ hybridization; ※ primary breast cancer patients.

upregulation of EGFR mRNA and protein may partially explain the overexpression of EGFR in TNBC.

EGFR expression and mutation in human TNBC tissues

Compared to other subtypes of breast cancer, EGFR is more frequently overexpressed in TNBC [11], and EGFR expression has been recognized as a factor of poor prognosis for TNBC [26]. EGFR expression, gene amplification, and mutation status have been broadly studied in TNBC. IHC-based interrogation of the frequency of EGFR protein expression in TNBC indicated a range between 13-76%, largely depending on the methods of the evaluation and antibodies (Table 1) [27-34]. For example, Choi et al. [27]

and Rakha et al. [29] reported a frequency of 13% and 37% of EGFR overexpression, respectively, in TNBC using an antibody from Novocastra (2+ to 3+ membranous staining in $\geq 10\%$ tumor cells for evaluation). Tan et al. reported a rate of 52% of EGFR overexpression in TNBC using an antibody from Zymed (2+ to 3+ membranous staining in $\geq 10\%$ tumor cells) [30]. Using EGFR PharmDx Kit (Dako), which is widely used to assess EGFR expression in CRC, Martin et al. have indicated a frequency of 76% of EGFR overexpression in TNBC [31]. EGFR protein overexpression has been detected in 72% of TNBC by EGFR PharmDx, but a frequency of 11% and 47%, respectively, of EGFR mRNA overexpression and normal expression was also observed in the same study [32], suggesting that EGFR protein overexpression in TNBC

is partly due to post-transcriptional regulation, such as protein stabilization or enhanced recycling. Amplification of *EGFR* gene was observed in 2-24% of TNBC patient tumor tissue samples whereas high polysomy of *EGFR* gene was reported to be between 8-27% (Table 1) [31, 35-38]. Therefore, gene amplification only partly accounts for expression of EGFR protein.

Many studies have been conducted to investigate the activating mutations in *EGFR* genes, but the results are controversial (Table 1). Santarpia *et al.* have documented that the EGFR-activating mutation (L858R) exists in 3.4% of TNBC (4 out of 116) [39]. However, several reports from Europe and Australia indicated that no activating mutations were identified in TNBC patient samples [31, 40, 41]. In contrast with the European and Australian studies, report from Asia by Teng *et al.* indicated the presence of activating mutations (4 of exon 19 deletion and 1 of L858R out of 70 TNBC samples) in a patient cohort in Singapore [42]. Furthermore, Lv *et al.* reported that two activating mutations, exon 19 deletion and L858R mutation, were detected in all tumor samples from a breast cancer patient cohort (N=143) in China [43] while several studies did not find any activating mutations in EGFR in Japanese and Chinese cohorts [44-46]. It is well known that the incidence of EGFR activating mutations in NSCLC is significantly higher in Asian population [47]. Although some studies have indicated that the incidence of EGFR mutations in TNBC may be related to ethnicity, it is clear that more studies will be required to demonstrate the significance of ethnicity in EGFR-activating mutation(s) in TNBC. A recent Norwegian breast cancer study revealed a 12% frequency (2 out of 17) of the T790M mutation in the tumors of TNBC patients [48], but because these tumors do not have primary activating mutations, there may not be any clinical significance.

Anti-EGFR therapies for treatment of various cancers

Specific anti-EGFR agents currently used in clinic include TKIs for NSCLC (gefitinib, erlotinib, afatinib, and osimertinib) and pancreatic cancer (erlotinib) as well as mAbs for CRC (cetuximab and panitumumab), head and neck squamous cell carcinoma (HNSCC) (cetuximab),

