Pilot study of bevacizumab in combination with docetaxel and cyclophosphamide as adjuvant treatment for patients with early stage HER-2 negative breast cancer, including analysis of candidate circulating markers of cardiac toxicity: ICORG 08–10 trial

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Ther Adv Med Oncol

2019, Vol. 11: 1–9

DOI: 10.1177/1758835919864236

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Abstract

Background: Combining bevacizumab and chemotherapy produced superior response rates compared with chemotherapy alone in metastatic breast cancer. As bevacizumab may cause hypertension (HTN) and increase the risk of cardiac failure, we performed a pilot study to evaluate the feasibility and toxicity of a non-anthracycline-containing combination of docetaxel with cyclophosphamide and bevacizumab in early stage breast cancer patients.

Methods: Treatment consisted of four 3-weekly cycles of docetaxel and cyclophosphamide (75/600 mg/m^2). Bevacizumab was administered 15 mg/kg intravenously on day 1, and then every 3 weeks to a total of 18 cycles of treatment. Serum biomarker concentrations of vascular endothelial growth factor (VEGF), cardiac troponin-I (cTnI), myeloperoxidase (MPO), and placental growth factor (PlGF) were quantified using enzyme-linked immunosorbent assay (ELISA) in 62 patients at baseline and whilst on treatment to determine their utility as biomarkers of cardiotoxicity, indicated by left ventricular ejection fraction (LVEF).

Results: A total of 106 patients were accrued in nine sites. Median follow up was 65 months (1–72 months). Seventeen protocol-defined relapse events were observed, accounting for an overall disease-free survival (DFS) rate of 84%. The DFS rates for hormone receptor positive (HR+) and triple-negative (TN) patients were 95% *versus* 43%, respectively. The median time to relapse was 25 (12–54) months in TN patients *versus* 38 (22–71) months in HR+ patients. There have been 13 deaths related to breast cancer . The overall survival (OS) rate was 88%. The 5-year OS rate in HR+ *versus* TN was 95% *versus* 57%. None of the measured biomarkers predicted the development of cardiotoxicity.

Conclusions: We observed a low relapse rate in node-positive, HR+ patients; however, results in TN breast cancer were less encouraging. Given the negative results of three large phase III trials, it is unlikely that this approach will be investigated further.

Trial Registration

ClinicalTrials.gov Identifier: NCT00911716.

Keywords: bevacizumab, breast cancer, cardiotoxicity biomarker, docetaxel/cyclophosphamide

Received: 14 August 2018; revised manuscript accepted: 29 May 2019.

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Introduction

Angiogenesis is one of the hallmarks of cancer, and anti-angiogenic drugs have been the focus of intense study in clinical trials. Vascular endothelial growth factor (VEGF) occupies a central regulatory role in the process of angiogenesis. High levels of circulating VEGF predict a poor prognosis in cancer patients.[1] The VEGF family consists of several signal protein variants and their receptors. Among them, the interaction between VEGF-A and VEGF receptor (VEGFR) subtype 2 is predominant in the formation of new blood vessels. Several studies have found a significant correlation between VEGF expression and clinical outcome in breast cancer (BC), with disease-free survival (DFS) and overall survival (OS) being significantly worse among those patients who have cancers overexpressing VEGF.[2] Micro-metastases seem to depend on angiogenesis,[3] therefore, targeting the anti-angiogenic switch before tumour vascularization, in the adjuvant setting when few pro-angiogenic factors are involved, has been hypothesized as an appropriate setting for anti-angiogenic therapy.

Bevacizumab is a humanized monoclonal antibody that binds and neutralizes the biological activities of the human VEGF isoforms, resulting in a steric blocking of the binding of VEGF to both VEGFR 1 and VGFR 2. The positive results of the first random assignment trial on HER2-negative metastatic BCs led to the approval of bevacizumab in combination with paclitaxel chemotherapy. However, following the conflicting results of trials published subsequently, in 2011 the US Food and Drug Administration (FDA) withdrew their approval of bevacizumab, whereas the European Medicines Agency maintained their approval. Meta-analyses and subgroup analyses of trials conducted in the advanced setting have indicated that the triple-negative (TN) metastatic population could derive a bigger benefit from the addition of bevacizumab to chemotherapy than the oestrogen receptor (ER) or progesterone receptor (PR) positive counterpart.[4] However, data from the BEATRICE,[5] BETH,[6] E2104[7] and E5103[8] trials showed no benefit from the addition of bevacizumab to adjuvant chemotherapy in TN or HER2+ early stage BC, respectively.

