Significantly higher levels of vascular endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) lacking expression of steroid receptors and human epidermal growth factor receptor 2, having chemotherapy as the only therapeutic option, is characterised by early relapses and poor outcome. We investigated intratumoural (i.t.) levels of the pro-angiogenic cytokine vascular endothelial growth factor (VEGF) and survival in patients with TNBC compared with non-TNBC.

Patients and methods: VEGF levels were determined by an enzyme immunoassay in a retrospective series consisting of 679 consecutive primary breast cancer patients.

Results: Eighty-seven patients (13%) were classified as TNBC and had significantly higher VEGF levels; median value in TNBC was 8.2 pg/µg DNA compared with 2.7 pg/µg DNA in non-TNBC (P < 0.001). Patients with TNBC had statistically significant shorter recurrence-free survival [hazard ratio (HR) = 1.8; P = 0.0023], breast cancer-corrected survival (HR = 2.2; P = 0.004) and overall survival (HR = 1.8; P = 0.005) compared with non-TNBC. Patients with TNBC relapsed earlier than non-TNBC; mean time from diagnosis to first relapse was 18.8 and 30.7 months, respectively. The time between first relapse and death was also shorter in TNBC: 7.5 months versus 17.5 months in non-TNBC (P = 0.087).

Conclusions: Our results show that TNBC have higher i.t. VEGF levels compared with non-TNBC. Ongoing clinical trials will answer if therapy directed towards angiogenesis may be an alternative way to improve outcome in this poor prognosis group.

Key words: angiogenesis, survival, triple-negative breast cancer, VEGF

introduction

Breast cancer (BC) is a heterogeneous disease and has previously been divided into subgroups according to histopathological features [1]. Gene expression analysis using DNA micro arrays has revealed five subgroups of BC (luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)/neu overexpressing, basal like and normal like) with different prognosis [2–5]. Luminal, steroid receptor-positive tumours have a superior outcome compared with HER2/neu or basal-like subtypes. Basal-like BC has been reported more frequently in younger women and in BRCA1 carriers. The majority of basal-like BC lacks expression of estrogen and progesterone receptors (ER and PgR) and HER2, as do the group of triple-negative breast cancer (TNBC) classified by use of immunohistochemistry (IHC) and FISH. Although the overlap is not complete [6], basal-like cancers have been estimated to contribute to ~80% of the overall triple-negative group [7, 8], and the latter has become a convenient surrogate for basal-like BC as standard biomarkers already in clinical routine are used. TNBC contributes to ~15% of all BC and the aggressive nature of TNBC had been described by early relapses [9, 10], more commonly development of visceral metastasis [11], and poor outcome comparable to or even worse than that of HER2-positive patients before the introduction of trastuzumab [12, 13]. Basel-like BC has been characterised by expression of basal cytokeratins (CK 5/6 and 17), epidermal growth factor receptor 1 (EGFR1), and c-kit and a higher incidence of p53 mutations [5, 7, 13]. Identification of new biological key pathways driving TNBC might aid to find targets of potential interest for blockade. For example, increased expression of nestin, as well as reduced levels of E-cadherin, have been reported in basal-like BC and TNBC [14, 15]. Vascular endothelial growth factor (VEGF) has been indicated as the major angiogenic factor in human cancer [16], with numerous studies showing reduced survival times for patients with high

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levels of VEGF in the primary tumour. Increased VEGF expression is seen under hypoxic conditions and in inflammatory processes. Moreover, steroid hormones increase VEGF expression in part through an estrogen receptor response element-binding site in the promoter region of the VEGF gene \[17\]. VEGF promotes neoangiogenesis and invasion and increases vascular permeability. We and others have shown high VEGF levels within the primary breast tumour to be correlated with shorter survival times \[18, 19\] and overexpression of HER2 \[20\]. To our very best knowledge, data on expression of angiogenic factors in TNBC are so far not published. The aim with the present study was to compare intratumoural (i.t.) VEGF levels and survival for patients with primary operable TNBC defined as negative for ER, PgR and HER2 with non-TNBC. The same analyses were also carried out with the patients divided into three groups: TNBC, HER2 positive and ER/PgR positive but HER2 negative.

