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Cell-specific biomarkers and targeted biopharmaceuticals for breast cancer treatment

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Abstract

Breast cancer is the second leading cause of cancer death among women, and its related treatment has been attracting significant attention over the past decades. Among the various treatments, targeted therapy has shown great promise as a precision treatment, by binding to cancer cell-specific biomarkers. So far, great achievements have been made in targeted therapy of breast cancer. In this review, we first discuss cell-specific biomarkers, which are not only useful for classification of breast cancer subtyping but also can be utilized as goals for targeted therapy. Then, the innovative and generic-targeted biopharmaceuticals for breast cancer, including monoclonal antibodies, non-antibody proteins and small molecule drugs, are reviewed. Finally, we provide our outlook on future developments of biopharmaceuticals, and provide solutions to problems in this field.

1 | INTRODUCTION

Breast cancer is the most common cancer among women in the world, which is reported to be a heterogeneous tumour with well-defined molecular subtypes.¹ In 2015, an estimated 231 840 new cases of invasive breast cancer were diagnosed in women in the United States, along with 40 290 new cases of breast cancer death.² In China, breast cancer has also become a major health problem of women especially those living in urban areas with an overall morbidity of 24.20 per 100 000.^{3–6} Furthermore, the incidence of breast cancer is unfortunately increasing. Despite the extraordinary progress in understanding the molecular mechanisms underlying carcinogenesis, tumour promotion, and mammographic screening for the early detection and treatment of breast cancer, the global mortality of breast cancer patients is still unacceptably high, and the 5-year survival rate of metastatic breast cancer (stage IV) is below 15%.⁵

So far, the common breast cancer treatment is surgery supplemented with radiation therapy, various forms of systemic adjuvant chemotherapy, and hormonal therapy, which may also kill the normal cells as a side effect. Targeted therapy has shown great promise as a precision cancer therapy by binding to cancer cell-specific biomarkers.

In comparison with the traditional medicine, the targeted therapy for breast cancer seems to be more effective and lower toxic. For the development of such targeted therapy, it is necessary to investigate the biology of breast cancer cells and especially understand the cell-specific biomarkers expression and tumour subtyping in advance. Nowadays, many efforts have been made to study the genes, proteins, metabolites, and signalling pathways, which are related to the growth and metastasis of breast cancer.⁷ Several cell-specific biomarkers of breast cancer cells, such as oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor (VEGF), and Ki-67, have been thus discovered.^{8–11} These cell-specific biomarkers can not only be utilized as targets for novel drugs delivery but also be used for early detection and classification of breast cancer, which will greatly accelerate the development of the targeted therapies for breast cancer.^{8–11}

A biopharmaceutical, also known as a biological medical product, biological or biologic, is any medicinal product obtained from biological sources. Different from chemically synthesized pharmaceuticals, they possess high affinity, specificity, selectivity, low toxicity as well as perfect efficacy. Since the first approval of trastuzumab by the United States Food and Drug Administration (FDA) in 1998,

TABLE 1 The currently approved and emerging biopharmaceuticals for breast cancer targeted therapy

Name	Target	Type	Mechanism of action	References
Trastuzumab	HER2	Humanized IgG1	Triggers HER2 internalization and degradation, and inhibits HER2-related cell signalling; ADCC.	20–25
Pertuzumab	HER2	Humanized IgG1	Inhibits the heterodimerization of HER2 with other HER family, and hinders HER2-related cell signalling; ADCC.	26–30
T-DM1	HER2	Humanized IgG1	Targets intracellular delivery of cytotoxic emtansine; Triggers HER2 internalization and degradation; Inhibits HER2-related cell signalling.	31–35
Denosumab	RANKL	Humanized IgG2	Inhibits the receptor RANK on osteoclasts and blocks the downstream signalling pathways of breast cancer-mediated bone diseases.	36–38
Ertumaxomab	HER2 and CD3	Murine and rat biospecific IgG2	ADCC and ADCP	39–41
Bevacizumab	VEGF	Humanized IgG1	Inhibits VEGF-mediated angiogenesis	42–46
Ramucirumab	VEGFR2	Humanized IgG1	Inhibits VEGFR2-mediated angiogenesis	47,48
Aflibercept	VEGF	Recombinant peptide-antibody fusion protein	Inhibits VEGF-mediated angiogenesis	49–52