Based on the positive results of clinical trials conducted in the metastatic disease, we designed the ICORG 08–10 trial as a feasibility pilot study of bevacizumab in addition to adjuvant chemotherapy for node-positive or high-risk node-negative HER2-negative BC. Interestingly, no randomized clinical trial of bevacizumab in the adjuvant setting has been published in the HER2-negative, ER-positive or PR-positive population. Furthermore, a rationale exists to study biomarkers of cardiotoxicity (CT) in this setting as bevacizumab causes hypertension (HTN) and may increase the risk of cardiac failure.[9] Moreover, taxanes may (rarely) perturb cardiac rhythm[10] and cyclophosphamide (at high doses) can cause myocarditis, pericarditis and heart failure.[11] The use of serum biomarkers as indicators of CT is therefore a burgeoning field in cardio-oncology, not least as the diagnosis and management of CT in patients being treated with cytotoxic and biological agents is difficult.[12] As part of ICORG 08–10 we also studied changes in a panel of previously implicated serum biomarkers of CT [cardiac troponin-I (cTnI), placental growth factor (PlGF), myeloperoxidase (MPO)][12,13] and vascular endothelial growth factor (VEGF) as putative biomarkers of CT.

Patients and methods

ICORG 08–10 was a single-arm nonrandomized pilot study designed to investigate the safety and feasibility of the combination of docetaxel and cyclophosphamide (TC) plus bevacizumab in the adjuvant treatment of patients with early stage HER2-negative, high-risk BC.

The primary objective of the study was to investigate the feasibility of the combination, especially in relation to cardiac toxicity, HTN and bleeding complications. Secondary objectives were to conduct translational studies to investigate serum biomarker levels during and after treatment with bevacizumab, and noncomparative efficacy measures, including DFS and OS.

The main eligibility criteria were: early stage BC that was HER2 negative, node-positive or high-risk node-negative defined as tumour size >2 cm and ER/PR negative, or tumour size >3 cm irrespective of ER/PR status. Patients must have normal cardiac ejection fraction (EF), no active or uncontrolled cardiovascular disease, normal organs and marrow function. For full inclusion exclusion criteria please see the Supplemental Material. The name of the Ethics Committee for this study is: The study was conducted according to the principles of Good Clinical Practice, the provisions of the Declaration of Helsinki, and other applicable local regulations. The study was

approved by Tallaght University Hospital/St. James's Hospital Joint Research Ethics Committee (REC; formerly SJH/AMNCH Research Ethics Committee). The approval number is not applicable as this REC did not provide a reference for the trial. Written informed consent was obtained from each participant enrolled onto the study. The approval number is not applicable as this REC did not provide a reference for the trial. Written informed consent was obtained from each participant enrolled onto the TC-Avastin study.

Primary end point and statistical justification

The primary endpoint of TC-Avastin study was to identify the percentage of patients with congestive heart failure (CHF), defined as left ventricular ejection fraction (LVEF) below the lower limit of normal (<55%) and ⩾20% decrease from baseline. The primary evaluation was based on the percentage of patients experiencing CHF. A true CHF rate of <4% is considered acceptable and a true CHF rate of >10% would be unacceptably high. The Simon single-stage design with a probability of 0.05 of incorrectly rejecting the unacceptable rate and a probability of 0.2 of incorrectly rejecting the acceptable rate, requires enrolling 101 patients meeting this criteria. Allowing up to 5% of the patients to not start treatment or to not have adequate cardiac function at baseline, a total of 106 patients were entered over a period of 1 year.