patients and methods

patients

A total of 679 consecutive patients with primary operable invasive BC from 1 January 1993 through 31 December 1996 at two institutions in Stockholm were included. Patients were identified by use of the Breast Cancer Registry database at the Regional Oncologic Centre, Karolinska University Hospital, Stockholm, Sweden. Patients with locally advanced BC, presence of distant metastasis at diagnosis or those subjected to neoadjuvant therapy were not included.

tumour tissue preparation

After pathological perioperative examination, representative tumour tissue was cut out and frozen in liquid nitrogen for later analysis of estrogen and progesterone levels. Frozen tumour tissue was homogenised in a micro Dismembrator (Braun, Melsungen, Germany) and suspended in cold standard receptor buffer \[10\ mmol/l Tris (pH 7.4), 1.5\ mmol/l EDTA, 10\ mmol/l sodium molybdate and 1.0\ mmol/l monothioglycerol\]. Supernatants were collected after refrigerated centrifugation at 20 000 \times g and stored at \(70^\circ\)C. The pellet fractions were analysed for DNA content by the method of Burton, in order to evaluate cell concentrations in samples.

ER and PgR analyses

ER and PgR levels were determined by cytosols by an enzyme immunoassay (Abbott Laboratories, Diagnostic Division, Abbott Park, IL). Receptor concentrations were expressed as femtomole of receptor per microgram of DNA. The cut-off value used separating ER or PgR positive from negative was 0.05 fmol receptor/\mu g DNA. Steroid receptor levels were used in clinical routine and carried out once or twice weekly pending on number of patients subjected to surgery.

VEGF analysis

A VEGF assay was carried out using a commercial quantitative immunoassay kit for human VEGF A (Quantikine human VEGF; R&D Systems, Minneapolis, MN) as earlier described \[19\]. VEGF levels in patient samples were expressed as pg/\mu g DNA. Analyses of VEGF in all samples were carried out by one person within a short time period.

HER2 analysis by IHC

In 160 cases, overexpression of HER2 was evaluated with IHC with the mAb CB11 (NovoCastra) in representative paraffin sections using the peroxidase-antiperoxidase technique for immunohistochemical assays.

HER2 analysis by enzyme-linked immunosorbent assay

The amount of HER2 protein in cytosols was determined using an enzyme immunoassay kit (Oncogene Science, San Francisco, CA) as earlier described \[20\]. Amount of HER2 was expressed in pg/\mu g DNA. Analyses of HER2 were carried out for research purpose only by one person within a short time period.

statistical methods

Pearson chi-square test was used to investigate associations between TNBC and HER2 with VEGF and other routine clinical and biological BC parameters (tumour size, nodal status, histopathological type and grade and age). VEGF was also tested as a continuous variable. Twenty-four of the 160 cases (15%) analysed with IHC were HER2 positive and consequently the 15% of patients with the highest HER2 protein levels by enzyme-linked immunosorbent assay (ELISA) were considered HER2 positive. VEGF was analysed as a dichotomous variable with the median value in the total patient population (2.8 pg/\mu g DNA) as cut-off point. Survival was estimated using the Kaplan–Meier method, and comparison between study groups was carried out with the log-rank test. Relapse-free survival (RFS), distant disease-free survival (DDFS), overall survival (OS) as well as breast cancer-corrected survival (BCCS) were calculated. To evaluate the simultaneous effect of different factors on survival, the Cox’s proportional hazard model was used. Factors were tested and included in a forward stepwise procedure. The survival time was measured from the date of diagnosis to first documented relapse or death. In all tests, the significance level was set to 0.05, and all tests were two sided. Analyses were carried out with a two-group comparison (TNBC versus non-TNBC) and a three-group comparison (TNBC versus HER2 positive versus ER and/or PgR positive but HER2 negative). The latter group is referred to as ER/PgR positive.