T-DM1: Ado-trastuzumab emtansine; RANKL: Receptor activator of NF- κ B ligand; TKI: Tyrosine kinase inhibitor; ADCC: Antibody-dependent cell-mediated cytotoxicity; ADCP: Antibody-dependent cellular phagocytosis.

several biopharmaceuticals, including monoclonal antibodies (mAbs), non-antibody proteins and other small molecules, have been extensively developed to target specific antigens during cancer progression.^{12–19} Table 1 lists the innovative biopharmaceuticals currently approved by the FDA and the European Medicine Evaluation Agency for the treatment of breast cancer.

In the past decades, targeted therapy of breast cancer was one of the hottest areas of cancer research, and hundreds of articles, related to cell-specific biomarkers or therapies, have been published. Although rich and colourful reviews are available on both cell-specific biomarkers and correlated biopharmaceuticals for the targeted therapy of breast cancer, they have been seldom discussed together as one topic. To better understand the development of targeted therapy in breast cancer, in this article, we first discuss the cell-specific biomarkers for biopharmaceuticals targeting, then review the innovative and generic-targeted biopharmaceuticals for breast cancer treatment, and finally make an outlook of the future development of targeted biopharmaceuticals for breast cancer as well as provide the solutions to the problems in this field.

2 | CELL-SPECIFIC BIOMARKERS FOR BREAST CANCER SUBTYPING AND TARGETED BIOPHARMACEUTICALS DELIVERY

Cell-specific biomarkers refer to specific molecules that are expressed or overexpressed on particular cancer cells, which provide the foundation of breast cancer detection, classification, risk assessment, and treatment response prediction and also play an important role in targeted approaches in both primary and metastatic breast cancer therapy.⁵³ In order to achieve personalized therapy, prior

knowledge of breast cancer subtyping based on cell-specific biomarkers is required. Currently, the methods for the determination of cell-specific biomarkers mainly consist of immunohistochemistry, enzyme-linked immunosorbent assay, polymerase chain reaction and fluorescence in situ hybridization.^{54–62} After years of research and testing, several cell-specific biomarkers, including ER, PR and HER2, have been discovered and further used as gold standard biomarkers for both breast cancer classification and targeted therapy. Based on above three cell-specific biomarkers, breast cancer can be classified into four major intrinsic subtypes: Luminal A, Luminal B, HER2-overexpressed and basal-like.^{53,63–66} The treatments corresponding to four different breast cancer molecular subtypes are showed in the Table 2. Additionally, VEGF, which plays a very important role in cancer angiogenesis, can also be regarded as a cell-specific target for breast cancer treatment. However, owing to the molecular heterogeneity and considerable histopathology, the molecular fingerprints for breast cancer classification are still unaccomplished, and new cell-specific tumour biomarkers for both breast cancer classification and targeted therapy need to be discovered.

2.1 | ER and PR

Oestrogen receptor and PR are expressed in the cell nuclei of about 60%–70% breast cancer cells.^{63–65} ER, which was first identified in the late 1960s, plays an important role in breast cancer progression.⁶⁷ ER-positive breast cancer is thought to be the best variant, with effective strategies to block, downgrade or deprive the ER of its efficacy, when compared with other subtypes.⁶⁸ As a nuclear transcription factor, ER participates at the regulation of transcriptional activity of breast tissues, which is termed nuclear-initiated steroid signalling. When ER interacts with oestrogen, they

TABLE 2 Immunohistochemical criteria for breast cancer intrinsic subtyping and corresponding treatment^{53,63–66}

Subtype	ER	PR	HER2	Treatment
Luminal A	Either ER or PR positive		Negative	Endocrine therapy
Luminal B	Either ER or PR positive		Positive	Endocrine therapy + anti-HER2 therapy + Chemotherapy
HER2 overexpressed	Negative	Negative	Positive	Anti-HER2 therapy + Chemotherapy
Basal-like	Negative	Negative	Negative	Chemotherapy

