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Monoclonal Antibody-Based Targeted Therapy in Breast Cancer

Current Status and Future Directions

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Contents

Abstract

The recent development of monoclonal antibodies targeting growth factor receptors in cancer treatment represents a milestone for both researchers and physicians. Advances in the understanding of key molecular pathways for tumour growth and survival have facilitated the development of these targeted therapies, in particular in breast cancer. This review focuses on the three most important recombinant humanised monoclonal antibodies that have shown activity in women with breast cancer: trastuzumab, pertuzumab and bevacizumab. Trastuzumab, an anti-erbB2 (human epidermal growth factor receptor) monoclonal antibody, is currently routinely used in both the metastatic and adjuvant settings for patients with erbB2-positive tumours. Pertuzumab, a monoclonal antibody binding to a different epitope on erbB2 than trastuzumab, is under early clinical evaluation. This drug has been developed for breast cancer patients, whether overexpressing erbB2 or not. Bevacizumab, a monoclonal antibody directed

against vascular endothelial growth factor-A, is being evaluated in the metastatic setting for its antiangiogenic properties, and is showing promising results.

One of the major advances in breast cancer treatment in the last decade has been the development of monoclonal antibodies targeting growth factor receptors. Indeed, traditional anticancer drugs are generally non-selective, inducing toxicity in normal as well as malignant cells. In developing novel anticancer agents, the goal is to target specific molecular abnormalities within tumour cells, leading to improved cure rates and reducing cytotoxicity in normal cells.[1] Advances in the understanding of tumour pathobiology and molecular biology have facilitated the development of these targeted therapies, in particular in breast cancer.[2] The human epidermal growth factor (EGF) receptor (HER; erbB) family are receptors with tyrosine kinase activity and are expressed in various tumours, including breast cancer. The vascular endothelial growth factor (VEGF) receptor has also emerged as a target for breast cancer treatment, as angiogenesis plays an important role in tumour growth and metastasis.[3] Antibodies directed against the extracellular domain of erbBs and against VEGF are the main topics of this review. Articles cited in this review were identified through a search using PubMed software and key words such as 'targeted therapy', 'trastuzumab' and 'pertuzumab'. In addition, the ASCO (American Society of Clinical Oncology) and the San Antonio Breast Cancer Symposium websites were searched for abstracts relevant to the topic.

1. Rationale for Targeting Growth Factors or Growth Factor Receptors

1.1 The Epidermal Growth Factor Receptor Family

The EGF receptor was the first tyrosine-kinase receptor to be linked directly to human tumours.[4,5] Activated erbBs stimulate many intracellular signalling pathways, mainly the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K)-Akt pathways.[6] In preclinical models, the treatment of tumour cells with erbB-targeted antibodies rapidly downregulates PI3K-Akt and MAPK signalling and, as a consequence, blocks the proliferation of the tumour.[7] For example, overexpression of erbB2 in 20–25% of invasive breast cancer is typically a consequence of amplification at the DNA level (evaluated by fluorescence *in situ* hybridisation [FISH]), and is associated with an increased risk of relapse and death for patients with early-stage breast cancer.[1,8] This poor prognosis is the clinical manifestation of the many biological actions of erbB2: cell growth, division, differentiation, migration, adhesion and angiogenic activity.[9-11]

In addition to directly inducing cancer cell death, monoclonal antibodies also lead to immune activation, resulting in indirect tumour cell cytotoxicity.[12-18] They also potentiate the cytotoxic effects of certain DNA-damaging chemotherapeutic agents or ionising radiation in tumour cell lines and xenografts that overexpress particular growth factor receptors.[19-25] The mechanism of this synergy involves attenuation of DNA repair activity and indicates that there is an interaction between cell surface growth factor receptor signal transduction pathways and DNA repair processes.[20,21]

