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Therapy of breast cancer with molecular targeting agents

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Invasive breast cancer is a heterogeneous disease sustained by intercorrelated and complex growth pathways. Classically, human breast carcinoma has been classified for therapeutic purposes in two distinct categories: one hormone-correlated, the other hormone-uncorrelated. However, the recent advancements of the technology applied to molecular biology by genomic and proteomic analyses have suggested that many more factors are involved in breast cancer growth and progression and that some clusters of these distinguish subgroups of patients at different prognosis. The knowledge of the diversities between tumor and normal tissue of origin is the key to identify novel targets for new selective therapeutic strategies. In fact, the principal goal of molecular-targeted therapy is the suppression of the transformed phenotype minimally affecting normal cells. This review focuses on the molecular targeting compounds directed against the known molecular pathways involved in breast cancer such as: type I growth factors (HER-2/*neu*; epidermal growth factor receptor [EGFR]), angiogenesis, cyclooxigenase-2 (COX-2) and farnesylation. Presently, trastuzumab is the first agent approved for therapy of HER-2/*neu* overexpressing tumors. Several other compounds directed against different targets have entered clinical evaluation and the preliminary results are here presented and commented. The major challenges on the clinical development of targeted therapy include the proper selection of patients, the identification of the optimal dosage and schedule of administration, the combinations with conventional treatments and the more appropriate therapeutic strategy.

Key words: targeted therapy, breast cancer

Introduction

Tumorigenesis is a multistep process that involves genetic alterations driving the progressive transformation of normal cells into the malignant phenotype. It is characterized by a dysregulation of numerous molecular pathways, such as cell cycle progression, angiogenesis, and apoptosis that represent rational targets for more selective therapeutic approaches (Figure 1).

The recent advancements of molecular technology have allowed for a better understanding of the mechanisms sustaining breast cancer (BC) transformation and progression. Proteomic and genomic analyses will allow these to be further assessed.

The aim of targeted therapy is the selective inhibition of the transformed phenotype, minimally affecting normal tissues, so that the main goal is to target specific molecular lesions within tumor cells, leading to improved cure rates with limited toxicity.

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BC is the most common female tumor with an increased morbidity in Western countries. The trend in 5-year survival rate (years 1992–1998) is 86%, but differs significantly in patients at different stages and between patients with a very poor prognosis and those with less aggressive disease.

Both chemotherapy and hormone therapy have significantly impacted on survival of these patients, however, therapy of metastatic disease still remains palliative and also adjuvant treatments do not guarantee optimal results. In the past few years, an improved understanding of the peculiar molecular pathways involved in BC growth and progression allowed the identification of novel targets that can be selectively inhibited by new generations of anticancer compounds.

This review aims to provide an overview of the principal targeted agents in clinical testing for BC treatment.

Targeting type I growth factors

The epidermal growth factor receptor (EGFR, HER1) and HER-2/*neu* proteins are transmembrane tyrosine kinase cell surface growth receptors expressed on normal epithelial cells. The EGFR and HER-2/*neu* oncoproteins are composed of three membrane portions: the internal tyrosine kinase is responsible for signal transduction; a short transmembrane part, and the extracellular domain (ECD); the latter being