and squamous cell lung cancer (necitumumab). EGFR TKIs bind to the ATP binding site of the EGFR tyrosine kinase to compete with ATP, thereby inhibiting EGFR kinase activity [49]. EGFR TKIs are currently approved for and highly effective against NSCLC with EGFR-activating mutations, e.g., exon 19 deletion and L858R mutation [50, 51]. Although some patients with NSCLC with wild type *EGFR* gene amplification and wild type *KRAS* also respond to EGFR TKIs, the TKIs have yet to receive approval for those patient [52, 53]. NSCLC patients with EGFR mutations dramatically respond to initial TKIs treatment but most patients eventually develop acquired resistance after long-term treatment. Consequently, understanding the mechanisms of EGFR TKI resistance in NSCLC is an important subject being studied extensively in preclinical and clinical studies. About half of acquired resistance to gefitinib and erlotinib is attributed to a secondary EGFR mutation in exon 20, T790M. A third-generation EGFR TKI, osimertinib, has demonstrated efficacy against EGFR with primary exon 19 deletion or L858R and secondary T790M mutations. Nonetheless, more recent studies have identified yet another mutation (C797S) contributing to the resistance of NSCLC to osimertinib [54]. A newer generation TKI was recently reported to inhibit EGFR with T790M and C797S mutations in NSCLC [55]. In addition to secondary mutations, there are many possible mechanisms of resistance that have been reported, including activation of alternative receptor kinases (c-Met, HER2, FGF and Axl overexpression), activation of downstream bypass signaling pathways (*BRAF* mutation, *PTEN* loss, NF- κ B activation, etc.), and phenotypic changes (small-cell lung cancer transformation and epithelial-to-mesenchymal transition) [56-64]. Although these mechanisms partially explain TKI-resistance, the clinical significance of some of these mechanisms is not well established. Since activating mutation of EGFR in breast cancer is rare, it is uncertain whether some of the above-mentioned mechanisms in NSCLC are involved in the failure of clinical trials of TKI in TNBC.

EGFR mAbs bind to the ligand binding site on the cell surface EGFR in such a manner to compete with EGFR ligands, thereby inhibiting EGFR activation and dimerization [65]. Following antibody binding, cell surface EGFR undergoes

Anti-EGFR therapies in TNBC

Table 2. Clinical studies of EGFR inhibitors in TNBC

Year	Author	Phase	Regimen	Outcome	Patient Number and Population
2012	Bernsdorf et al. [88]	II	NAC: EC vs EC + Gefinitib	ORR: No significance	ER negative early BC (n=181)
2012	Schuler et al. [90]	II	Afanitib alone	ORR: 0%	Metastatic TNBC (n=29)
2013	Layman et al. [87]	I	Bendamustine and erlotinib	ORR: 9%	Metastatic TNBC (n=11)
2012	Carey et al. [91]	II	Carboplatin vs Carboplatin + Cetuximab	ORR: 6% (Carb), 16% (Carb + cetux), TTP - 2.1 month	Stage IV TNBC (n=102)
2013	Baselga et al. [92]	II	Cisplatin vs Cisplatin + Cetuximab	ORR: 10% (cis), 20% (cis + cetux) P=0.032	Metastatic TNBC (n=115)
2014	Nabholtz et al. [95]	II	NAC: EFC100 + DOC100 + Panitumumab	pCR rate 47%	Operable TNBC (n=60)
2015	Tredan et al. [93]	II	Ixabepilone vs Ixabepilone + Cetuximab	ORR: No significance	Advanced/Metastatic TNBC (n=79)
2016	Nabholtz et al. [96]	II	NAC: Docetaxel + Cetuximab	pCR: 24%	Operable TNBC (n=33)
2016	Crozier et al. [94]	II	Irinotecane + Cetuximab	ORR: 11%	Metastatic TNBC (n=19)

Abbreviations: EGFR: epidermal growth factor receptor; NAC: neoadjuvant chemotherapy; EC: epirubicin and cyclophosphamide; EFC: epirubicin, fluorouracil and cyclophosphamide; DOC: docetaxel; ORR: overall response rate; TTP: time to progression; pCR: pathological complete response; PFS: progression-free survival; ER: estrogen receptor; BC: breast cancer; TNBC: triple negative breast cancer.