results

patients’ characteristics

Information regarding patients’ characteristics was obtained from the Breast Cancer database at the Regional Oncologic Centre, Karolinska University Hospital, Stockholm, Sweden. This database reveals information on all patients with primary BC regarding several parameters at the time of diagnosis as age, way of detection (mammogram screening or not), type of surgery, pathologic tumour size in millimetre, number of removed axillary lymph nodes, number of axillary metastases, as well as levels of ER and PgR. The database also includes the adjuvant therapy recommended (radiotherapy and adjuvant systemic therapy), date and type of first relapse and date and cause of death. For classification of TNBC, levels of ER and PgR from the registry in combination with HER2 status previously determined for research purpose were used. No regrading of tumours was done. Patients’ records were used to collect data on histopathological type and grade, to ensure correct data on adjuvant therapy actually given. The median age of patients included was 63 years (range 29–92), and the median follow-up time 92 months (range 59–119). The median tumour size was 16 mm, 420 patients had a node-negative BC and 195
node-positive BC, while in 56 patients axillary dissection was not carried out in elderly patients with concomitant diseases. Patients subjected to breast-conserving surgery received postoperative radiotherapy against remaining breast parenchyma ($n = 358$). Adjuvant therapy was given to a total of 592 patients. Different types of adjuvant chemotherapy (combination chemotherapy with cyclophosphamide, methotrexate and fluorouracil, standard or dose-escalated 5-fluorouracil, epirubicin, cyclophosphamide) were given to 69 patients (13%). Adjuvant endocrine therapy, alone or combined with chemo- or radiotherapy, was given to 457 patients, mainly as tamoxifen for 2 or 5 years. Despite negative steroid receptors, forty-four TNBC patients received tamoxifen (Table 1). The medical ethical committee of the Karolinska Institute, Stockholm, Sweden, approved the study design.

Table 1. Clinicopathological characteristics of TNBC ($n = 87$) and non-TNBC ($n = 592$) patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>TNBC, n (%)</th>
<th>non-TNBC, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients enrolled</td>
<td>87</td>
<td>592</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal invasive</td>
<td>72 (83)</td>
<td>442 (75)</td>
</tr>
<tr>
<td>Lobular invasive</td>
<td>1 (1)</td>
<td>53 (9)</td>
</tr>
<tr>
<td>Ductal and lobular</td>
<td>2 (2)</td>
<td>24 (4)</td>
</tr>
<tr>
<td>Others</td>
<td>5 (6)</td>
<td>45 (7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (8)</td>
<td>28 (5)</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range), mm</td>
<td>16 (3–75)</td>
<td>15 (2–92)</td>
</tr>
<tr>
<td>T1</td>
<td>33 (38)</td>
<td>183 (31)</td>
</tr>
<tr>
<td>T2–4</td>
<td>54 (62)</td>
<td>409 (69)</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Node negative</td>
<td>61 (70)</td>
<td>362 (61)</td>
</tr>
<tr>
<td>Node positive</td>
<td>20 (30)</td>
<td>180 (29)</td>
</tr>
<tr>
<td>1–3</td>
<td>14 (16)</td>
<td>46 (16)</td>
</tr>
<tr>
<td>4–9</td>
<td>4 (5)</td>
<td>11 (4)</td>
</tr>
<tr>
<td>≥10</td>
<td>2 (2)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (7)</td>
<td>50 (8)</td>
</tr>
<tr>
<td>Histopathological grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3 (3)</td>
<td>56 (10)</td>
</tr>
<tr>
<td>II</td>
<td>33 (38)</td>
<td>200 (34)</td>
</tr>
<tr>
<td>III</td>
<td>40 (46)</td>
<td>221 (37)</td>
</tr>
<tr>
<td>Not analysed</td>
<td>11 (12)</td>
<td>115 (19)</td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (&gt;0.05 fmol/μg DNA)</td>
<td>0 (0)</td>
<td>511 (86)</td>
</tr>
<tr>
<td>Negative (&lt;0.05 fmol/μg DNA)</td>
<td>87 (100)</td>
<td>81 (14)</td>
</tr>
<tr>
<td>PgR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (&gt;0.05 fmol/μg DNA)</td>
<td>0 (0)</td>
<td>480 (81)</td>
</tr>
<tr>
<td>Negative (&lt;0.05 fmol/μg DNA)</td>
<td>87 (100)</td>
<td>112 (19)</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>0 (0)</td>
<td>101 (15)</td>
</tr>
<tr>
<td>VEGF median (range)</td>
<td>8.2 (0.0–661.3)</td>
<td>2.7 (0.0–502.0)</td>
</tr>
<tr>
<td>Adjuvant systemic therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine therapy only</td>
<td>34 (39)</td>
<td>375 (63)</td>
</tr>
<tr>
<td>Chemotherapy only</td>
<td>6 (7)</td>
<td>35 (6)</td>
</tr>
<tr>
<td>Both chemo- and endocrine therapy</td>
<td>1 (1)</td>
<td>47 (8)</td>
</tr>
<tr>
<td>No systemic adjuvant therapy</td>
<td>46 (53)</td>
<td>135 (23)</td>
</tr>
</tbody>
</table>