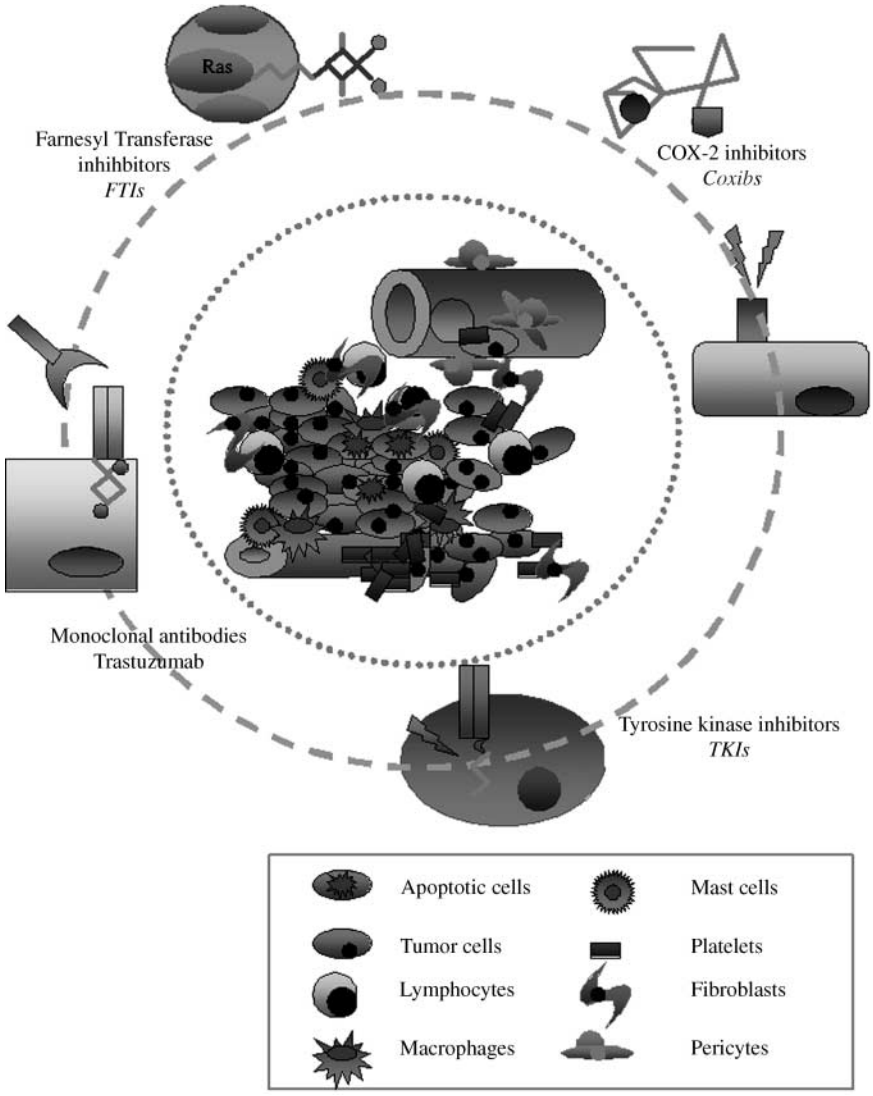

Figure 1. Principal targeting approaches in BC treatment.

the site of binding for the ligand growth factors [1]. The development of EGFR and HER-2/*neu* antagonists represents a promising novel anticancer therapeutic approach. EGFR and HER-2/*neu* are overexpressed or dysregulated in approximately 50% and 25%, respectively, of BC tumors [1]. Their activation is associated with increased cell proliferation, tumor cell motility and invasiveness, angiogenesis, and inhibition of apoptosis [1, 2].

Overexpression of HER-2/*neu* identifies a subgroup of patients with aggressive disease, frequently hormone receptors negative and with poor prognosis [3, 4]. Furthermore, tumor amplification of the HER-2 gene has been associated with resistance to a variety of cytotoxic agents and tamoxifen [5, 6].

Trastuzumab

Trastuzumab is a humanized monoclonal antibody with high specificity for the HER-2 protein [7], that demonstrated activity when used as a single agent in first- or second-line treatment of metastatic breast cancer (MBC) [8, 9]. In a pivotal randomized prospective controlled trial of first-line therapy in HER-2/*neu* positive MBC, the combination of trastuzumab and chemotherapy significantly improved time to progression (TTP), response rate (RR), duration of response and overall survival (OS) as compared to chemotherapy alone [10]. In addition, the combination also determined significant improvements in quality of life compared with standard chemotherapy [11]. However, there was an unacceptable high rate of cardiotoxicity in the subgroup of patients treated concurrently with doxorubicin, which limited the use of such a combination in clinical practice.

Several clinical studies have assessed the antitumoral activity and the tolerability as front-line therapy of the combination of trastuzumab with platinum salts [12], paclitaxel [13–15], docetaxel [16–18], vinorelbine [19–21], or of triplets with taxanes and platinum salts [22, 23] (Table 1).