Procedures

Study treatment consisted of four 3-weekly cycles of intravenous (iv) docetaxel 75 mg/m^2 and cyclophosphamide 600 mg/m^2. Patients commenced bevacizumab 15 mg/kg iv on day 1 of cycle 1, and continued every 3 weeks to a total of 18 cycles. For full treatment details, see the Supplemental Material.

Cardiac safety monitoring

As assessing cardiovascular toxicity was a primary objective of the ICORG 08–10 trial, we measured LVEF by Echocardiogram (ECHO) and/or Multigated acquisition (MUGA) at baseline, after the second and fourth cycle of chemotherapy and then at 6, 9 and 12 months from registration. Four further LVEF measurements were taken, one per year. The same method of assessment was used throughout the study. Standard 12-lead electrocardiogram (ECG) and assessment of serum troponin and brain natriuretic peptide (BNP) were carried out at the same time as each LVEF assessment. The definitions of cardiac events are included in the Supplemental Materials.

Serum biomarker analysis

As part of the clinical trial design, serum was collected at baseline (prior to treatment), after cycle 4 (around 3 months) and at 6 months. In the initial study protocol, serum samples were taken only for VEGF analysis, however the protocol was later amended to include PlGF, MPO and cTn1 analysis in surplus serum samples. Only patients for whom baseline serum was available could be included in this study ($n = 62$).

Briefly, blood was collected in a nonheparinized serum tube, allowed to clot for 30 min to 1 h at room temperature and centrifuged at 1000*g* for 15 min at room temperature. Serum was removed into fresh cryovials, immediately frozen at −20°C and subsequently stored at −80°C. All measurements were performed on previously unthawed samples. VEGF levels were measured within the context of the clinical trial, at baseline and 6 months. Subsequently, serum from baseline and cycle 4 (3 months) was available for MPO, cTnI and PlGF analysis. VEGF analysis was performed using a human VEGF enzyme-linked immunosorbent assay (ELISA; R&D Systems) according to the manufacturer's instructions. The lower limit of detection of the VEGF ELISA is 9 pg/ml, therefore this was selected as a threshold for VEGF positivity. Plasma from a healthy volunteer, and serial dilutions of recombinant VEGF125 (R&D Systems), incubated for 2 h with 0.35 mg/ml of bevacizumab (obtained from St Vincent's University Hospital), were used as controls. For cTnI, MPO and PlGF, biomarker levels were also assessed using ELISA assays (BlueGene Biotech Ltd. Shanghai, China) following manufacturer's instructions. Coefficient of variation values (CVs) for all assays were less than 10% and sensitivities were 0.1 ng/ml for cTnI and MPO and 1.0 pg/ml for PlGF.

To assess the utility of MPO, cTnI, PlGF and VEGF as early toxicity biomarkers, patients were initially stratified into two groups, those that presented with CHF and those who did not, as per the primary end point of the trial itself. However due to low numbers in the CHF group available

for serum analysis ($n=3$) this comparison was underpowered to determine a difference between groups. As such, patients were restratified into two groups where CT was instead defined (as per previously published studies[13,14]) as a reduction in LVEF of ⩾5% to <55% with symptoms of heart failure or an asymptomatic reduction in LVEF of ⩾10% to <55%. Group 1 consisted of patients who presented with CT during the study and Group 2 consisted of those patients who did not [no cardiotoxicity (NCT)].

Statistical considerations and sample size

A total of 106 patients were consented and entered the ICORG 08–10 TC-Avastin study.

DFS was defined as the interval between the date of registration and first invasive recurrence of BC, contralateral invasive BC, second (primary) non-breast invasive cancer, or death from any cause, whichever occurred first. OS was calculated from the date of registration to the date of death from any cause, or date of last recorded follow up.