TNBC, triple-negative breast cancer; ER, estrogen receptor; PgR, progesterone receptor; VEGF, vascular endothelial growth factor.

patients’ outcome

With a median follow-up of 92 months, a total of 151 recurrences (58 local, 73 distant and 20 both local and distant) and 160 deaths (69 due to BC and 91 unrelated to BC (i.e. in patients without any documented relapses) have been recorded in all patients. Eighty-seven (13%) patients were classified as TNBC and 101 as HER2 positive (15%).

expression of VEGF

The median level of VEGF in the total patient population was 2.8 pg/μg DNA (range 0.0–661.3). The VEGF levels in TNBC were statistically significantly higher: median 8.2 pg/μg DNA (range 0.0–661.3) compared with 2.7 pg/μg DNA (range 0.0–502.1) in non-TNBC ($P < 0.001$). When non-TNBC was split into HER2-positive and ER/PgR-positive patients, the median values were 5.9 pg/μg DNA (range 0.0–502.1) and 2.4 pg/μg DNA (range 0.0–5.9), respectively ($P = 0.002$). Fifty-four of 87 patients (62%) with TNBC had a VEGF expression above the median value compared with 281 out of 592 patients (47%) with non-TNBC, Pearson chi-square ($P = 0.036$).

expression of HER2 determined by IHC and ELISA

Twenty-four of 160 patients (15%) were found to overexpress HER2 with IHC. A wide range of HER2 content was found when analysed quantitatively with ELISA, and the majority of patients had low levels. The median value in the total patient population was 0.18 pg/μg DNA (range 0.02–4.96). A statistically significant correlation between HER2 status determined by IHC and ELISA, respectively, was seen ($P < 0.001$). Of patients classified as 3+ with IHC, 88% were classified as positive with the ELISA method.

absence of correlations between TNBC and prognostic or biologic factors

TNBC were not significantly correlated with tumour size (T1 versus T2–3) ($P = 0.07$), nodal status (node negative versus node positive) ($P = 0.11$), histological grade (I + II versus III) ($P = 0.17$), type of relapse (distant versus local) ($P = 0.82$) or age (≤50 versus >50 years) ($P = 0.18$).

associations between HER2 and other prognostic or biologic factors

Overexpression of HER2 protein by ELISA was significantly associated with higher VEGF expression (Smedian versus >median) ($P < 0.01$), negative ER ($P = 0.04$), presence of lymph node metastases ($P = 0.01$) and poorly differentiated tumours ($P = 0.04$). No correlation was seen between HER2 and PgR status, tumour size or age (not shown).