Anti-EGFR therapies in TNBC

Table 3. Ongoing clinical trials of EGFR inhibitors in breast cancer

Phase	Intervention	Target	NCT Number	Patient Number and Disease
I	Metformin Erlotinib	AMPK etc. TKI (1st)	NCT01650506	8 (TNBC)
II	Paclitaxel Afatinib	Chemo TKI (2nd)	NCT02511847	40 (TNBC)
II	Icotinib	EGFR TKI	NCT02362230	67 (TNBC)
II	Nab-paclitaxel Erlotinib Bevacizumab	Chemo EGFR TKI VEGF mAb	NCT00733408	63 (TNBC)
II	Ixabepilone Cetuximab	Chemo EGFR mAb	NCT01097642	40 (TNBC)
II	Nab-paclitaxel Carboplatin 5-Fluorouracil Epirubicin Cyclophosphamide Panitumumab	Chemo Chemo Chemo Chemo Chemo EGFR mAb	NCT01036087	40 (IBC, include TNBC)
II	Docetaxel Capecitabine Nimotuzumab	Chemo Chemo EGFR mAb	NCT01939054	90 (TNBC)

Abbreviations: EGFR, epidermal growth factor receptor; Chemo, Chemotherapy; TKI, tyrosine kinase inhibitor; mAb, monoclonal antibody; mTORi, mammalian target of rapamycin inhibitor; VEGF, vascular endothelial growth factor; NCT, national clinical trial; TNBC, triple negative breast cancer; IBC, inflammatory breast cancer.

internalization and subsequent degradation [66]. Interestingly, in addition to inhibition of EGFR signaling pathway, EGFR mAbs have been shown to induce antibody-mediated immune response to cancer cells, such as antibody-dependent cell-mediated cytotoxicity and T-cell-mediated immune response, which are essential in the efficacy of the EGFR mAb therapy [67, 68]. Aside from EGFR-specific inhibitors, EGFR/HER2 dual kinase inhibitor lapatinib and VEGFR/EGFR/RET inhibitor vandetanib are also being used to treat patients with HER2 positive breast cancer and medullary thyroid cancer, respectively.

The mechanisms underlying EGFR mAb resistance have been widely studied in CRC. Mutant RAS, which is able to bypass the EGFR signaling pathway, is reported to be the primary mechanism driving resistance to EGFR mAbs. Consequently, EGFR mAbs are only used to treat CRC patients with wild type *KRAS* and *NRAS* tumors [69]. In addition to *RAS* mutations, various intrinsic resistance mechanisms have been reported, including activation of EGFR downstream signaling by activating *BRAF* and *PIC3CA* mutation, and *PTEN* deletion, as well as alternative receptor activation, such as

HER2 and c-Met [70-73]. Furthermore, multiple mechanisms of acquired resistance, such as acquired mutations in *KRAS*, *NRAS*, and *BRAF*, and amplification of *HER2* and *c-Met* genes have been reported [73-76]. Besides upregulation of EGFR downstream signaling, the mechanisms that affect mAb binding to EGFR have been identified, including the S492R mutation and arginine methylation (R198/200) in extracellular domain of EGFR [77, 78]. As for predictive factors for therapeutic efficacy of EGFR mAb, so far, the status of *RAS* mutation is the only one used in the clinic. However, among nonresponders to

EGFR mAb, only 30-40% of patients with CRC have *KRAS* mutations, suggesting that other mechanisms also play an important role in cetuximab response, and that identification of additional predictive markers is urgently needed. Although several clinical studies shown that increased *EGFR* gene copy number correlates with the clinical outcome of EGFR mAb therapy in CRC patients, the significance of *EGFR* gene copy number in the efficacy of EGFR mAb therapy remains controversial [79-81]. In TNBC patients, mutations in *KRAS* gene are rare (0-7.7%) [36, 40, 82, 83], suggesting that other mechanisms may be involved in EGFR mAb sensitivity.