A subcohort of 62 patients were included in the toxicity biomarker study. For each patient, the biomarker fold-change (ratio) was determined at 3 months relative to the baseline measurement (MPO, cTnI, PlGF) or at 6 months (VEGF). The log base-2 of the ratio of 3 months to baseline, which represents a doubling in biomarker level, of fold-change was analysed for each biomarker using simple logistic regression [adjusting for age, body mass index (BMI) and angiotensin-converting enzyme (ACE) inhibitor intake] with presence of CT (as defined by a reduction in LVEF of ⩾5% to <55% with symptoms of HF or an asymptomatic reduction in LVEF of ⩾10% to <55%) at any visit during follow up as the primary outcome. In addition, biomarker levels between the CT and NCT groups were compared using two-sample t tests. Ignoring CT status, simple regression analyses were used to determine possible (linear) relationships between biomarker levels and LVEF. In these exploratory analyses, adjustments for multiple testing were not performed. Data management, screening for anomalous data values and statistical modelling were performed using SAS Version 9.3.

Results

A total of 106 female patients were accrued in nine ICORG sites in Ireland between December 2008 and July 2010. Patients' characteristics are summarized in Table 1. The great majority of patients enrolled had axillary lymph node involvement (93%) and ER- and/or PR-positive status (78%). A total of 22% of patients had TN BC.

The study database was locked and analysed as of 31 March 2016. Median duration of follow up from registration was 65 months (range 1–72 months).

Adverse events

A total of 26 patients (24.5%) were removed from the study due to HTN ($n=9$), intestinal/gastrointestinal perforation ($n=2$), pain ($n=2$), nausea, abdominal pain, sinusitis, tooth abscess, breast abscess, incision site cellulitis, decrease in white cell count, neutropenia, hypersensitivity, proteinuria, anal fistula, headache and arthralgia (all $n=1$); Supplemental Table 1. The two intestinal perforations occurred at cycles 1 and 16, respectively, and neither patient had prior abdominal surgery or a previous history of intestinal disease.

There were no deaths attributable to the study treatment, however 22 patients had a serious adverse event related to treatment. Only 4 patients had a discontinuation of the chemotherapy in their drug regimen relating to an adverse event, whereas 16 women had discontinuation of bevacizumab (Supplemental Table 2).

The estimated proportion of patients with CHF was 3.8% ($n=4$; 95% CI 1.5–9.3% using Wilsons method), noting that upper bound of 9.3% is below the 10% mark considered unacceptable in the protocol. Three of the women with asymptomatic CHF had right-sided BC and received radiotherapy as part of their treatment. The only women to have symptomatic CHF had a left-sided BC, but also received radiotherapy as part of her treatment. In this case, however, she also had a history of diabetes. Moreover, we observed a LVEF drop >10% from baseline during study treatment in 41 patients (39%). In 8 patients (7.5%) LVEF declined below 50%: 6 are documented to have recovered to normal, 2 had no further LVEF measurements (1 patient declined, 1 unknown reason). A total of 92 patients were evaluated by ECHO, 3 patients were evaluated by ECHO and MUGA, and a further 11 patients we evaluated by MUGA. Normal troponin and BNP levels have been observed in all 57 patients with serial measurements.

HTN of any grade occurred in 56 out of 106 patients (53%) during treatment period. Grade 3 HTN

Table 1. Baseline clinicopathological characteristics treated in ICORG 08–10 TC-Avastin study.

	N **= 106**
Median age (range)	52 (25–75)
Tumour size	
T1 (>0 cm to <2 cm)	33 (31%)
T2 (2 cm to <5 cm)	65 (61%)
T3 (>5 cm)	4 (4%)
T4	2 (2%)
Unknown	2 (2%)
Hormone receptors (HR) status	
ER and/or PR positive	83 (78%)
ER and PR negative (TN)	23 (22%)
Positive axillary lymph nodes	
0	7 (7%)
1–3	66 (62%)
>4	33 (31%)
ER+ BC positive axillary node	80/83 (96%)
TN BC positive axillary node	19/23 (83%)
Histology	
Invasive Ductal	91 (86%)
Invasive Lobular	13 (12%)
Other subtypes	2 (2%)

BC, breast cancer; ER, oestrogen receptor; PR, progesterone receptor; TN, triple negative.

occurred in 26 patients (25%), no Grade 4 HTN was recorded. A total of 48 patients (45%) required medical treatment for HTN, whereas 7 patients with HTN did not start treatment. Among patients who developed HTN while on study treatment and who completed bevacizumab as per protocol, 40 (82%) were still on at least one antihypertensive 4 weeks after last infusion of bevacizumab.