1.2 The Vascular Endothelial Growth Factor (VEGF) Family

The VEGF family consists of six growth factors (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor) and three receptors, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4). The three VEGFRs are transmembrane tyrosine kinases that are predominantly found on endothelial cells. The activation of VEGFR-2 by its ligands results in enhanced permeability of the vasculature and increased migration and proliferation of endothelial cells, making it also a major target for therapy.[26,27] Elevated levels observed in solid tumours are correlated with worse

clinical outcomes.[28-30] As a result of high VEGF expression, the vasculature in tumours is chaotic and abnormal. By neutralising local VEGF, anti-VEGF agents can induce the normalisation of these vessels, resulting in a potential decrease in vascular volume and interstitial fluid pressure within the tumour, thereby allowing enhanced delivery of oxygen and cytotoxic therapies to the tumour.[31] A recent meta-analysis has shown that a high microvessel density, which is a surrogate marker of tumoural angiogenesis, predicts poor survival.[32] It has also been suggested recently that antiangiogenic treatments may be most effective in micrometastatic disease, and that anti-VEGF treatments could inhibit tumour growth most effectively at the onset of the angiogenic process.[33]

1.3 Resistance Mechanisms

It is unlikely that targeting only one alteration is sufficient to kill metastatic tumour cells. For example, for erbB2-overexpressing metastatic breast cancer, response rates of approximately 35% are observed in trastuzumab-treated patients.[34] Several theories, ranging from the existence of compensatory pathways to downstream signalling aberrations of erbB2, have been proposed to explain the suboptimal clinical results. It has been shown experimentally that trastuzumab cannot block the proliferation of tumour cells that have autocrine EGFR activation,[35] and it cannot prevent the ligand-induced formation of erbB2-containing heterodimers or the activation of downstream signalling pathways.[36,37] The fact that trastuzumab binds to domain IV of erbB2, a region not involved in receptor dimerisation,[38] explains why erbB ligands can induce the formation of erbB2-containing heterodimers in the presence of the antibody. In contrast, pertuzumab binds erbB2 near the centre of the domain II dimerisation arm,[39] thereby preventing the formation of ligand-induced erbB2-containing heterodimers.[40] This characteristic might partly explain why pertuzumab inhibits the growth of tumours that express low erbB2 levels, whereas trastuzumab does not.[37] In relation to the activation of downstream signalling pathways, it has been suggested that persistent activation of the MAPK and PI3K-Akt pathways caused by downstream signalling aberrations of the receptors might also play a role in resistance to trastuzumab, as well as to EGFR-directed inhibitors.[41,42] Consequently, to avoid these potential resistance mechanisms, logical combination strategies are awaited using agents also targeting mammalian target of rapamycin (mTOR),[43] RAF kinase[44] or insulin-like growth factor receptor (IGRF)-1.[45]

Through our increased understanding of the tumour microenvironment, the concept of targeting several key kinases has emerged. On the basis of the importance of tumour vasculature in the process of cancer growth and spread, inhibitors that block endothelial cell survival have gained in importance. Preclinical studies have demonstrated encouraging combination effects with erbB- and VEGFR-directed inhibitors in experimental animal tumour models.[46]

2. Anti-ErbB2

2.1 Trastuzumab

Trastuzumab, the first approved monoclonal antibody for erbB2-overexpressing metastatic breast cancer, provided the proof of principle that targeting specific growth factor receptors results in clinical benefit.