ER: Oestrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2.

will form a co-regulator complex, and regulate transcription of specific genes, such as PR, TFF1, GREB1 and PDZK1.^{68–70} There are two isoforms of ER: ER α and ER β . Normally, the expression of ER α is essential for the development of normal mammary gland, while a prominent expression is often observed in breast carcinoma sites.^{67,68} ER β is highly homologous to ER α both in the DNA-binding domain and ligand-binding domain. However, for both ER α -positive breast cancer and ER α -negative one, ER β can be observed, which plays a key role in breast cancer classification and endocrine therapy.⁶⁷

Progesterone receptor, which regulates ER expression, is another important hormone receptor biomarker in breast cancer classification and targeted therapy. Similar to ER, it also exists in two kinds of forms: PRA and PRB.⁶⁷ PR plays a very important role in the lobuloalveolar development of breast. However, an overexpression of PRA always means the elevated risk of breast cancer.⁶⁸ So PR is usually used as an indicator of nuclear ER pathway and tumour aggression and serves as an instruction for endocrine therapy.⁷¹ Furthermore, breast cancer with a remarkable overexpression of PR is always less aggressive compared with PR-negative breast cancer.^{68,71}

2.2 | HER2

Human epidermal growth factor receptor 2 is a 185 kDa transmembrane protein belonging to the ErbB family of receptor tyrosine kinases.^{72–75} It can regulate a series of cellular processes such as cell proliferation, differentiation and survival.^{21,76} As a perfect predictive biomarker associated with anti-HER2 biopharmaceutical targeted therapies, HER2 protein is overexpressed in approximately 20%–25% of all invasive breast cancer patients.^{21,77,78} Consequently, a couple of anti-HER2 biopharmaceuticals, such as trastuzumab,^{20,79} pertuzumab²⁸ and ado-trastuzumab emtansine (T-DM1), have been developed and commercialized.^{26,80} The detail mechanisms of these HER2-targeted biopharmaceuticals are displayed in Fig. 1.²⁶ Moreover, it is noted that the overexpression of HER2 protein is much due to gene mutation of HER2, which happens in about 1%–2% of breast cancer.⁸¹

Due to the fact that HER2 status is an important biomarker in guiding personalized HER2 therapy, it is of significance to accurately determine its status in certain breast cancer tissues. In clinical treatment, HER2 targeted therapy should be recommended only when the HER2 test result is positive. The HER2-positive criteria by immunohistochemistry is 3+ based on complete circumferential membrane

staining or the HER2/CEP17 ratio ≥ 2.0 by fluorescence in situ hybridization.⁸² However, these two techniques can give false-positive or false-negative results to some extent. Therefore, new methods for clinical evaluation of HER2 expression should be developed.

2.3 | VEGF pathways

Vascular endothelial growth factor and its correlated signalling pathways play key roles in tumour angiogenesis.⁴⁴ The VEGF family consists of several polypeptides, namely VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF, among which VEGF-A is not only an endothelial growth factor but also a regulator of vascular permeability.^{44,83,84} VEGF-A always interacts with VEGFR1 or VEGFR2, two transmembrane receptor tyrosine kinases (RTKs), to promote endothelial cells migration and mitosis and withstand their apoptosis or senescence.^{84–86} The detail mechanisms of VEGF-mediated signalling pathways are as follows: when VEGF-A interacts with VEGFR2, the VEGFR2 PTKs site will autophosphorylate, which produces inositol 3,4,5-trisphosphate and diacylglycerol. They are two kinds of second messenger molecules that can activate the protein kinase C and the extracellular signal-regulated kinase pathway, which eventually result in endothelial cells multiplication, migration and invasion.^{85–87} VEGF-mediated angiogenesis can not only affect reproduction, embryogenesis and skeletal growth in the physiology conditions but also seriously influence pathological angiogenesis. VEGF-targeted biopharmaceuticals are thus demonstrated to decrease tumour capillary density and suppress tumour growth by specifically inhibiting VEGF-mediated signal transduction pathway.^{88–91}