Clinical outcome

Seventeen protocol-defined relapse events have been observed, accounting for an overall DFS rate of 84%. The DFS rates for hormone receptor (HR)-positive and TN patients are 95% and 44%, respectively. The median time to relapse from registration was 25 (12–54) months in TN patients *versus* 38 (22–71) months in HR-positive patients. There have been 13 deaths, all related to relapsed BC. The OS rate was 88%. Five-year OS rates in HR-positive and in TN patients were 95% *versus* 57%. In Figure 1 we report the Kaplan–Mayer curves for DFS and OS for the overall study population and for the two subgroups: HR-positive and TN, respectively.

MPO, cTnI, PlGF and VEGF as early biomarkers of CT

In the cohort of patients that could be included in the serum biomarker study ($n = 62$), 12 patients were categorized into the CT group whereas 50 were NCT. Early biomarker fold change from baseline to 3 months (MPO, cTnI, PlGF) or baseline to 6 months (VEGF) was calculated. Patients were excluded where no available measurement of serum biomarkers were available at either baseline or follow-up months.

MPO and cTnI levels were significantly higher after 3 months in this patient cohort, whereas there was no significant difference in levels of PlGF at these time points. VEGF was significantly lower at 6 months compared with baseline levels (Supplemental Table 3). Klettner and Roider[15] suggested that binding of bevacizumab to VEGF sterically inhibits recognition of VEGF by ELISA. To determine whether the decrease in VEGF observed after 6 and 12 months of treatment was due to bevacizumab, serial dilutions (2000 to 31.25 pg/ml) of recombinant VEGF were incubated for 2 h with a clinically relevant concentration of bevacizumab (0.35 mg/ml)[16] and VEGF levels were determined by ELISA. VEGF was undetectable in the presence of bevacizumab (data not shown). Therefore, our results suggest that bevacizumab has efficiently neutralized VEGF after 12 months of treatment in 50 patients (serum VEGF levels ⩽9 pg/ml), whereas in 25 patients VEGF remained detectable (range 11.5–332.4 pg/ml), suggesting that bevacizumab has not completely bound all VEGF in these cases.

Data showing comparison of biomarkers between CT and NCT groups are presented in Supplemental Table 4. No differences between the CT and NCT group biomarker levels were apparent for any of the four biomarkers. The