Trastuzumab is a recombinant humanised anti-erbB2 monoclonal antibody that binds the extracellular domain of the receptor and blocks intracellular signalling. Amplification of the erbB2 gene occurring in 20–25% of human breast cancer is an independent adverse prognostic factor;[47] it is also a predictive factor for increased response to doxorubicin- and taxane-based combination chemotherapy, as well as for decreased response to tamoxifen.[48-51]

2.1.1 Preclinical Studies

The antiproliferative activity of trastuzumab has been largely tested *in vitro* and in animal models. Both cytostatic and cytotoxic mechanisms of action of trastuzumab were identified in preclinical studies.[1] *In vitro*, downregulation of erbB2 disrupts receptor dimerisation and signalling through the

downstream PI3K cascade. Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle, with a concomitant induction of the cdk inhibitor p27kip1 and increased formation of p27kip1-cdk2 complex.[35] Trastuzumab was also shown to be antiangiogenic and to downregulate proteins involved in angiogenesis.[52] It effectively prevents cell proliferation of the erbB2-overexpressing breast cancer SK-BR-3 cell line.[23]

As trastuzumab is a monoclonal antibody that binds to the surfaces of erbB2-overexpressing cancer cells, it has been postulated that antibody-dependent cellular cytotoxicity (ADCC) may play an important role in the mechanism of action of this drug.[12-18] *In vitro* and *in vivo* xenograft studies[12-15] have demonstrated that natural killer (NK) cells are able to kill trastuzumab-coated erbB2-overexpressing cells via a FcγRIII receptor (CD16)-mediated ADCC mechanism. *In vivo* studies[16-18] have confirmed that trastuzumab in combination with interleukin-2 or chemotherapy led to NK cell expansion and NK cell-mediated ADCC against erbB2-overexpressing cells. An interesting area for future investigation could be combination therapy with trastuzumab, chemotherapy and immunomodulators.

Furthermore, *in vitro* experiments have shown synergistic and additive interactions between trastuzumab and cytotoxic agents. The interaction between trastuzumab and chemotherapy to promote apoptosis is not well understood, but it is a phenomenon that occurs both *in vitro* and *in vivo*. The potential antitumour activity of trastuzumab has also been confirmed *in vivo*, both alone and in combination with numerous cytotoxic drugs.[24,53,54] Synergistic interaction occurs between trastuzumab and carboplatin, cyclophosphamide, gemcitabine and taxanes (more with docetaxel than with paclitaxel).[54] The combination of trastuzumab with doxorubicin and epirubicin has been shown to have an additive interaction both *in vitro* and *in vivo*.[23,55] The interaction between trastuzumab and different vinca-alkaloids is specific to each drug (synergistic with vinorelbine but additive with vinblastine).[24] The synergy of trastuzumab with cytotoxic drugs is specific to erbB2-overexpressing tumour cells and is not seen in cells with normal erbB2 expression levels. The three-way interaction between trastuzumab, carboplatin and docetaxel is highly synergistic, even at very low drug concentrations.

2.1.2 Clinical Studies in Advanced Disease

Single-Agent Use

Trastuzumab, administered as single-agent, first-line therapy in women with erbB2-overexpressing metastatic breast cancer,[34] and in women with erbB2-overexpressing metastatic breast cancer that has progressed after chemotherapy,[56] produces durable objective responses (see table I) with a median duration of response of 9.1 months.[56] Retrospective studies have shown that trastuzumab efficacy is correlated with erbB2 gene amplification (FISH-positive tumours) and that clinical benefit for patients with tumours evaluated by immunohistochemistry with a score of 3+ (IHC 3+) is similar to that of FISH-positive patients.[57]

Overall, clinical studies have shown that trastuzumab administered as a single agent is well tolerated and is associated with a low incidence of adverse effects. In particular, trastuzumab is not associated with the toxic effects typically produced by chemotherapy, such as nausea, vomiting, hair loss and bone marrow toxicity.[34,56-58]

Combination with Chemotherapy

In 1998, trastuzumab was approved by the US FDA for clinical use largely on the basis of a randomised clinical trial (H0648g) that compared chemotherapy with chemotherapy plus trastuzumab as a front-line treatment for patients with metastatic breast cancer.[63] The addition of trastuzumab to chemotherapy with either doxorubicin plus cyclophosphamide or paclitaxel resulted in improvements in response rate, time to progression and

Table I. Single-agent activity of monoclonal antibodies in advanced breast cancer