In patients with extensively pretreated MBC the combination of trastuzumab with cisplatin resulted in a response rate of 24% [12], while triplets of platinum-based combined with docetaxel and trastuzumab showed RRs of 58–79% as

Table 1. Clinical trials with trastuzumab-containing regimens

Phase	Schedu	Pts	Results	Toxicity (%)	Ref
First-line					
III	$A_{(60)}$ $C_{(600)}$ Ptx $_{(175)}$	143 92	RR: 56 %; OS: 26.8 m RR: 41 %; OS: 22.1m	Cardiotoxicity (27) Cardiotoxicity (13)	10
II	Ptx $_{(90)}$	33	RR: 62 % OS: ND	Alopecia (33) G4 Neutropenia (9) Neuropathy (3) Stomatite (3)	15
IIb	Dtx $_{(100)}$	92	RR: 61 % OS: 27.7 m	Neuropathy (32) Asthenia (45) Diarrea (43)	18
II	Vnr $_{(30)}$	40	RR: 78 % OS: >20m	G3–4 Neutropenia (20) Neuropathy (8)	20
II	Vnr $_{(25)}$	54	RR: 68 % OS: ND	G4 Neutropenia (17) Neuropathy (44) Diarrhea (35)	21
II (BCIRG 101)	Dtx $_{(75)}$/Cddp $_{(75)}$	62	RR: 79 % OS: 16+ m	G4 Neutropenia (16) Neuropathy (52) Diarrhea (73) Asthenia (94)	22
II (UCLA-ORN)	Dtx $_{(75)}$/Cbdca $_{(AUC:6)}$	62	RR: 58 % OS: 20+ m	G4 Neutropenia (65) Neuropathy (42) Diarrhea (52) Asthenia (81)	22
II	Ptx $_{(70)}$/Cbdca $_{(AUC:2)}$	61	RR: 84 % OS: 22.2 m	G3–4 Neutropenia (28) fatigue (67) Diarrhea (38)	23
IIb	Ptx $_{(80)}$	64	RR: 78 % OS: ND	Neuropathy (60) Asthenia (54)	26
Second/Third-line					
II	Cddp $_{(75)}$	37	RR: 24 % OS: ND	Cytopenias (27) Asthenia (13)	12
II	Ptx $_{(90)}$	95	RR: 56.8 % OS: ND	Neuropathy (92)	13
II	Ptx $_{(60\text{-}90)}$	25	RR: 56 % OS: ND	Cardiotoxicity (8) Onycholysis (20)	14
II	Dtx $_{(35)}$	30	RR: 63 % OS: ND	G4 Neutropenia (10) Cardiotoxicity (29) Diarrhea (66) Fatigue (82)	16
II	Vnr $_{(25)}$	40	RR: 75 % OS: ND	G4 Neutropenia (10)	19
II	Gmz $_{(800)}$/Vnr $_{(25)}$	31	RR: 51.9 % OS: 13+ m	Asthenia (48.6) Neuropathy (14.8)	28

Cbdca: carboplatin; Gmz: gemcitabine; Ptx: paclitaxel; Cddp: cisplatin; A: adriamicin; C: cyclophosphamide; RR: response rate; OS: overall survival; m: months; ND: Not Done.

first-line therapy [22]. These regimens induced severe non-hematologic toxicities, including fatigue, nausea, vomiting and neurotoxicity, which limit the use of such combinations in clinical practice.

Recently, preliminary analysis of a randomized study of trastuzumab and paclitaxel *versus* the same regimen in combination with carboplatin demonstrated an improvement in TTP with the triplet association [24]. However, the demonstration whether triplets give superior benefit against doublets needs further prospective clinical trials.

The combination of trastuzumab and taxanes is supported by preclinical data demonstrating a synergistic cytotoxicity in BC cell lines [25]. Paclitaxel/docetaxel-trastuzumab regimens induce a RR of 41–78% as first-line therapy [10, 15, 18, 26] and a high percentage of objective responses has been observed also in pretreated patients [13, 14, 16]. In particular, the weekly schedule of administration of both paclitaxel or docetaxel and trastuzumab has been successfully evaluated in several clinical trials [13–15, 26] and offers the potential to improve certain toxicities associated with tri-weekly taxanes administration. Weekly schedules of taxanes are characterized by moderate hematological toxicity, allowing their administration for prolonged period of time, and by several non-hematological toxicities, mainly consisting of fatigue, myalgia and neurotoxicity [27]. Due to the high activity and the good tolerability of weekly schedules of chemotherapeutic agents, the overall finding regarding the administration of trastuzumab as front-line therapy is favoring the regimens based on combinations with weekly schedules of docetaxel, such as paclitaxel or vinorelbine.