survival—univariate analysis—TNBC

Patients with TNBC had a significantly worse prognosis than non-TNBC (two-group comparison): RFS [hazard ratio (HR) = 1.8; $P = 0.002$], OS (HR = 1.8; $P = 0.005$) as well as BCSS (HR = 2.2; $P = 0.005$). For DDFS, a similar trend was seen although not reaching statistical significance (HR = 1.6; $P = 0.088$) (Figure 1A–D and Table 2). When HER2-positive patients were separated from the non-TNBC group, we found
a similar inferior outcome for TNBC and HER2-positive patients compared with the ER/PgR-positive group (three-group comparison). The difference was seen in RFS (HR = 2.1; \( P < 0.001 \)), DDFS (HR = 1.8; \( P = 0.032 \)), OS (HR = 2.0; \( P = 0.001 \)) and BCCS (HR = 2.8; \( P < 0.001 \)) (Figure 2A–D and Table 2). Differences in survival remained when we investigated node-negative patients separately with an impaired survival for patients with TNBC. The corresponding figures were for node-negative patients: RFS (HR = 2.4; \( P = 0.004 \)), DDFS (HR = 3.0; \( P = 0.006 \)), OS (HR = 2.0; \( P = 0.026 \)) and BCCS (HR = 5.1; \( P < 0.001 \)). In the node-positive group, TNBC was statistically significantly correlated with RFS (HR = 2.2; \( P = 0.007 \)) and OS (HR = 2.1; \( P = 0.015 \)) while no difference was seen for DDFS (HR = 1.4; \( P = 0.426 \)) and BCCS (HR = 2.0; \( P = 0.074 \)).

In the total triple-negative group, node-negative as well as node-positive high VEGF expression was significantly correlated to impaired BCCS (HR = 5.1; \( P = 0.029 \)), a trend not reaching statistical significance was found for RFS (HR = 2.1; \( P = 0.066 \)).

time of recurrences with reference to TNBC
Distant recurrences for TNBC patients were registered early with a peak from 1 to 4 years of follow-up time. The mean time from diagnosis to first recurrence in TNBC was 18.8 months compared with 30.7 months in non-TNBC. Similarly, the mean time from diagnosis to distant metastasis was shorter for TNBC patients with a mean time of 19.0 months compared with 33.9 months for non-TNBC. The time between the first relapse and death was also shorter in TNBC: 7.5 months versus 17.5 months in non-TNBC (\( P = 0.087 \)) (Figure 3).

survival—univariate analysis according to HER2, VEGF and prognostic factors
Univariate analysis of the total patient population showed high HER2 content to be significantly correlated with shorter RFS (\( P = 0.011 \)), OS (\( P = 0.024 \)) and BCCS (\( P = 0.004 \)), respectively. Likewise, a higher VEGF content as well as the following routine prognostic factors were significantly correlated with shorter RFS, BCCS as well as OS: negative ER status, negative PgR status, histological grade III, tumour size >20 mm and presence of axillary metastases (Table 2).

survival—multivariate analysis
A Cox proportional hazard regression model was used to estimate HRs. Factors tested were those statistically significantly correlated with survival in univariate analyses and age. HR >1.0 indicated a greater risk of recurrence or death than for the comparative group set as reference. TNBC did not remain statistically significantly correlated with an
increased risk of RFS (HR = 1.7; \( P = 0.08 \)), DDFS (HR = 1.4; \( P = 0.41 \)), OS (HR = 1.5; \( P = 0.17 \)) or BCCS (HR = 1.6; \( P = 0.26 \)), respectively (two-group comparison) (Table 2).

Neither in multivariate analyses with the three-group comparison did TNBC remain significantly correlated with survival.

**Discussion**

The results from this retrospective study including a relatively large series of consecutive patients with operable primary BC confirms the inferior outcome for patients with TNBC, in our series as poor as for HER2-positive patients before the introduction of trastuzumab [12, 13]. As the only therapeutic option for patients with TNBC today is chemotherapy, research has during recent years focused on increased knowledge about key pathways in TNBC aiming at discovery of new treatment options. Our results show, to our very best knowledge for the first time, that TNBC has significantly higher i.t. levels of the angiogenic factor VEGF. The VEGF content was three times higher in TNBC compared with the ER/PgR-positive group and 1.5 times higher compared with the HER2-positive group. Interestingly, the region 6p21–p25 harbouring the VEGF gene as well as several candidate oncogenes as DEK, E2F3, NOTCH4, PIM1 and CCND3 has been reported as often amplified in basal-like BC [21]. Among the biological aberrations more frequently reported in basal-like BC or TNBC, abnormalities of p53 [3, 12, 22] deserve
a special focus in relation to VEGF. In cell lines, wildtype p53 has been shown to suppress angiogenesis through transcriptional activation of the thrombospondin-1 gene and by down-regulation of the VEGF promoter region [23]. We and others have shown a significant correlation between higher VEGF expression and overexpression and/or mutated p53 in patients with BC, indicating loss of wildtype p53 as in part responsible for increased angiogenesis [24–26]. In the pilot work of Sorlie et al. [3], p53 mutations were found in >80% of the basal-like subtype, compared with 15%–20% reported in general primary BC patient populations [24, 25] and almost 30% among relapsing patients [26].