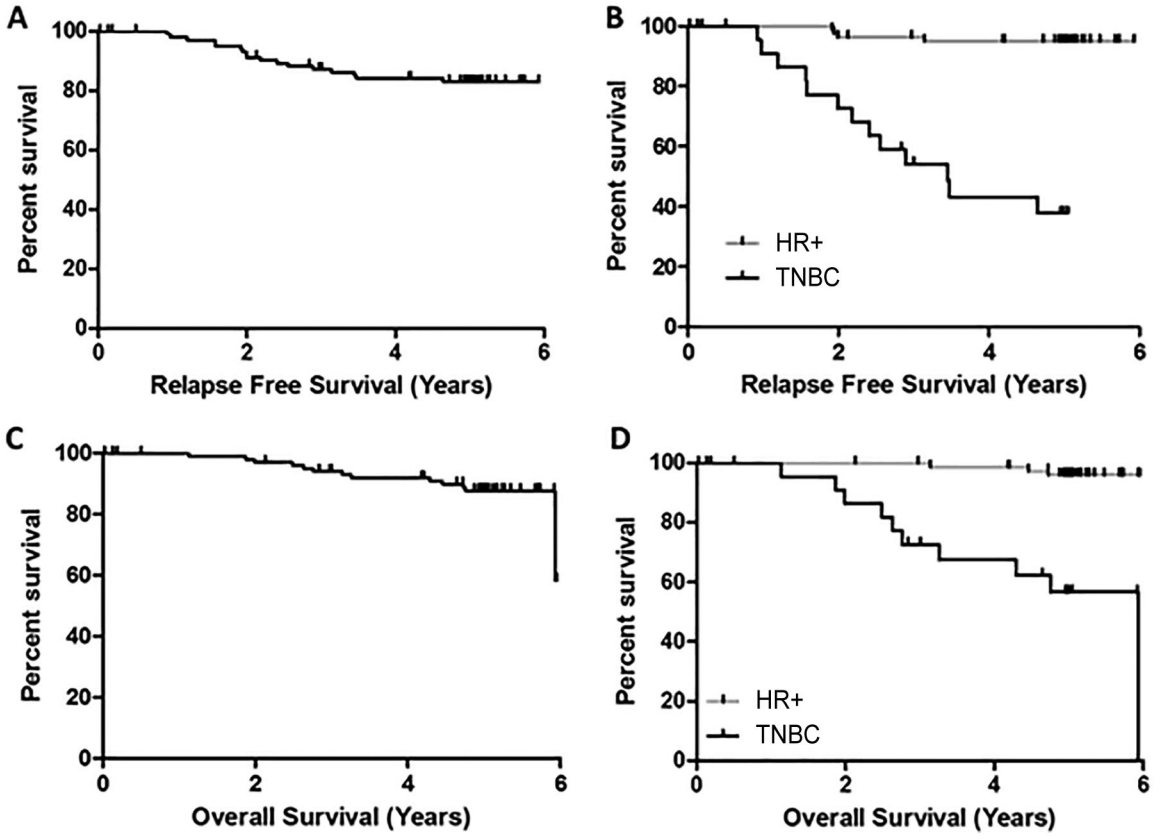

Figure 1. Survival analysis of the TC-Avastin 08–10 clinical study. (A) Relapse-free survival for all patients and (B) in those patients who were either triple-negative (TN) or Hormone receptor (HR)-positive (HR+). (C) Overall survival for all patients on the study and (D) in those patients who were either TN or ER-positive.

logistic regression analysis, adjusting for age, BMI and ACE inhibitor intake, showed similar results (data not shown). None of the biomarkers were predictive of later CT phenotype. In addition, multiple regression analysis indicated that a combination of these markers was not predictive of CT. Nevertheless, by examining potential linear relationships with LVEF (Figure 2) we observed that higher baseline PlGF was correlated to a lower overall LVEF at cycle 3 (Figure 2(A), $R=0.22$, $p=0.0002$) and a more pronounced decrease in LVEF compared with baseline LVEF at the same time point (Figure 2(B), $R=0.15$, $p=0.003$). Conversely, a larger decrease in circulating VEGF at 6 months compared with baseline was correlated with a higher LVEF at a number of subsequent cycles (Table 4 and Figure 2C, $p<0.05$).

Discussion

ICORG 08–10 was designed to assess the feasibility of adding bevacizumab to an adjuvant non-anthracycline-containing chemotherapy regimen for early stage HER2-negative BC. Our data suggest that while the regimen is feasible, it is associated with substantial toxicity. The overall toxicity profile of bevacizumab in our study is similar to that which had been reported for patients with metastatic BC, with HTN being the most frequent adverse event observed (54%). Bevacizumab discontinuation due to toxicity was relatively frequent (12%).

Despite the absence of an anthracycline, CHF was seen in 4% of patients. This is striking, and its occurrence may be contributed to by the use of radiotherapy or prior underlying conditions, for example, diabetes. Other clinically significant adverse events, including intestinal perforation and fistula, occurred in our early stage BC patients, even during the postchemotherapy phase of bevacizumab therapy.

To investigate whether serum biomarkers could predict the development of CT in patients enrolled on ICORG 08–10, levels of four biomarkers at baseline and during early treatment