Drug	Response rate range (%)	References
Trastuzumab	12–40	34,56-58
Pertuzumab	7–29	59,60
Bevacizumab	6–17	61,62

Table II. Combination activity of monoclonal antibodies in advanced breast cancer

Drug	Response rate range (%)	References
Trastuzumab		
+ Paclitaxel	27–81	63-73
+ Docetaxel	44–83	66,74-87
+ Capecitabine	45–47	88,89
+ Vinorelbine	38–86	90-100
+ Gemcitabine	27–44	101-103
+ Cisplatin	48	76
+ Docetaxel + cisplatin	79	104
+ Docetaxel + carboplatin	56	104
+ Docetaxel + gemcitabine	56	105
+ Paclitaxel + carboplatin	52–77	65,106
+ Paclitaxel + gemcitabine	53–62	107,108
+ Epirubicin + docetaxel	69	109
+ Liposomal doxorubicin	59	110
+ Liposomal doxorubicin + paclitaxel	92	111
+ Pegylated liposomal doxorubicin	52	112
+ Pegylated liposomal doxorubicin + docetaxel	45	113
Pertuzumab		
+ Docetaxel	30	114
Bevacizumab		
+ Vinorelbine	31	115
+ Docetaxel	52	116
+ Capecitabine	19	117
+ Paclitaxel	28	118

survival for women with erbB2-positive metastatic breast cancer.[63] Combinations of trastuzumab plus chemotherapeutic agents are summarised in table II.

The synergy between trastuzumab and chemotherapy occurs only in erbB2-positive tumours as assessed by both FISH and IHC analysis, regardless of the chemotherapeutic agent.[63,64,74,119] The most troubling effect of the H0648g study was cardiac dysfunction. Concurrent treatment with an anthracycline, cyclophosphamide and trastuzumab significantly increased the risk of cardiac dysfunction, as compared with treatment with only an anthracycline and cyclophosphamide (27% and 8% of patients, respectively). A smaller increase in risk also occurred with treatment with paclitaxel and trastuzumab, as compared with treatment with paclitaxel alone (13% and 1% of patients, respectively), but all these patients had previously received an anthracycline. The only significant risk factor associated with cardiac dysfunction was older age.[63]

Cardiac toxicity resulting from an interaction between trastuzumab and doxorubicin was not detected in preclinical studies,[120] but erbB2 proved to have an important anti-apoptotic role in normal cardiac myocytes, interruption of which leads to increased stress-related cardiac damage.[121] The cardiac safety of trastuzumab combined with anthracyclines known to be less cardiotoxic than doxorubicin, for instance epirubicin or liposomal formulations of doxorubicin, is currently under evaluation. Preliminary analysis of the HERCULES trial, comparing the cardiac safety of trastuzumab plus epirubicin and cyclophosphamide (EC) versus EC alone as first-line therapy for metastatic breast cancer, did not show an excess of cardiac events in the trastuzumab-containing arm.[122] Trastuzumab has also been combined with the following: liposomal doxorubicin alone[110] or with paclitaxel,[111] and with the pegylated liposomal formulation of doxorubicin alone[112] or with docetaxel.[113] Both regimens have shown interesting overall response rates along with acceptable rates of cardiac events.

In a randomised phase III trial, a triple combination of trastuzumab, paclitaxel and carboplatin has shown to improve overall response rates and time to progression with a trend to improved survival, as compared with trastuzumab plus paclitaxel.[65]

Combination with Endocrine Treatment

ErbB2 positivity predicts a relative resistance to endocrine therapy, particularly tamoxifen,[48-51] as a result of crosstalk between the erbB2 and the estrogen receptor intracellular signalling pathways. ErbB2 signalling stimulates the estrogenic, proliferative (agonist) effect of tamoxifen.[123] Preliminary evidence suggests that aromatase inhibitors may be more effective than tamoxifen in erbB2-positive tumours.[124,125] Randomised clinical trials are investigating combinations of trastuzumab and letrozole, anastrozole or exemestane.[126-128]

Practical Use

Neither the optimal schedule nor the optimal duration of trastuzumab therapy has yet been clearly defined in controlled clinical trials.