Patients with TNBC were found to have an inferior outcome compared with non-TNBC. Relapses occurred significantly earlier in the TNBC group than in non-TNBC and the majority of recurrences were seen within 1–4 years of follow-up time. Similar results have been reported in two large studies, both showing early recurrence peaks before 3 years of follow-up time [8, 9]. Contrary to our results, one of these found larger tumour size and a higher frequency of lymph node involvement in TNBC compared with non-TNBC [8]. In our study, a trend not reaching statistical significance was seen between TNBC and smaller tumours, while no difference was found regarding nodal status. Shorter survival times have been reported in node-negative [27, 28] as well as node-positive TNBC [7]. We found the TNBC subtype to be correlated with an inferior survival in above all node-negative patients supporting the hypothesis that TNBC may be predisposed to haematological spread. In concordance with previous studies, we found shorter survival times following first relapse for patients with TNBC: 7.5 months compared with 17.5 months in the non-TNBC group [8, 10].
Contrary to others [8, 29] we did not find a correlation between TNBC subtype and histological grade. In this retrospective study, we used information from pathology reports in patients’ records and did not carry out regrading according to Elston and Ellis used today. This together with the fact that knowledge of grade was lacking in 20% of patients may in part explain why in this material, a correlation was not found. Another drawback with the present study is the method for HER2 determination. During the years patients included in our study were diagnosed with BC, HER2 status was not part of clinical routine. For research purposes only, we used an ELISA in the total population with concomitant IHC data in a quarter of the patients. However, a large study has shown determination of HER2 by an enzyme immunoassay as a possible alternative to IHC [30]. A high correlation between the methods as well as the anticipated reduced survival times for patients classified as HER2 positive with the ELISA method were reported and also found in the present study. Taking into account that some patients are misclassified, we believe that our results can be guarded as hypothesis generating.

New strategies in the search for effective treatment options for patients with TNBC have focused on both new chemotherapy regimens and targeted therapies. Despite that overexpression of EGFR1 is reported more frequently in TNBC, EGFR1 blocking therapy in the neoadjuvant setting was found less effective in TNBC compared with receptor-positive patients [31]. TNBC patients who do not reach a pathological complete remission (pCR) after neoadjuvant chemotherapy have a high risk for early recurrences [9, 32]. However, an Italian study in which TNBC patients received a low-dose metronomic therapy for 4–6 months as maintenance after neoadjuvant chemotherapy and surgery has so far resulted in a 2-year RFS of 88%, which is a duplication of patients receiving a pCR (40%) [33]. Metronomic chemotherapy consisting of daily cyclophosphamide and methotrexate twice per week (CM) has successfully been investigated in metastatic BC. Patients responding to therapy showed a significant decrease in serum levels of VEGF, indicating its function to be in part by inhibition of angiogenesis [34]. Results from the Eastern Cooperative Oncology Group E2100 trial comparing weekly paclitaxel alone or in combination with bevacizumab, a humanised antibody directed towards VEGF has shown effect in advanced BC, also in the subgroup of TN patients [35]. In summary, we demonstrate for the first time significantly higher i.t. levels of VEGF in TNBC compared with non-TNBC. Whether blockade of angiogenesis will improve the outcome in this group of patients is under investigation in ongoing adjuvant phase III trials investigating the addition of 1 year of maintenance therapy with CM (International Breast Cancer Study Group-22-00) as well as 1 year of bevacizumab (BEATRICE) to standard chemotherapy.

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**references**