Anti-EGFR therapies in breast cancer clinical trials

Several clinical trials investigating the toxicity and efficacy of TKIs in breast cancer have been conducted, but the results thus far have been disappointing for both TKI monotherapy and in combination with chemotherapy (Table 2). Outcomes in phase II clinical trials of gefitinib and erlotinib as a monotherapy in metastatic and recurrent breast cancer demonstrated only a partial response (PR) of 0-3% [84-86]. The

phase I clinical trial of erlotinib combined with chemotherapy (Bendamustine) for stage III and IV TNBC patients was conducted with results indicating an objective response rate (ORR) of 0% but with severe lymphopenia as an adverse effect [87]. A clinical trial to test the additive effect of gefitinib in preoperative chemotherapy in ER-negative breast cancer patients reported no significant difference in pathologic complete response (pCR) between patients treated with gefitinib (17%, 7 out of 41 patients) and placebo (12%, 5 out of 41 patients). Interestingly, however, a significant difference between TNBC and non-TNBC ($P=0.03$) was observed for gefitinib [88]. In a phase II trial of lapatinib monotherapy for patients with recurrent and anthracycline-refractory inflammatory breast cancer (IBC), the response rate was 50% among the 30 patients with HER2-positive tumors but only 7% among the 15 patients with HER2-negative IBC [89]. In a phase II trial with second-generation irreversible EGFR TKI afatinib in patients with metastatic TNBC, no objective responses were observed [90]. Currently, five EGFR-TKI clinical trials are ongoing in the United States, including TKI monotherapy and TKI in combination with chemotherapy as well as the combination of mTOR inhibitor, AMPK activator (Metformin), or an anti-VEGF mAb with chemotherapy.

To date, 6 phase II clinical trials to investigate the efficacy and safety of anti-EGFR mAbs in patients with TNBC have been reported (**Table 2**). Carey *et al.* compared cetuximab and cetuximab plus carboplatin in a metastatic advanced recurrent breast cancer clinical trial [91]. The response rate (RR) was 6% with cetuximab alone, and 16% with cetuximab plus carboplatin. During the course of this study [91], a repeat biopsy at the tumor sites was carried out in 16 patients 1 week after the initiation of the treatment; EGFR activation was detected in tumor specimens from 13 patients, and the treatment-mediated inhibition of the EGFR pathway was observed in 5 of these patients. However, the PR outcome was obtained in only one treated patient. Meanwhile, because cetuximab failed to inhibit EGFR signaling in 72% (13 of 18) of the patients, the authors of the study suggested that an alternative pathway may be present. Baselga *et al.* reported that an RR of 20% in the cisplatin-cetuximab combination group and an RR of 10% in the cisplatin alone group in a clinical trial for advanced TNBC [92].

However, no statistically significant difference was found between the two treatment groups. Progression-free survival (PFS) was 1.5 months for the cisplatin alone group and 3.7 months for the cisplatin plus cetuximab group, showing an extension of 2.2 months. Similarly, Tredan *et al.* conducted a phase II trial of ixabepilone alone and ixabepilone plus cetuximab in patients with advanced/metastatic TNBC with results indicating no improvement in the RR or PFS [93]. Meanwhile, a slightly higher response rate to irinotecan and cetuximab was reported by Croziert *et al.* in patients with TNBC compared to one with other subtypes of breast cancer but the difference was not of statistical significance (TNBC 18% vs non-TNBC 0%; $P=0.49$) [94].

Two studies have investigated EGFR mAbs in neoadjuvant setting in operable TNBC patients [95, 96]. One study examined the standard FEC (5-fluorouracil, epirubicin, and cyclophosphamide) therapy and preoperative chemotherapy with docetaxel combined with panitumumab in TNBC patients, and the other cetuximab combined with docetaxel. Both of those single-arm studies reported modest activities of the therapies administered [95, 96]. Interestingly, the studies also showed that high CD8⁺ tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment can predict response to EGFR mAb neoadjuvant therapy. Although it has been known that the status of TILs is a general prognostic factor in cancer, including TNBC [97], it may be particularly critical for EGFR mAb therapies since T-cell-mediated immune response plays an essential role in the efficacy of the EGFR mAb therapy [67]. Overall, in TNBC, the outcomes of clinical trials of EGFR mAbs seem slightly better than the ones of EGFR TKIs. In addition to the above-mentioned trials, there are currently 3 ongoing clinical trials evaluating anti EGFR-mAbs combined with chemotherapy in TNBC (**Table 3**).