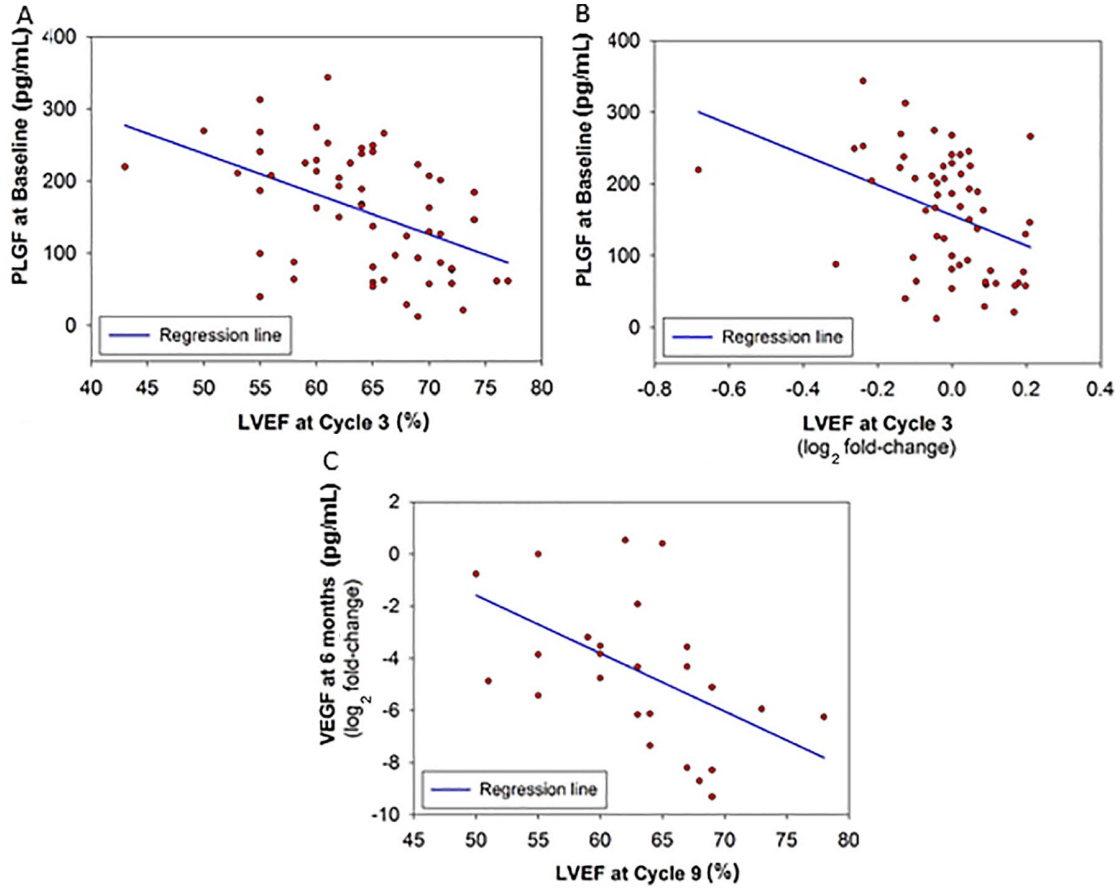

Figure 2. Linear regression analysis between biomarker levels and left ventricular ejection fraction (LVEF) at follow-up visits. Representative linear regression analyses to determine relationships between biomarker levels and LVEF at early and late follow-up visits: (A) baseline placental growth factor (PlGF) *versus* LVEF at cycle 3 ($R = 0.22$, $p < 0.01$), (B) baseline PlGF *versus* log2 fold change in LVEF at cycle 3 ($R = 0.15$, $p < 0.01$) and (C) log2 fold change in vascular endothelial growth factor (VEGF) at cycle 6 *versus* LVEF at cycle 9 ($R = 0.27$, $p < 0.01$).
Note: Missing values occur where LVEF measurements were not available at that visit or where biomarker levels were below the limit of detection.

were evaluated. Although we found a linear correlation between high baseline PlGF and a more pronounced decrease in LVEF at cycle 3 (Figure 2 and Supplemental Table 4) this was not predictive of CT phenotype as defined. It has previously been proposed that increased levels of PlGF may predict CT following doxorubicin and trastuzumab therapy.[13] Moreover, increased PlGF level has been observed in patients with a cardiovascular event history.[17] In this study increased baseline PlGF may indicate a subclinical phenotype that renders patients more prone to cardiovascular insult. Interestingly, a more pronounced VEGF decrease in VEGF levels at 6 months compared with baseline correlated ($p < 0.05$) with a higher LVEF during subsequent cycles (Supplemental Table 4 and Figure 2). However, these results are preliminary and further investigation of these two putative CT biomarkers in larger patient cohorts are warranted.

