Focus On

Anti-angiogenic therapy: from laboratory to the patient

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Abstract Angiogenesis, or new vessel formation, plays a key role in the process of tumor growth and metastasis in breast cancer. Recent large phase-III trials (E2100 and AVADO) demonstrate the clinical utility of bevacizumab in combination with front-line taxane-based chemotherapy in patients with metastatic breast cancer, leading to its recent approval in the United States and Europe. Several other anti-angiogenic strategies against vascular endothelial growth factor are in various phases of clinical development. This paper reviews the data on bevacizumab in metastatic breast cancer and other anti-angiogenic strategies on development in breast cancer.

Keywords: Bevacizumab; Breast cancer; Tyrosine kinase inhibitor; VEGF

Introduction

Despite advances in the diagnosis and treatment of breast cancer, the disease remains a common cause of death. An estimated 184 450 new cases and 40 930 deaths due to breast cancer have occurred in the United States in 2008 [1]. Angiogenesis – as a crucial component of cancer growth and metastasis – was initially described by Folkman in 1971 [2]. At that time, he proposed that the release of certain growth signals from a malignant cell results in angiogenesis, and that without this process tumors (both primary and metastatic) will be unable to grow past $\sim 3\,mm$ in size. The 'angiogenic switch', allowing progressive growth, occurs when a neoplasm initiates new blood vessel supply with appropriate stimuli by shifting the balance between proangiogenic and anti-angiogenic factors in favor of proangiogenesis [3]. Hypoxia, which is a stimulus, induces expression of hypoxia-induced factor 1α leading to downstream increased expression of vascular endothelial growth factor (VEGF). The VEGF family consists of at least seven different proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factors 1 and 2) [4]. When VEGF binds to its receptors (VEGFR-1, VEGFR-2, VEGFR-3), several pathways are activated leading to angiogenesis [5]. Thus, targeting angiogenesis through VEGF inhibition strategies has recently been the subject of intense research in breast cancer.

The role of angiogenesis in breast cancer is supported by preclinical and clinical models. Hyperplastic murine breast papillomas and normal breast next to malignant tissue induced angiogenesis in a site distant from the breast [6]. In clinical breast cancer models, microvessel density (MVD) and microvessel counts in the area of most intense neovascularization were predictive of metastatic disease in either axillary lymph nodes or at distant metastatic sites, suggesting that tumor angiogenesis was correlated with the development of metastatic disease in breast cancer [7]. In addition, other researchers have shown that the risk of

developing invasive breast cancer is linked to a higher MVD in patients with benign fibrocystic breast disease [8]. Higher MVD are also associated with more aggressive ductal carcinoma in situ lesion, higher risk of metastatic disease, and poorer clinical outcomes [7,9,10]. A higher VEGF level has been shown to correlate with a shorter overall survival even when patients receive adjuvant endocrine therapy or chemotherapy, and is also a particularly strong marker for early breast cancer relapses [11,12]. Higher VEGF levels are also found in patients with visceral, brain, and soft tissue recurrences as opposed to those with bone metastasis [12]. These studies all support the role of angiogenesis in breast cancer.

Anti-angiogenic agents in breast cancer

Bevacizumab

Bevacizumab is a humanized monoclonal antibody that binds and neutralizes all VEGF-A isoforms. Among the VEGF proteins, VEGF-A is the most potent in inducing pathological angiogenesis [5,13]. A phase-I/II trial evaluating the dose and safety of bevacizumab in 75 patients with metastatic breast cancer (MBC), determined an overall response rate (ORR) of 9.3% with a median duration of response of 5.5 months. The optimal dose was 10 mg/kg every 2 weeks [14]. This study demonstrated that bevacizumab had activity in MBC, supporting the initiation of phase-III studies. The first randomized phase-III trial evaluated bevacizumab in combination with capecitabine vs. capecitabine alone in heavily pre-treated patients and showed response rates of 19.8% vs. 9.1% ($P = 0.001$), favoring the combination arm. Unfortunately, this did not translate to a superior progression-free survival (PFS) (the trials' primary endpoint), overall survival, or quality of life (QOL) [15]. A subsequent phase-II trial of capecitabine plus bevacizumab in the front-line metastatic disease setting (the XCaliBr trial) has been recently reported by Sledge et al. [16]; 106 patients with MBC were given capecitabine and bevacizumab. Patients had HER2 negative disease and no prior chemotherapy except in the neoadjuvant/adjuvant setting at an interval of greater than 6 months from study entry. Median time to progression was met at 5.7 months with capecitabine plus bevacizumab. The overall response rate was 38%. In a subset analysis, there appeared to be better outcomes in the estrogen receptor (ER) positive group vs. the ER negative group.