Conclusions and future directions

TNBC patients initially respond to conventional chemotherapy, but the disease frequently relapses and leads to worse outcome than patients with other subtypes of breast cancer. Therefore, effective therapeutic strategies for TNBC are urgently needed. A significant number of TNBC is associated with EGFR overexpression, and EGFR-targeted therapies, includ-

ing TKIs and mAbs, are already available to treat various types of cancers, making EGFR inhibitors attractive options for treatment of TNBC patients. While results from clinical trials of EGFR TKI have been disappointing for breast cancer, a small portion of patients may still respond to EGFR inhibitors. It is unfortunate that some breast cancer patients who actually respond to EGFR inhibitors cannot benefit from the therapies already used in clinic due to the low overall response rate of the entire population of breast cancer patients in the clinical trials. Thus, it would be crucial to identify the sub-population of EGFR-overexpressing breast cancer patients that will respond to treatment with EGFR inhibitors so that they can benefit immediately from existing drugs without waiting for development of new drugs that usually takes over a decade.

One plausible reason for the lack of response to current targeted therapies is that most TNBCs are not exclusively dependent on EGFR signaling for their survival. EGFR TKIs are effective for NSCLC with activating mutations in EGFR, whose survival is largely dependent on EGFR. Although EGFR activating mutations in TNBC are rare, these tumors with EGFR activating mutations may still respond to EGFR TKIs via the aforementioned mechanisms of resistance for EGFR inhibitors other than secondary mutations, including activation of alternative receptors and pathways. Because some patients with NSCLC with wild type *EGFR* gene amplification and wild type *KRAS* demonstrate response to EGFR TKIs [52, 53], these alternative resistant pathways may need to be blocked in wild type EGFR-overexpressing TNBC to increase efficacy. Identification of the biomarkers that are associated with the mechanisms of resistance and potential combination of EGFR TKIs and other inhibitors that attenuate these mechanisms are required.

For anti-EGFR mAbs, certain levels of therapeutic effects have been reported in some studies, but the efficacy has not been satisfactory. Similar to TKIs, it is necessary to dissect the potential pathways associated with intrinsic resistance to EGFR mAbs. Recently, we have reported that extracellular domain of EGFR is methylated by protein arginine methyltransferase 1 (PRMT1), and that EGFR methylation is involved in mAb resistance in CRC [77]. It would

be of interest to determine whether EGFR methylation also plays a role in primary resistance to EGFR mAbs in TNBC. If so, EGFR methylation may help stratify patients to maximize response to EGFR mAb. Inhibitors against PRMT1 are currently under development [98], and the combination of PRMT1 inhibitors and EGFR mAbs may be the effective therapeutic strategy for TNBC with high levels of EGFR methylation.

In addition, EGFR inhibitors may be combined other types of targeted drugs. Currently, the most promising clinical target for TNBC is PARP. Inhibitors against PARP are known to induce synthetic lethal effects in cancer cells with *BRCA* mutations [99]. There are multiple PARP inhibitors that are in clinical trials, and one PARP inhibitor, olaparib, has been approved for the treatment of *BRCA*-deficient metastatic ovarian cancer. Interestingly, inhibition of EGFR was reported to sensitize TNBC and HNSCC cells to PARP inhibitors [100, 101]. Moreover, we recently demonstrated that PARP is directly phosphorylated by c-Met kinase, and that the PARP phosphorylation is involved in PARP inhibitor resistance [102]. Thus, EGFR may also be involved in PARP inhibitor resistance in TNBC, and the combination of EGFR inhibitors and PARP inhibitors may be an effective therapeutic strategy against TNBC.

Finally, immune checkpoint inhibitors such as PD-1 and CTLA-4 antibodies have drawn considerable attention in cancer treatment development due to the outstanding efficacy in some cancers, such as melanoma and lung cancer [103]. There are multiple trials of immune checkpoint inhibitors in TNBC ongoing. Given that T-cell-mediated immune response is associated with EGFR mAb efficacy [68], the combination of EGFR mAb and immune checkpoint inhibitors may be promising therapeutic approaches for TNBC with high *EGFR* copy numbers.

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