An Italian multicenter randomized Phase IIb trial evaluated the combination of trastuzumab with weekly paclitaxel (80 mg/m^2) *versus* weekly paclitaxel alone as first-line therapy of HER-2 over-expressing (HercepTest 2+/3+) MBC: the intent-to-treat overall RR in the first 85 evaluable patients was of 78% for the combined treatment *versus* 60% of paclitaxel alone and the median TTP was 52+ weeks *versus* 28+, respectively [26]. Both the treatment arms were feasible and well tolerated, but trastuzumab significantly improved the clinical end-points, particularly in those patients with HercepTest 3+, visceral disease or pretreated with adjuvant anthracyclines.

Taking into account that the combination of trastuzumab with paclitaxel is the only one approved by the FDA, it is reasonable to compare such a schedule with new promising regimens. Burstein et al. are currently conducting a randomized trial comparing a taxane/trastuzumab regimen with vinorelbine/trastuzumab (TRAVIOTA trial).

Trastuzumab plus vinorelbine has determined one of the most interesting tolerability and efficacy profiles, with RRs greater than 70% in Phase II clinical trials of first- or second-line therapy [19–21]. The most common toxicity observed in these clinical trials was manageable neutropenia, while few

non-hematological toxicities were reported and the incidence of neuropathy was limited.

Since the use of combination chemotherapy in MBC is palliative, patients with HER-2/*neu* positive MBC may benefit mostly from combining trastuzumab with agents with moderate toxic effects and satisfactory activity, rather than toxic agents. Therefore, taking into account the low systemic toxicity and the good tolerability, vinorelbine and weekly schedules of taxanes seem to exhibit, at the moment, one of the best therapeutic indexes when associated with trastuzumab.

Another attractive schedule for taxanes-pretreated patients with HER-2/*neu* positive tumors is the combination of trastuzumab with gemcitabine and vinorelbine, that has been evaluated by our group, as second-line therapy in patients pretreated with anthracyclines and/or taxanes and/or trastuzumab. The results of our phase II study suggest that this combination is characterized by a favorable toxicological profile, absence of cardiac toxicity, and that the efficacy of such a schedule is particularly satisfactory in patients with HercepTest 3+, showing a RR of 73.3% in this subgroup of patients [28].

The positive results of trastuzumab alone or in combination with cytotoxic agents provide a rational for its use in the adjuvant setting (Figure 2). However, the optimal schedule and duration of therapy and whether benefit can be achieved with continued treatment after tumor progression have not been defined. Other crucial questions on trastuzumab therapy concern: a) the usefulness in responsive patients of a maintenance therapy; b) the opportunity in patients with progressive disease to follow trastuzumab combined with a non cross resistant chemotherapeutic regimen.

Gefitinib (ZD1839) and Erlotinib

Gefitinib (ZD1839) is a low-molecular weight EGFR tyrosine kinase selective inhibitor that acts by blocking the signal transduction pathways that promote cancer cell growth [29]. In preclinical studies gefitinib demonstrated antitumor activity against ovarian, colon and BC cell lines overexpressing EGFR [30]. The drug can be favorably combined with several cytotoxic drugs or radiation therapy, leading to enhanced tumor growth inhibition in vitro [31]. Data from a Phase I study documented that the maximum tolerated dose of gefitinib is >700 mg/day and that the recommended daily dose in non-small-cell lung cancer (NSCLC) is 250 mg [32–34]. The activity and tolerability of gefitinib as monotherapy has been evaluated in MBC [35–37]. Baselga et al. reported a clinical benefit of 61.4% in 34 patients with daily dose of 500 mg. Only one patient had a grade 3 skin toxicity [35]. Robertson et al. demonstrated activity of gefitinib in tamoxifen resistant estrogen receptor (ER)-positive and (ER)-negative BC patients [36]. Gefitinib was administred at 500 mg/day and it was generally well tolerated, with mild adverse events including rash, diarrhea, nausea, vomiting and lethargy. A negative result has been recently reported by von Minckwitz et al., in a multicentre Phase II study in 58 taxane- and anthracycline-pretreated MBC [37]. Only 1 patient obtained a partial response (1.7%) and 2 patients reported a significant improvement in pain at metastatic sites. The authors [37] concluded that gefitinib monotherapy is well tolerated, but it does not appear to be efficacious in heavily pretreated patients. The combination of gefitinib with cytotoxic agents has been evaluated in some Phase I-II studies [38–40]. Fountziles et al. evaluated the activity of the combination of paclitaxel, carboplatin and gefitinib as first-line chemotherapy [38]. Gefitinib was administered at the dose of 250 mg/day orally. An objective RR of 46% was reported and the major toxicities were grade 3–4 neutropenia (16%), thrombocytopenia (6%), anemia (10.5%), peripheral neuropathy (6%), allergic reaction (6%) and diarrhea (7.5%). Ciardiello et al. investigated the combination of gefitinib and docetaxel as first-line therapy [39]. The schedule was well tolerated, with grade 3 and 4 neutropenia in