Subsequent to the presentation of the capecitabine phase-III trial, investigators in The Breast Cancer Intergroup presented data from a randomized open-label phase-III trial (E2100) evaluating the efficacy and safety of the combination of bevacizumab and paclitaxel vs. paclitaxel alone as initial chemotherapy in patients with MBC. This trial was based on preclinical data suggesting synergistic activity for the combination of taxanes with bevacizumab against endothelial cells [17]. Patients in the chemotherapy alone arm received paclitaxel alone, while the experimental arm received the same dose and schedule of paclitaxel plus bevacizumab, on days 1 and 15. Paclitaxel plus bevacizumab prolonged PFS as compared with paclitaxel alone (11.8 vs. 5.9 months; hazard ratio = 0.60, $P < 0.001$) and significantly increased the objective response rate (36.9% vs. 21.2%; $P < 0.001$) [18]. Subset analyses of E2100 failed to reveal any population of patients receiving preferential benefit, and toxicities were similar to those seen in prior phase-III trials in other diseases. Based on this landmark trial, the Food and Drug Administration granted accelerated approval for bevacizumab in combination with paclitaxel as a first-line regimen in patients with HER2 negative MBC.

More recently, a phase-III trial of docetaxel with or without bevacizumab (the AVADO trial) was reported at the 2008 American Society of Clinical Oncology (ASCO) annual meeting. In this trial [19], 736 patients received docetaxel at 100 mg/m^2 plus placebo vs. docetaxel plus either bevacizumab 7.5 mg/kg or bevacizumab 15 mg/kg. Docetaxel was administered every 3 weeks for up to nine cycles while placebo or bevacizumab were given every 3 weeks till progression or intolerable toxicities. The median PFS was 8 months for docetaxel alone vs. 8.7 months for 7.5 mg/kg of bevacizumab and 8.8 months for 15 mg/kg of bevacizumab. The response rates for the three groups were 44.4%, 55.2%, and 63.1%, respectively. Survival data for AVADO are still immature. Although the median PFS data from AVADO are less impressive than E2100, these results provide confirmatory evidence supporting the use of bevacizumab in MBC. It is possible that the weekly taxane regimen used in E2100 is superior to q3w taxane therapy used in AVADO because of the anti-angiogenic properties of a 'metronomic' chemotherapy approach causing a dual-hit on the angiogenic pathway. Patients on the AVADO trial were also exposed to a shorter duration of chemotherapy than patients on E2100. These may have accounted for differences in results seen between the two trials.

A phase-III trial evaluating the addition of bevacizumab to standard chemotherapy in the adjuvant setting is currently ongoing (E5103). The primary aim of this trial is to compare the disease-free survival of patients treated with adjuvant therapy comprising doxorubicin, cyclophosphamide, and paclitaxel plus bevacizumab vs. without bevacizumab.

Small molecule tyrosine kinase inhibitors (TKIs)

Sunitinib is an oral, small molecule, multi-targeted TKI which blocks VEGF, c-kit, Flt-3, RET, PDGFR-α, and PDGFR-β. A phase-II trial on 64 heavily pre-treated patients with MBC using sunitinib mono-therapy demonstrated an ORR of 11% [20]. Median time to progression and overall survival were 10 and 38 weeks, respectively. A phase-III trial comparing paclitaxel plus sunitinib to paclitaxel plus bev-acizumab is ongoing. Sorafenib, another TKI, has also been tested in patients with MBC. In a phase-II trial [21], 54 hormone- or trastuzumab-refractory patients received sorafenib 400 mg orally, twice daily. One patient had a partial response and 20 (37%) patients had stable disease as their best response, with prolonged stabilization observed in 11% at 6 months. These data indicate that sorafenib monotherapy is tolerable but has limited activity as monotherapy in MBC; combination studies are being planned to further determine efficacy. Axitinib, which is another oral TKI that inhibits VEGF and PDGF (platelet-derived growth factor), has been tested in a randomized double-blind phase-II trial in patients with MBC [22]. Patients received axitinib with docetaxel or placebo with docetaxel. The time to progression was 8.2 vs. 7 months favoring the axitinib arm. Similarly, the response rates were 40% vs. 23%. There are several other TKIs against VEGF, such as cediranib, pazopanib, motesanib, and valatanib, which are currently being evaluated in patients with breast cancer.