Although trastuzumab is currently usually administered on a weekly intravenous schedule, evidence suggests that a triple dose of the drug given once every 3 weeks has a pharmacokinetic profile expected to be equally efficacious.[67]

Since the synergistic effect of trastuzumab with cytotoxic drugs acts by interfering with the DNA damage repair mechanism, trastuzumab administration could theoretically be continued, despite disease progression, to take advantage of a potential interaction with second-line chemotherapy. Sufficient data exist on the safety of continuing trastuzumab administration at the time of disease progression. Extended trastuzumab administration up to 40 months after progression in the pivotal phase III trial H0648g was well tolerated with no cumulative toxicities.[129] Furthermore, two retrospective studies have reported encouraging overall response rates of subsequent lines of trastuzumab-chemotherapy combinations.[130,131] It will nevertheless be interesting to see the results of the BIG (Breast International Group) 3-05/GBG (German Breast Group) 26/TBP (Treatment Beyond Progression) trial, which randomises patients not responding to a taxanes-plus-trastuzumab combination to receive capecitabine alone or with trastuzumab.[132]

2.1.3 Clinical Studies in Early Disease

In the last few years, five trials have been initiated to investigate trastuzumab as part of adjuvant therapy for patients with high-risk erbB2 overexpressing early breast cancer (table III). These trials address the question of the benefits of trastuzumab given concomitantly or sequentially with different chemotherapy regimens in the adjuvant setting, and the question of duration of trastuzumab therapy (1 year vs 2 years). The NSABP (National Surgical Adjuvant Breast and Bowel Project) has already published the results of the cardiac safety analysis of the B-31 protocol.[133] A joint analysis of the NSABP B-31 and of the North Central Cancer Treatment Group Intergroup Trial N9831[134] and the first results of the HERA (Herceptin® Adjuvant) trial[135] have been recently published. The results of the BCIRG 006 (Breast Cancer International Research Group) and Finnish trials have been recently presented at the 2005 San Antonio meeting.[136,137] All are concordant and show an impressive 39–52% reduction in the rates of recurrence at median follow-up times ranging from 1 year to 36 months. Noticeably, the majority of recurrences in the erbB2-positive population occur early and at distant sites. It is their incurable nature that prompted the release of the results at unusually short follow-up times. There is already a significant difference in overall survival in the joint American trial analysis, with a 33% reduction in the risk of death, and similar observations are expected for the other trials as more events occur. Incidence of class III/IV congestive heart failure or cardiac death in trastuzumab arms has ranged from 0.3% (non-anthracycline-based regimen arm of BCIRG 006) to 4.1% (NSABP B-31).

The neoadjuvant approach allows for the monitoring of biological phenomena occurring during the administration of trastuzumab. In the majority of the reported studies (table IV), trastuzumab was administered for 12 weeks before surgery and continued post surgery to complete 1 year of treatment in responding patients. Combinations of trastuzumab with taxanes and other cytotoxic agents have achieved pathological complete response rates of 12–66% and clinical response rates of 70–100%, including 24–67% clinical complete response rate.[138-147] In the only randomised study, the pathological complete response rate was 66% for patients treated with trastuzumab plus chemotherapy compared with 26% for patients treated with chemotherapy alone.[147] Common adverse effects were manageable with no unexpected toxicities.