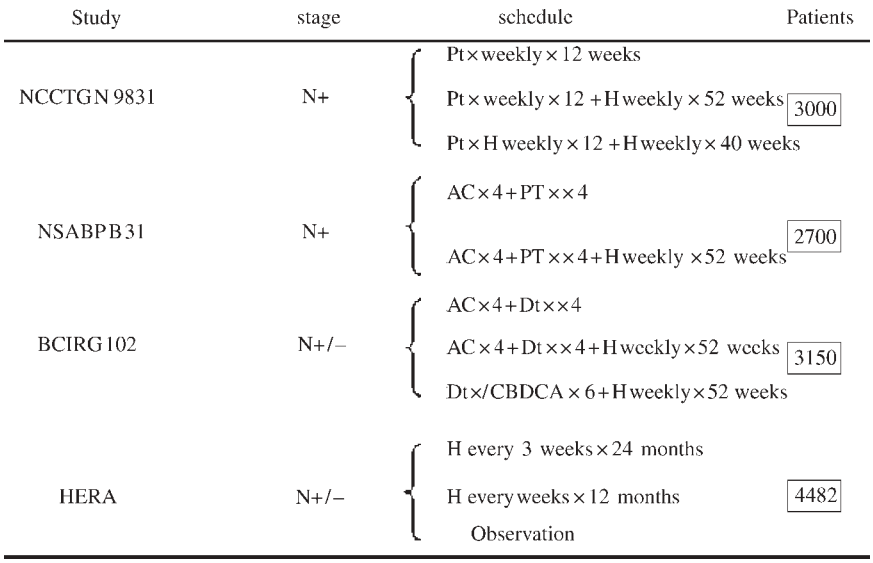

Figure 2. Principal ongoing trials with adjuvant trastuzumab.

21% and 36% of patients, respectively, grade 3 diarrhea in 2 patients and grade 4 skin rash in 1 patients. Among the first 14 patients treated, a RR of 64% was reported. A dose-finding study was performed by Gasparini et al. to evaluate the optimal schedule of combination of gefitinib with weekly epirubicin in patients pretreated with taxanes [40]. The recommended dose of epirubicin for Phase II studies was 30 mg/m^2 in combination with gefitinib at the daily dose of 250 mg. The maximum tolerated dose was achieved at 35 mg/m^2 of epirubicin, with 2 patients experiencing grade 4 dyspnea and asthenia, grade 3 diarrhea and thrombocytopenia. This combination was well tolerated with moderate hematological and non-hematological toxicities, being asthenia, skin rash, nausea, dyspnea, conjunctivitis and diarrhea the most frequent, but moderate, adverse events. Of the 14 cases assessable for response, partial response was documented in 2 patients, and stable disease in 7, for an overall disease control rate of 64.2% [40].

Ongoing research is aimed to identify predictive markers of response. Two recent papers suggest that mutations of the EGFR tyrosine kinase domain can be predictive of response to gefinitib [41, 42], whilst previous studies did not find a clear relationship between RR and EGFR overexpression. The mutation rate in NSCLC is approximately 10%, but the real value of these data needs to be confirmed in larger clinical studies [41].