Due to the fact that angiogenesis plays a very important role in the progression of breast cancer, the development of VEGF-targeted biopharmaceuticals has been attracting significant attention from scientists and clinic doctors. Currently, there are a couple of VEGF-targeted biopharmaceuticals under investigation for breast cancer treatment, including bevacizumab,⁹² ramucirumab⁹³ and aflibercept.^{86,94} The detail mechanisms of such VEGF-targeted biopharmaceuticals are illustrated in Fig. 2.⁸⁶ Moreover, other potential VEGF-targeted biopharmaceuticals for breast cancer are also being investigated, and more tests need to be done to evaluate their effects. Most recently, the potential synergistic therapies between VEGF-targeted biopharmaceuticals-based therapy and other forms of treatment are in full swing, which holds new promise for breast cancer therapy and prognosis.

FIGURE 1 Molecular mechanisms of novel biopharmaceuticals for HER2-targeted therapy. Reprinted with permission from reference 26. Copyright© Nature Publishing Group

2.4 | Other cell-specific biomarkers for breast cancer targeted therapy

Besides the above biomarkers and pathways, other biomarkers, such as HER3,⁹⁵ MUC1⁹⁶ and insulin-like growth factor receptor,⁹⁷ also have the potential for breast cancer targeted therapy. However, up to now, few biopharmaceuticals based on such biomarkers have been developed.

3 | TARGETED BIOPHARMACEUTICALS FOR BREAST CANCER TREATMENT

3.1 | Monoclonal antibodies against HER2

3.1.1 | Trastuzumab

Trastuzumab, used as the first-line treatment for HER2-positive metastatic breast cancer, is a fully humanized version of a murine HER2-targeted IgG1 monoclonal antibody with a molecular mass of 148 400 Da.^{98–101} Since it was first approved by FDA in 1998, trastuzumab has achieved great clinical success and saved thousands of lives of breast cancer patients. The functions of trastuzumab for breast cancer treatment mainly involve HER2 monoubiquitination, anti-angiogenic properties, HER2 cleavage and extracellular domain shedding, DNA repair inhibition and antibody-dependent cell-mediated cytotoxicity (ADCC).^{102–106}

Large amounts of clinical trials indicate that trastuzumab is well tolerated and can work effectively either in monotherapy or in combination with other cytotoxic agents on HER2-positive metastatic breast cancer. However, the patients initially treated with trastuzumab would suffer from an infusion reaction of either fevers or chills, which may be an obstacle to its application.^{106–109} Additionally, the development of the related drug resistance in patients would make HER2-positive breast cancer more difficult to cure. The proposed mechanisms of trastuzumab resistance mainly include the prevention of antibody-receptor interaction, phosphatase deficiency, inhibition of cell proliferation, activation of PI3K/AKT pathway, damages of insulin-like

growth factor-1 receptor (IGF-1R) signalling pathways, and sheltering of HER2 receptor or epitope by mucin 4 (MUC4).^{96,110–116} Meanwhile, both the upregulation of HER2 expression and cyclin E overexpression could lead to trastuzumab resistance as well.^{115,117} Finally, owing to the fact that the expression of HER2 in cardiac myocytes plays a significant role in both embryogenesis and myocyte repair, trastuzumab can slightly increase the risk of symptomatic cardiac events (about 4% every 4 years),¹¹⁸ which is another shortcoming of trastuzumab in breast cancer treatment.

Nowadays, combination therapy has become the hottest field in tumour treatment.¹¹⁹ At the beginning of trastuzumab treatment, the overall response rate (ORR) of HER2-positive metastatic breast cancer patients can reach 26%,^{100,120} while the combination therapy of trastuzumab with other chemical agents can reach up to approximately 50%.¹⁰⁷ The chemical agents currently suggested for the combination therapy of HER2-positive breast cancer include paclitaxel,¹²¹ docetaxel,^{122,123} carboplatin,^{124,125} capecitabine,¹²⁶ gemcitabine^{127,128} and cisplatin.¹²⁹