2.1.4 Remaining Questions

Despite all these data, a huge amount of work still needs to be done in erbB2-positive breast cancer. Indeed, unanswered questions include (i) the optimal timing for initiating trastuzumab; (ii) the optimal duration (1 year, 2 years or 6 months); (iii) whether the strong trastuzumab effect will

Table III. Adjuvant trastuzumab trials

Trial (accrual)	Patient characteristics	Treatment regimens	First endpoint	Median follow-up	DFS HR (95% CI; p-value)	OS HR (95% CI; p-value)	Class 3–4 CHF (%)
NSABP B-31[133,134] (2043)	Node positive	AC x 4 → P x 4 AC x 4 → P x 4 + T (P 3 x wk)	OS[a]	2y	Pooled analysis: 0.48 (CI 0.39, 0.59; p < 0.0001)	Pooled analysis: 0.67 (CI 0.48, 0.93; p = 0.015)	0.3 4.1
Intergroup N9831[134] (2766)	Node positive	AC x 4 → P x 4 AC x 4 → P x 4 + T starting concurrently with P AC x 4 → P x 4 + T starting after P[b] (P wkly)	DFS				0 2.4 1.4
HERA[135] (5090)	All except small (<1cm) node negative	Any accepted CT alone (observation) T for 1y after completion of CT T for 2y after completion of CT[b]	DFS	1y	0.54 (CI 0.43, 0.57; p < 0.0001)	0.74 (CI 0.47, 1.23; p = 0.26)	0 0.5
BCIRG 006[136] (3222)	Node positive or high-risk node negative	AC x 4 → D x 4 AC x 4 → D x 4 + T starting concurrently with D D + CBDP x 6 + T (D 3 x wk)	DFS	≈2y	0.49 (CI 0.37, 0.65; p < 0.0001) 0.61 (CI 0.47, 0.79; p = 0.0002)	NA	0.2 1.5 0.3
FinHer[137] (232)	Node positive or high-risk node negative	V or D x 3 with or without T 9wk followed by FEC x 3	RFS	38mo	0.46 (p = 0.0078)	0.43 (p = 0.08)	0

a DFS for pooled analysis.

b Not included in analysis.

A = adriamycin; **BCIRG** = Breast Cancer International Research Group; **C** = cyclophosphamide; **CBDP** = carboplatin; **CHF** = congestive heart failure; **CT** = chemotherapy; **D** = docetaxel; **DFS** = disease-free survival; **E** = epirubicin; **F** = fluorouracil; **HERA** = Herceptin® Adjuvant Trial: **HR** = hazard ratio; **NSABP** = National Surgical Adjuvant Breast and Bowel Project; **NA** = not available; **OS** = overall survival; **P** = paclitaxel; **RFS** = relapse-free survival; **T** = trastuzumab; **V** = vinorelbine; → indicates followed by.

Table IV. Neoadjuvant trastuzumab trials

Drug combination	Response rates (%)	Pathological complete response rates (%)	References
Paclitaxel + trastuzumab	75	18	138
Docetaxel + trastuzumab	70–95	12–28	139-142
Vinorelbine + trastuzumab	88	19	143
Docetaxel + cisplatin + trastuzumab	NR	26	144
Docetaxel + epirubicin + trastuzumab	100	22	145
Docetaxel + vinorelbine + trastuzumab	88	39	146
Paclitaxel → fluorouracil + epirubicin + cyclophosphamide + trastuzumab	NA	67	147

NA = not available; **NR** = not reached; → indicates followed by.

weaken over time; and (iv) whether a subset of patients do not benefit from trastuzumab and, conversely, whether chemotherapy could be avoided in selected women.

Prospective confirmation of the results of two retrospective studies presented at the 2005 San Antonio meeting is awaited in order to better individualise trastuzumab therapy. Tumours with co-amplification of c-myc and erbB2 seem to derive the greatest benefit from adjuvant trastuzumab.[148] Co-amplification of topoisomerase IIα may confer a therapeutic advantage to an anthracycline-based regimen.[136]

2.2 Pertuzumab

Because only about 25% of breast cancers are eligible for trastuzumab, i.e. erbB2 overexpressed (3+ by IHC) or amplified (FISH-positive) breast cancers, novel targeted therapeutics for the remaining tumours are needed. Pertuzumab, a recombinant humanised monoclonal antibody 2C4, binds to a different epitope on erbB2 than trastuzumab, and inhibits both homodimerisation and heterodimerisation with other erbB receptors.