Cotargeting VEGF and HER2

Angiogenesis involves many redundant cascades that results in the same downstream events. Pre-clinical models have shown a link between the expression of human epidermal growth factor receptor 2 (HER2) and increased transcriptional regulation of VEGF [23]. VEGF protein synthesis via activation of mTOR/p70S6K cap-dependent trans-lational pathways is also increased by HER2 [24]. Creating a 'dual anti-angiogenic' effect by cotar-geting HER2 and VEGF has been the subject of recent research efforts. A phase-I trial on nine patients with HER2 overexpressing MBC or recur-rent breast cancer, using combined HER2 and VEGF blockade found preliminary results of one complete response, four partial responses, and two patients with stable disease [25]. The combination of trastuzumab and bevacizumab did not alter pharmacokinetic parameters of either drug; however, an updated report on a follow-up phase-II trial with 37 evaluable patients receiving trastuzumab plus bevacizumab is concerning for cardiac toxicity, with one patient developing a grade IV event and an
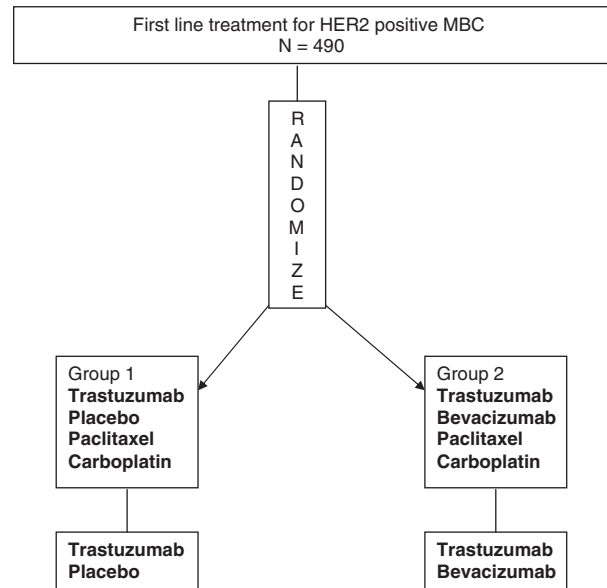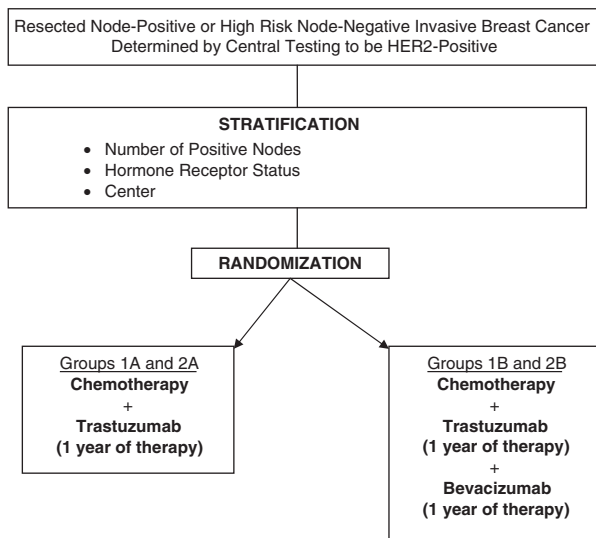

Figure 1.
E1105 schema.