These results have important clinical implications, including: patient selection, definition of diagnostic predictive tests, design of second-generation inhibitors, understanding of the resistance mechanisms, and selection of other solid tumors with the same mutations.

Only a single published study has evaluated erlotinib, an orally EGFR tyrosine kinase inhibitor, in MBC. In 18 patients treated with erlotinib as monotherapy at 150 mg/day, no responses were observed [43].

Other clinical studies are needed to evaluate the role of this agent in BC and other solid tumors.

Targeting Angiogenesis

Angiogenesis, the process of new capillary formation from pre-existing vessels, is necessary for tumor growth and metastasis. The initiation of the angiogenic program, the angiogenic switch' requires the acquisition of the angiogenic phenotype through a series of molecular events leading to increased expression of angiogenic factors and/or down-regulation of naturally occurring inhibitors [44].

Vascular endothelial growth factor (VEGF) is the most specific and powerful angiogenic factor. Also angiopoietin-2, transforming growth factor-β1 (TGF-β1), basic fibroblast growth factor (bFGF) and matrix metalloproteinases (MMPs) play a major role in angiogenesis. The biologic effects of VEGF are mediated through the binding to three specific endothelial surface cell receptors VEGF-R1 (flt-1), VEGF-R2 (flk-1/kdr), and VEGF-R3. VEGF-R1 promotes differentiation and vascular maintenance, VEGF-R2 induces endothelial cell mitogenesis and vascular permeability, whilst VEGF-R3 stimulates lymphangiogenesis [45]. *VEGF* gene expression may be upregulated by a number of stimuli, including: hypoxia, nitric oxide, various growth factors, estrogens, progestins, loss of *p53,* activation *ras*, v-*src*, and HER2*/neu* [46].

In BC, initiation of the angiogenic phenotype is correlated with progression from DCIS to invasive carcinoma [44]. In premalignant lesions, VEGF-R1 (Flt-1) is absent and VEGF-R2 (KDR/Flk-1) is minimally expressed. Expression of VEGFR is enhanced in invasive cancer and endothelial cells.

VEGF and HER2 signaling pathways are interlinked at molecular level and both cooperate to promote cell proliferation. Many studies indicated VEGF as an independent prognostic marker [47]. Indeed, intratumor VEGF levels seems related to chemotherapy and tamoxifen resistance [48].

A number of antiangiogenic agents are being tested in Phase I/II clinical trials for the treatment of BC, either alone or in combination with other therapies, including carboxyamidotriazole, interleukin-12, thalidomide, celecoxib, soy isoflavone, anti $\alpha_v\beta_3$ integrin monoclonal antibody and MMPs inhibitors.

Two phase I clinical studies demonstrated that bevacizumab can be administered safely, without dose-limiting toxicities, up to the dose of 10 mg/kg, and that it could be combined with chemotherapy without apparent synergistic toxicity.

A phase II study of bevacizumab monotherapy at escalating doses was conducted in 75 patients with previously treated MBC [49]. A 9.3% objective RR with 17% of patients responding or stable at 22 weeks was reported; four (7%) patients continued therapy without progression for over 12 months. 20 mg/kg was considered the toxicity limiting dose. Another phase II trial on 55 metastatic pretreated patients evaluated the safety and activity of bevacizumab (10 mg/kg every two weeks) and vinorelbine (25 mg/mq/week) combination, showing a RR of 31% with one complete response. Treatment was well tolerated, with only minor occurrence of hypertension, proteinuria, and epistaxis. No major bleeding or thrombotic events were registered [49].

A recently reported phase III trial randomly assigned 462 patients with anthracycline- and taxane-refractory disease to receive capecitabine with or without bevacizumab [50]. As expected, in bevacizumab arm was registered hypertension, proteinuria, and minor mucosal bleeding, but these toxicities rarely were severe. In both the arms 12% of patients discontinued therapy because of toxicity. The combination therapy significantly increased RRs (9.1% *versus* 19.8%; $P = 0.001$), but not TTP (4.17 *versus* 4.86 months; hazard ratio = 0.98) [50].

A phase III trial (E2100) comparing weekly paclitaxel with or without bevacizumab in chemo-naïve patients is ongoing. In this trial, correlative studies on potential predictive factors are foreseen. Combination of bevacizumab with biological agents including trastuzumab and erlotinib (an inhibitor of the EGFR-1 tyrosine kinase) are also being evaluated [49].