2.2.1 Preclinical Studies

In preclinical studies, pertuzumab has an inhibitory effect on breast, prostate and non-small-cell lung cancer cell lines, whether overexpressing erbB2 or not. In contrast to trastuzumab, 2C4 disrupts erbB2's association with other erbB receptors and inhibits ligand-stimulated signalling in tumour cells that express lower erbB2 levels.[37] The potential therapeutic benefit of 2C4 treatment is that it does not require the antibody to contain an intact Fc region and is observed in human breast and prostate cancer models, which do not overexpress erbB2. Therefore, the presence of erbB2-containing heterodimers appears to predict responsiveness to pertuzumab *in vivo* in non-small-cell lung cancer and breast cancer models.[149]

2.2.2 Clinical Studies

Phase I data showed that pertuzumab single agent therapy is well tolerated and clinically active.[59,60,150] In a phase I study, 21 patients with incurable metastatic solid tumours unselected for erbB2 overexpression were recruited to a dose-escalation study of pertuzumab (0.5 to 15 mg/kg) given intravenously every 3 weeks (table I).[59] Rash, diarrhoea and fatigue, mainly grade 1 and occasionally grade 2, were the most common adverse effects. Two patients achieved a partial response, both of whom had erbB2 non-overexpressing tumours, and stable disease lasting for more than 2.5 months was observed in six patients (29%). Pertuzumab had a pharmacokinetic profile that supports 3-week dose administration.[150] A phase I study of pertuzumab and docetaxel is currently ongoing.[114]

In a phase II trial, administration of pertuzumab every 3 weeks has been shown to be safely used and well tolerated, but single-agent treatment is correlated with limited activity.[60] Indeed, 79 metastatic breast cancer patients with low erbB2 expression were treated with two different dosages of pertuzumab. No obvious relationship between dose and activity or toxicity was observed. In the lower-dose arm, six patients showed a partial response and 18 a

stable disease. In the higher-dose arm, 14 patients experienced stable disease. The most frequent adverse effects were diarrhoea, fatigue and nausea.

Prospective randomised trials are currently being conducted in patients nonresponsive to trastuzumab.[151]

It is likely that results with pertuzumab will improve in the future with administration earlier during the course of the disease, and even further when used in combination with chemotherapeutic drugs.

3. Anti-VEGF

Bevacizumab, a humanised monoclonal antibody directed against VEGF-A, is believed to globally prevent the binding of all VEGF isoforms to all VEGFRs.[20]

3.1 Bevacizumab

3.1.1 Preclinical Studies

Bevacizumab can reduce tumour angiogenesis and block the growth of human tumour xenografts.[152] Moreover, bevacizumab can reduce tumour interstitial fluid pressure, which causes poor delivery of large therapeutic molecules to solid tumours.[153] The combination of this anti-VEGF antibody and chemotherapy in nude mice with human cancer xenografts has greater activity than chemotherapy alone or the antibody alone.[154]

3.1.2 Clinical Studies

As a single agent (table I) or in combination with chemotherapy (table II), bevacizumab has produced encouraging results in phase I–II clinical trials in patients with refractory metastatic breast cancer. These studies showed important clinical benefits in these pretreated metastatic breast cancer patients, of whom up to 33% experienced stable disease[61,62,115-118,155] with a median duration of response of 5.5 months.[62] The adverse-effect profile of bevacizumab differed from that of cytotoxic chemotherapy and includes hypertension, proteinuria, thrombosis and epistaxis.