3.1.2 | Pertuzumab

Pertuzumab is a humanized IgG1 monoclonal antibody that binds to the extracellular domain II of HER2. It is the new class of HER family (HER1, HER2 and HER3) dimerization and ligand-activated signalling inhibitor.^{28,130} The mechanism of pertuzumab for breast cancer therapy is that pertuzumab can specifically bind with the cysteine-rich region of HER2 extracellular domain II and sterically block the dimerization of HER2 with HER1, HER3 or HER4, which leads to the inhibition of intracellular signalling pathway and further preventing the proliferation of tumour cells.^{131–134}

Different from trastuzumab, pertuzumab binds to the domain II of HER2 receptor.^{135,136} Therefore, the combined therapy of trastuzumab and pertuzumab will block the HER2-mediated signal transduction more completely by receptor crosstalk.^{132,137} To date, pertuzumab has been assessed both in monotherapy and in combination therapy with trastuzumab for HER2-positive breast cancer treatment.^{28,122,136–139} Clinical trials indicate that combination therapy of

FIGURE 2 Schematic of novel anti-angiogenesis biopharmaceuticals targeting VEGF pathways in breast cancer. Reprinted with permission from reference 86. Copyright@ Informa Healthcare

trastuzumab and pertuzumab synergistically suppresses the growth of HER2-overexpressed breast cancer cell lines.^{73,140} An anti-tumour activity study of trastuzumab and pertuzumab alone as well as in combination for HER2-positive breast cancer xenografts was conducted by Scheuer and his colleagues.¹³⁷ The results indicate that the combination therapy of trastuzumab and pertuzumab shows more robust anti-tumour effect than each agent applied separately.^{137,138} In addition, pertuzumab is also well tolerated and shows very little haematological and cardiac toxicity.

Other strategies of synergy therapy using pertuzumab in combination with other chemical agents have also been investigated.^{24,28,131} For example, the combination therapy of pertuzumab and lapatinib performed a greater tumour suppression effect than the results obtained in monotherapy.¹⁴¹ The efficacy of pertuzumab in combination with trastuzumab and docetaxel in neoadjuvant strategies for HER2-positive breast cancer treatment was evaluated in a phase II, and the results showed that a combination of three chemicals had better effect in HER2-positive breast cancer treatment than other dual combination therapies.¹⁴² Moreover, a phase III study was conducted to investigate the combination of trastuzumab and docetaxel with or without pertuzumab, and the results indicated that breast cancer patients who received pertuzumab had a greater progression-free survival (PFS) (12.4 months for control arm vs 18.5 months for pertuzumab arm, HR 0.62; 95% CI, 0.51–0.75; $P < .001$) and a stronger trend for improved survival.^{123,143,144}

3.1.3 | Ertumaxomab

Ertumaxomab is a trifunctional bispecific monoclonal antibody binding to HER2/neu and CD3. It specifically binds to Fcγ type I/III receptors to form a tri-cell complex among tumour cells, T cells and accessory cells, which eventually leads to the tumour cell death.^{145,146} It is produced by a quadroma cell line resulted from the fusion of a specific anti-CD3 rat hybridoma cell line and an anti-HER2 mouse hybridoma cell line.¹⁴⁵ The function of ertumaxomab is related to immune response such as ADCC and

antibody-dependent cellular phagocytosis.^{145,147} The combination therapy of ertumaxomab with other chemical drugs has also been studied, which indicates that ertumaxomab is a new promising monoclonal antibody for HER2-positive breast cancer targeted therapy.¹⁴⁸

3.2 | Monoclonal antibodies against VEGF

3.2.1 | Bevacizumab

Bevacizumab is a recombinant humanized IgG1 monoclonal antibody that binds and devitalizes soluble VEGF-A molecules.^{42–46} It is the first anti-VEGF drug used for the breast cancer treatment, which shows great promise in breast cancer targeted therapy. Although bevacizumab has been approved by the European Medicines Agency for the first-line treatment of metastatic breast cancer in 2007,^{149,150} there are still many controversies in utilizing bevacizumab for breast cancer treatment due to its adverse effects of hypertension, bleeding events, proteinuria and sensory neuropathy.^{151,152} Owing to the failure in demonstrating the clinical benefits of bevacizumab in combined therapy with other chemical agents, in 2011, the FDA banned the use of this drug for metastatic breast cancer treatment after its first approval in 2008.^{153,154} Fortunately, the latest research has verified the usefulness of bevacizumab in first-line standard chemotherapy for metastatic breast cancer treatment.⁴⁵