Our Center is involved in an international Phase II trial investigating the role of AG-013736, an orally active VEGF tyrosine kinase inhibitor, combined with docetaxel *versus* docetaxel and placebo, in first-line treatment of MBC.

A Phase II study conducted to verify the activity of thalidomide in heavily pretreated patients gave negative results [51].

Recently, a few studies have provided evidence supporting the concept that BC may utilize a number of different angiogenic molecules during tumor progression and that VEGF acts as mitogen in the earliest stages, but as cancer progresses, angiogenesis is supported by bFGF, TGFβ-1, platelet-derived endothelial cell growth factor, and pleiotrophin [52].

Preclinical studies suggest that antiangiogenic therapy probably need to be targeted to all the specific factors acting in each single tumor and in different stages of tumor initiation and progression.

Targeting cyclooxigenase-2 (COX-2)

COX-2 expression in BC is variable but it is associated with parameters of aggressivity, such as tumor size, axillary node metastasis, hormone receptor-negative disease, and HER-2/*neu* amplification [53]. In addition, moderate to high COX-2 expression is detectable in a significant proportion of preinvasive and invasive BCs and particularly those with aggressive or poor prognostic features [53]. Several *in vivo* experimental studies showed a pivotal role of COX-2 in various tumor processes, including apoptosis, angiogenesis, invasiveness, inflammation, and induction of aromatase, a cytochrome P 450 enzyme that catalyses estrogen production [54].

Selective COX-2 inhibitors significantly reduced carcinogen-induced rat mammary tumors [55] and may have a role in chemoprevention [53]. A meta-analysis of clinical studies indicates that the use of aspirin or nonsteroidal anti-inflammatory drugs can reduce the risk of BC by approximately 20% [56].

Celecoxib was tested in combination with trastuzumab in a phase II study conducted in HER-2/*neu* positive MBC with a good tolerability, but negative results [57]. In another phase II trial, the combination of celecoxib and exemestane showed promising activity without additional side effects [58]. In neoadjuvant treatment, celecoxib in combination with FEC regimen or exemestane was superior to either chemotherapy or hormone therapy alone [59, 60].

All these encouraging results might now be reconsidered taking into account the evidence that has emerged of the relevant cardiovascular and thrombo-embolic toxicity correlated to the prolonged use of coxibs, as shown in the APPROVe, APC studies with rofecoxib and celecoxib, respectively, as well as in a smaller trial with valdecoxib [61–63].

Several mechanisms may explain this unexpected toxicity. Coxibs reduce the levels of COX-2 mediated prostacyclin that inhibits platelets aggregation and vascular smooth muscle cells proliferation and induces vasodilatation, without affecting the levels of thromboxane A2, the key COX-1 mediated product of platelets that causes platelets aggregation, vasoconstriction, and vascular proliferation. In addition, coxibs increase blood pressure, decrease angiogenesis, and destabilize aterosclerotic plaques [54, 61–63].

Targeting farnesylation

Farnesylation is an essential step for activation of several proteins involved in cytoskeleton organization, apoptosis, gene transcription and cell proliferation. Activation of *Ras* oncogene is also farnesyl transferase dependent. However, continuous activation of Ras protein can occur as a result of permanent upstream growth factor stimulation independently of *Ras* mutation [64, 65]. Although farnesyl transferase inhibitors (FTIs) clearly inhibit Ras farnesylation, it is unclear whether their antiproliferative effects result exclusively from their inhibition of Ras activity [66]. Probably, other intracellular targets that include perioxoma membrane [67] and nuclear membrane associated proteins, such as lamins A and B [68], modulation of the PI3-K/Akt pathway [69] or GTP-binding proteins RHOB and RHOE regulating cell adhesion/motility [70] are involved.

A series of experiments documented an additive and/or synergistic effect of the combination of FTIs with cytotoxic agents [71]. In transgenic mice with spontaneous mammary tumors resistant to paclitaxel, lonafarnib was able to overcome resistance [72]. Based on these results, several clinical trials have been initiated to explore the combination of FTIs with taxane-containing regimens.