In a phase III trial, 462 patients with metastatic breast cancer previously treated with an anthracycline and a taxane were treated with capecitabine with or without bevacizumab.[117] The addition of bevacizumab produced a greater response rate (19.8 vs 9.1%) but without impact on progression-free survival, probably reflecting the late disease stage in the patient population. In contrast, the addition of bevacizumab to weekly paclitaxel as first-line therapy of 722 metastatic breast cancer patients increased overall response rates (28.2 vs 14.2%) and significantly prolonged progression-free survival (10.9 vs 6.1 months).[118] Further studies are warranted to determine the potential of bevacizumab in breast cancer, particularly in earlier stages of disease. In addition, methods of selecting patients who would most benefit from anti-VEGF therapy and optimal chemotherapy schedules are urgently needed. Bevacizumab is currently being tested in combination with letrozole in hormone receptor-positive metastatic breast cancer patients,[156] and a randomised Cancer and Leukemia Group B trial is planned to confirm the good preliminary results.

4. Combination with Other Molecular Targeted Therapies

The combination of trastuzumab and pertuzumab synergistically inhibits the survival of BT474 breast cancer cell lines, in part because of increased apoptosis. Trastuzumab increases 2C4-mediated disruption of erbB2 dimerisation with the epidermal growth factor receptor and erbB3. Combination drug treatment reduced levels of total and phosphorylated erbB2 protein and blocked receptor signalling through Akt, but did not affect MAPK. These results suggest that combining erbB2-targeting agents may be a more effective therapeutic strategy in breast cancer than treating with a single erbB2 monoclonal antibody.[157]

Erlotinib, a potent small molecule erbB1/EGFR tyrosine kinase inhibitor, has been combined with pertuzumab to evaluate whether the inhibition of both mechanisms is superior to monotherapy in tumour cell lines expressing different erbB levels.[158] Overall, erlotinib and pertuzumab are active against various human non-small-cell lung cancer and breast cancer xenograft models (Calu-3 and KPL-4 models), independently of erbB1/EGFR or erbB2

expression. A combination of these erbB-targeted agents resulted in additive or greater than additive antitumour activity. Both erlotinib and pertuzumab have different mechanisms of action. Erlotinib directly inhibits erbB1/EGFR tyrosine kinase activity, whereas pertuzumab blocks ligand-associated dimerisation of erbB2 with other erbBs. Active heterodimers stimulate at least two different signalling pathways. One pathway promotes cell growth (via MAPK) and the other pathway enhances cell survival (via Akt). Thus, inhibition of cell proliferation rates or induction of apoptosis may contribute to the additive effect with erlotinib and pertuzumab.[158]

A phase I/II study of bevacizumab combined with trastuzumab is currently ongoing in an effort to determine whether this combination can improve patient outcome.[159] This study is based on preclinical models demonstrating that erbB2 gene amplification is associated with an increase in VEGF gene expression.[7] VEGF expression alone was found to be predictive of survival in a study analysing the combined effects of erbB2 and VEGF on clinical outcome.[160] Additional prognostic information for survival was obtained, supporting the use of combination therapy with erbB2- and VEGF-targeted agents.

5. Conclusion

Targeted therapies against tumour biological properties are an essential part of the concept of individualising breast cancer treatment. Many new agents that block oncogenic pathways are currently being investigated. Trastuzumab represents a model of how the integration of targeted agents into therapeutic regimens may dramatically improve the outcome of a relevant subset of the breast cancer population. Our increased understanding of the molecular, structural and biological characteristics of tumours is essential for the rational development of targeted therapies. Monoclonal antibodies are highly specific therapies with moderate toxicities. The major limitations of monoclonal antibodies are their size, which may limit tumour penetration mainly into the brain, the heterogeneous antigen expression in tumours and the expression of tumour antigens in normal cells.

The development of accurate predictors of response to targeted therapies is clearly needed. The continued translation of knowledge that is emerging from the field of signal transduction will undoubtedly contribute to the development of novel agents complementary to the existing ones.

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