Bevacizumab was usually tested in combination with other chemotherapeutic agents or in the neoadjuvant therapy strategies,^{55,155–157} such as docetaxel,^{158,159} capecitabine^{160–162} and taxanes.¹⁶³ The results of first- and second-line trials demonstrate positive impact on the endpoints of PFS and ORR of breast cancer patients.^{164–166} The newest results of bevacizumab in combination with chemical drugs for breast cancer treatment indicate that there is a great improvement on endpoints of ORR, PFS (8.1 months vs 5.4 months), overall survival rates (39.0 months vs 23.7 months) and pathological complete response vs patients treated with chemical drugs alone.^{158,165–169}

3.2.2 | Ramucirumab

Ramucirumab is a fully humanized IgG1 monoclonal antibody, which specifically binds to the extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR-2). It can inhibit endogenous VEGF signalling of tumour angiogenesis.^{47,170} The early clinical trials of ramucirumab in various malignant tumours indicate that ramucirumab is well tolerated and produces satisfactory results in suppressing angiogenesis in about 69% of evaluated tumours.^{149,171} Similar to bevacizumab, the treatment of ramucirumab also leads to the occurrence of hypertension and venous thrombosis but such adverse effects could be alleviated by taking good care of patients.^{47,170} It should be noted that ramucirumab has been approved by FDA as the first monotherapy agent for the treatment of advanced or metastatic gastric cancer or gastroesophageal junction adenocarcinoma patients who had previously experienced disease progression on or after fluoropyrimidine- or platinum-containing chemotherapy.⁴⁸

3.3 | Antibody-drug conjugate

Antibody-drug conjugate (ADCs) is a kind of targeted drug that takes advantage of specifically binding properties of antibody and the high cytotoxicity of small molecule with the aim to improve the therapeutic efficacy of anti-cancer drugs and minimize adverse effects.^{172–174} They often consist of one monoclonal antibody as recognition part, and a linker and chemical or cytotoxic agents as functional part.

Up to now, several ADCs have been investigated in breast cancer treatment. Among them, T-DM1 is one of the most popular ADCs. In 2013, T-DM1 received FDA approval for the treatment of HER2-positive metastatic breast cancer or locally advanced breast cancer.^{31,32,175} T-DM1 is composed of trastuzumab, a stable linker (MCC), and the cytotoxic agent derivative of maytansine, emtansine (DM1), which specifically binds to HER2.^{172–177} It can reverse the trastuzumab resistance of breast cancer patients, showing great promising in the treatment of breast cancer patients who have failed on prior treatment with trastuzumab.³² T-DM1 first transports emtansine to target cells via the recognition of monoclonal antibody trastuzumab to the HER2 protein of breast cancer cells. Then it is internalized by endocytosis and degraded into trastuzumab and DM1 by the lysosomal enzyme. Eventually, the DM1 is delivered and binds to tubulin, leading to the cell cycle arrest and apoptosis by destroying the microtubules. Meanwhile, trastuzumab can also perform its properties of ADCC and inhibit HER2-mediated signalling pathways, providing a perfect double effect in breast cancer treatment.^{178–180} Several phase II studies of T-DM1 monotherapy indicate that T-DM1 will benefit metastatic breast cancer patients, who had failed at both HER2 targeted therapy and chemotherapy.^{181–183}

3.4 | Other targeted biopharmaceuticals for breast cancer treatment

With respect to the targeted biopharmaceuticals outlined above, other novel drugs such as denosumab,^{24–37} aflibercept,^{49–51} etc., also show great potential in breast cancer treatment.