additional five patients developing asymptomatic declines in left ventricular ejection fraction [26]. However, the preliminary response rate of 54% in this update is high and suggests that the combi-nation is quite active. Another study by Slamon *et al.* evaluating the efficacy and safety of dual HER2 and VEGF pathway inhibition in patients with HER2 positive advanced or MBC was recently presented at ASCO 2008 [27]. Patients received pazopanib plus lapatinib or lapatinib alone for 12 weeks. The rate of disease progression was 19% vs. 27% in the combination vs. the single agent arm, respectively; response rates were 44% vs. 30%. This rando-mized phase-II trial therefore suggested that the combination of pazopanib and lapatinib has activity in MBC. Trials using this strategy are currently ongoing, such as E1105, which is a randomized phase-III double-blind placebo-controlled trial of first-line chemotherapy and trastuzumab with or without bevacizumab for patients with HER2 over-expressing MBC (Figure 1). Similarly, in the adjuvant setting, 'BETH' is an ongoing multicenter phase-III randomized trial for patients with HER2 positive, node positive, or high risk node negative breast cancer, comparing chemotherapy plus trastuzumab with chemotherapy plus trastuzumab and bev-acizumab (Figure 2).

Toxicities with anti-angiogenic agents

The main toxicities seen with the administration of bevacizumab include hypertension; migraine-type vascular headaches; proteinuria, with or without

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┌─────────────────────────────────────────────────────────────┐
│ Resected Node-Positive or High Risk Node-Negative Invasive    │
│ Breast Cancer Determined by Central Testing to be HER2-Positive│
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                            │
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│                        STRATIFICATION                          │
│   • Number of Positive Nodes                                   │
│   • Hormone Receptor Status                                    │
│   • Center                                                     │
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                  ┌──────────────────┐
                  │  RANDOMIZATION   │
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┌──────────────────────┐   ┌──────────────────────┐
│ Groups 1A and 2A     │   │ Groups 1B and 2B     │
│ Chemotherapy         │   │ Chemotherapy         │
│      +               │   │      +               │
│ Trastuzumab          │   │ Trastuzumab          │
│ (1 year of therapy)  │   │ (1 year of therapy)  │
│                      │   │      +               │
│                      │   │ Bevacizumab          │
│                      │   │ (1 year of therapy)  │
└──────────────────────┘   └──────────────────────┘
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Figure 2.
BETH schema.

Table 1. Toxicities with bevacizumab.

Proteinuria, with or without nephrotic syndrome
Hypertension
Gastrointestinal perforation
Headaches
Bleeding
Thrombosis (venous and arterial) including myocardial infarction, cerebrovascular thrombosis, transient ischemic attacks, deep venous thrombosis

nephrotic syndrome [28]; and bleeding or thrombosis (Table 1). E2100 reported similar rates of hematologic, gastrointestinal, and musculoskeletal toxicities in the two groups in the trial [18]. Grade 3 or 4 neuropathy, infection, and fatigue were more frequent in the experimental arm. Hypertension, cerebrovascular ischemia, headaches, and proteinuria (though not clinically significant) were also more common in the combination group.

Common toxicities seen with TKIs include skin toxicities, hypertension, hand-foot syndrome, fatigue, and diarrhea. In the phase-II trial evaluating sunitinib monotherapy in patients with breast cancer [20], the most frequently reported toxicities were fatigue, nausea, diarrhea, mucositis, and anorexia. Grade 3 toxicities reported included neutropenia, fatigue, and hand-foot syndrome. Three patients developed grade 4 chemistry abnormalities: hyper uricemia, elevated alanine aminotransferase and alkaline phosphatase. Grade 4 neutropenia occurred in one patient, though there were no episodes of neutropenic fever. Many of these toxicities are not VEGF-related and presumably relate to off-target effects of these somewhat more 'promiscuous' TKIs.

Conclusion

Anti-angiogenic agents, particularly bevacizumab, are now recognized components for the medical treatment of patients with MBC, as angiogenesis plays a significant role in the development of metastasis. The exciting results of E2100 provide proof of concept for the use of anti-angiogenic agents in treating breast cancer. The recently presented AVADO trial now confirms that this treatment strategy is beneficial to patients with MBC.

Although this represents a significant advancement in the field of oncology, several challenges still exist such as the optimal duration and timing of administration. Also, determining the most appropriate biomarkers and predictors of response is a keen area of research at this time. Continued investigation of these agents and the challenges they pose by designing new generation trials with the incorporation of correlative studies will rapidly uncover the full benefits that these agents have for patients and clinicians who struggle on a day-to-day basis with MBC.

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