Recent experimental data support the combination of FTIs with endocrine therapy, suggesting a synergistic anti-tumor effect [73].

At least five FTIs are under clinical evaluation and preliminary results from nine Phase I-II studies are encouraging and suggest that continuous exposure is necessary to obtain the optimal efficacy. Dose-limiting toxicity includes myelosuppression, gastrointestinal side effects, peripheral neuropathy and fatigue.

In a phase II study in 76 patients with MBC, tipifarnib in two different oral dose administrations showed a moderate clinical efficacy and a good tolerability, the side effect profile being significantly improved by using an intermittent schedule. These results were independent of Ras mutations and hormone receptor status [74].

A number of published Phase I studies of FTIs in combination with cytotoxics agents with encouraging results and a predictable and manageable toxicity have been reported [73]. To date there is no published Phase II combination study, although studies with taxanes are in progress.

In addition, a number of Phase I-II trials have been initiated with FTIs (tipifarnib, lonafarnib) combined with endocrine therapies, including tamoxifen, fulvestrant, or aromatase inhibitors [73].

Despite these encouraging results, there are several unresolved questions, such as: (i) the optimal biological dose; (ii) surrogate biomarkers of activity, including inhibition of protein prenylation in peripheral blood lymphocytes and buccal mucosal cells; (iii) tumor histotypes and stage to treat; (iv)

optimal combinations with chemo-hormone therapy and/or radiotherapy, and (v) predictive markers of toxicity [73].

Open questions

It is unlikely that a single agent administered in the setting of advanced and pretreated tumors may result in the cure. Therefore, the usual method of testing a new molecular targeting agent in cancer patients who have failed conventional therapy is not the optimal strategy for the development of such compounds. In fact, most of the preclinical studies documented that these drugs are most effective in experimental models of minimal tumor burden and when administered by frequent low doses that maintain active and constant concentrations at the target level, rather than at high dosages with periods of resting between subsequent bolus injections. A second key question concerns the appropriate selection of patients as well as the identification of surrogate biomarkers predictive of response.

Experimental studies suggest additive and/or synergistic antitumor activity of combinations of molecular targeting agents to each other or with conventional anticancer treatments. The combination of multiple agents targeting a number of cell pathways may yield potent pro-apoptotic or growth inhibitory effects. Probably, the mechanisms for a synergistic effect of angiogenesis inhibitors with chemotherapy may be related to an increased access of the cytotoxic drug as a result of the enhanced permeability related to antiangiogenic effects on endothelial cells, increased blood flow, oxygen delivery and decreased interstitial pressure [75].

There are several obstacles to the use of targeted therapy in clinical trials: the identification of appropriate, biologically active, dosages from phase I studies, scheduling of drugs, and the optimal modalities of combination with cytotoxic agents, hormones, radiotherapy or other molecular targeted therapies.

The adjuvant setting is probably the best option to validate most of these compounds. Another potentially interesting setting is chemoprevention: coxibs and anti-estrogens showed promising results to prevent colorectal and breast cancers in high-risk subjects, respectively. The real efficacy of these agents should be validated in appropriately designed Phase III trials that must include: tissue or circulating surrogate biomarkers of efficacy, biologically-driven criteria of patient selection, and well-defined schedules of treatment.

Conclusions and future directions of research

BC is a heterogeneous disease characterized by tumor-specific mutations and dysregulated cellular pathways. Targeting these pathways with novel agents may be the key to enhance tumor control. Trastuzumab provides the proof of principle that active anticancer agents can interfere with selective molecular alterations of the disease and may be the compound of choice to be combined with other molecular targeted treatments in hormone uncorrelated tumors.

The characterization of the molecular alterations of each single tumor is at the basis of personalized anticancer approaches aimed to give each patient the most appropriate therapy, and, possibly, the least toxicity.

Most of the available molecular targeted compounds are not substitutive but rather integrating treatments to be combined with conventional anticancer drugs. Because the principal goal of novel therapeutic approaches for the palliative therapy of advanced disease is to obtain long-lasting disease control with acceptable quality of life, appropriate schedules of administration of cytotoxics should be tested (i.e. 'metronomic chemotherapy') in order to improve their efficacy and tolerability in combination with targeted agents [54].

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