3.4.1 | Denosumab

Denosumab is the newest fully human monoclonal antibody of IgG2 subtype, which specifically binds to the receptor activator of nuclear factor kappa B ligand (RANKL).^{24–37} It can inhibit the receptor RANK on osteoclasts and block the downstream signalling pathways of breast cancer-mediated bone disease.^{184–187} It was first developed for osteoporosis in postmenopausal women at increased risk of fractures and bone loss induced by hormone ablation in prostate cancer patients. Afterwards, it was also used for the treatment of breast cancer patients with bone metastases.³⁷ It had taken long time to conduct clinical trials of denosumab in breast cancer treatment before it got the FDA approval in 2010. Several randomized phase III trials of denosumab demonstrated that denosumab was non-inferiority with improved tolerability, which showed great potential for the treatment of advanced breast cancer with bone metastases.^{26–36,188–190}

3.4.2 | Aflibercept

Aflibercept is a recombinant peptide-antibody fusion protein consisting of the extracellular domains of VEGF and Fc portion of immunoglobulin G1, which shows high affinity for all ligands that bind to these receptors, including VEGF-A, VEGF-B and placental growth factors.^{49–51,191} The recent results demonstrated that aflibercept had got perfect anti-tumour effects and anti-angiogenic activity when used as a single agent or in combination therapy.^{52,192–194} Two phase I dose-escalation studies of aflibercept in patients with advanced solid tumours indicated that the dose recommended for further clinical development based on tolerability, anti-tumour activity and pharmacokinetics was 6 mg/kg.^{192,193} Notably, several phase II trials to evaluate aflibercept in patients with metastatic breast cancer treatment have been conducted.^{50,195} Further experiments should be done to certificate its activity and efficacy before it is clinically used for breast cancer targeted therapy.

4 | CHALLENGES AND STRATEGIES

In spite of significant progress towards breast cancer treatment made by targeted biopharmaceuticals in the past 20 years, breast cancer remains the second leading cause of death among women.^{2–4} Moreover, the side effects of biopharmaceuticals induced by the non-specificity of biomarkers, which cannot efficiently distinguish cancer cells and normal cells, have often been reported in their clinic utilization.¹⁹⁶ In addition, biopharmaceuticals could be degraded easily with a very short half-life under physiological conditions, making the optimal therapeutic doses larger than that of other agents, which will hugely increase the cost of treatment.^{196–198} Moreover, the present biopharmaceuticals are always heterogeneous products, which may cause serious immunological rejection response. The bioprocessing and purification technologies are another kind of obstacles that impede the development of biopharmaceuticals.^{199,200}

To circumvent those shortcomings of targeted biopharmaceuticals, all the therapeutical biopharmaceuticals should be humanized, redesigned or specifically modified, which are expected to be more tolerated and efficiently correlated with cancer targeted therapy. In order to improve the activity and affectivity of targeted biopharmaceuticals, more synergy therapies of biopharmaceuticals or in combination with cytotoxic agents should also be developed. In addition, developing optimal and innovative individual biotechnology for continuous bioprocessing and purification of biopharmaceuticals will greatly promote the industrialized production.^{201,202} These studies will certainly overcome the challenges confronted by the clinic utilization of targeted biopharmaceuticals for breast cancer treatment.

5 | CONCLUSION

Cell-specific biomarkers are the foundation of breast cancer targeted therapy. During the past years, targeted biopharmaceuticals in breast cancer treatment has been demonstrated to possess obvious advantages over traditional therapeutics due to their high affinity, specificity, selectivity, low toxicity as well as perfect efficacy. Since the first monoclonal antibody trastuzumab for the first-line HER2-positive metastatic breast cancer targeted therapy was approved by the FDA in 1998, there have been several targeted biopharmaceuticals developed for breast cancer treatment. Among all these novel biopharmaceuticals, some have been approved for clinical treatment such as trastuzumab, pertuzumab and T-DM1, while others are still under clinical research. With respect to monotherapy, trials of targeted biopharmaceuticals in combination with cytotoxic agents are growing vigorously. In order to enhance treatment efficiency, it is very imperative to figure out the occurrence, development and prognosis of breast cancer, which will benefit the discovery of highly cell-specific biomarkers of breast cancer.

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