After our study completed its recruitment, the results of a larger phase II trial (ECOG-2104)[7] and of three phase III trials (BETH, BEATRICE and ECOG-5103[5,6,8]) in all of which the addition of bevacizumab to standard adjuvant therapy was studied, presented and or published. The results of ECOG-2104[7] showed that the initiation of bevacizumab therapy either at the start of therapy or post-anthracycline was possible, and that although grade 3 HTN occurred in 12% of patients and approximately 4% of patients developed CHF, the regimen did not result in 'prohibitive cardiac toxicity'.

The three phase III trials addressed the question of efficacy. In the BETH trial[6] bevacizumab was added

to trastuzumab and chemotherapy for HER2-positive BC, and in the BEATRICE trial[5] to chemotherapy for TN BC. In ECOG-5103[18] bevacizumab was added to dose-dense doxorubicin-cyclophosphamide/paclitaxel. All of these studies were negative, that is, there was no benefit from adding bevacizumab. In all of these trials the addition of bevacizumab resulted in an increase in toxicity, especially HTN, leading to more treatment discontinuations. ICORG 08–10 was not designed to assess clinical outcome measurements.

These results are obviously disappointing, given that a clear biological rationale existed for the study of anti-angiogenic therapies in BC. It is clear from multiple adjuvant trials that bevacizumab fails to improve survival. It is possible that this lack of response is due to the limited vasculature present in the micro-metastases present in early stage disease, leading to an effective reduction in the anti-angiogenic therapeutic target.[19] Efforts to identify a clear bevacizumab-responsive BC subgroup is further complicated by the lack of robust patient selection biomarkers, despite intensive research.[20–22] No new phase III trials of bevacizumab are anticipated.

Nevertheless, as highlighted in a recent review,[23] in addition to its antiangiogenic activity, bevacizumab has a substantial immunomodulatory capacity; Specifically, VEGF-A has been shown to suppress dendritic-cell maturation, to inhibit proliferation of regulatory T cells and to attract myeloid-derived suppressor cells.[24,25] These immunosuppressive effects could be counteracted by bevacizumab. Indeed, several combination studies with checkpoint inhibitors and bevacizumab are ongoing across different cancer indications.[23]

Conclusions

We conclude that although bevacizumab can be added to a four-cycle docetaxel–cyclophosphamide regimen, toxicity, prominently HTN, results in frequent treatment discontinuation. Given the negative results of three large phase III trials, it is unlikely that this approach will be investigated further.

Acknowledgement

The authors would like to thank Roche Products Ireland for the provision of bevacizumab as the investigational medical product to conduct the study.

Author contributions

GG, AJE, NOD, ACOF, WMG, ATB and JC established the need for the clinical and biomarker studies, and were directly involved in the implementation, and execution of the clinical and biomarker studies and the interpretation of study data. They were also involved in writing the manuscript. AC, DMC, DM, AM, PD, MR and BK were involved in conducting and analysing the biomarker study. MJK, LG, OB, JM, MK, MM, RG, GL, MOC, PC, PD, JW, EM and DT were involved in establishing and completing the clinical trial and were also involved in the interpretation of the clinical data and writing of the manuscript. KS, AH, IP, VM and BM were part of the regulatory team required to complete the clinical trial and were involved in the collection and interpretation of the study data. All authors have reviewed the paper prior to submission.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The toxicity biomarker study was funded by AngioTox (www.angiotox.com), a European Commission FP7 Industry Academia Pathways and Partnerships Marie Curie Award (grant agreement 251528). AB, WMG, JC and NOD receive funding from the Irish Cancer Society Collaborative Cancer Research Centre under BREAST-PREDICT (www.breastpredict.com; grant number CCRC13GAL). Cancer Trials Ireland received financial support from Sanofi Aventis.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Availability of data and material

All data generated or analysed during this study are included in this published article (and its Supplemental information files)

Supplemental material

Supplemental material for this article is available online.

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