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Efficacy of neoadjuvant bevacizumab added to docetaxel followed by fluorouracil, epirubicin, and cyclophosphamide, for women with HER2-negative early breast cancer (ARTemis): an open-label, randomised, phase 3 trial

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Summary

Background The ARTemis trial was developed to assess the efficacy and safety of adding bevacizumab to standard neoadjuvant chemotherapy in HER2-negative early breast cancer.

Methods In this randomised, open-label, phase 3 trial, we enrolled women (≥18 years) with newly diagnosed HER2-negative early invasive breast cancer (radiological tumour size ≥20 mm, with or without axillary involvement), at 66 centres in the UK. Patients were randomly assigned via a central computerised minimisation procedure to three cycles of docetaxel (100 mg/m² once every 21 days) followed by three cycles of fluorouracil (500 mg/m²), epirubicin (100 mg/m²), and cyclophosphamide (500 mg/m²) once every 21 days (D-FEC), without or with four cycles of bevacizumab (15 mg/kg) (Bev+D-FEC). The primary endpoint was pathological complete response, defined as the absence of invasive disease in the breast and axillary lymph nodes, analysed by intention to treat. The trial has completed and follow-up is ongoing. This trial is registered with EudraCT (2008-002322-11), ISRCTN (68502941), and ClinicalTrials.gov (NCT01093235).

Findings Between May 7, 2009, and Jan 9, 2013, we randomly allocated 800 participants to D-FEC (n=401) and Bev+D-FEC (n=399). 781 patients were available for the primary endpoint analysis. Significantly more patients in the bevacizumab group achieved a pathological complete response compared with those treated with chemotherapy alone: 87 (22%, 95% CI 18–27) of 388 patients in the Bev+D-FEC group compared with 66 (17%, 13–21) of 393 patients in the D-FEC group (p=0.03). Grade 3 and 4 toxicities were reported at expected levels in both groups, although more patients had grade 4 neutropenia in the Bev+D-FEC group than in the D-FEC group (85 [22%] vs 68 [17%]).

Interpretation Addition of four cycles of bevacizumab to D-FEC in HER2-negative early breast cancer significantly improved pathological complete response. However, whether the improvement in pathological complete response will lead to improved disease-free and overall survival outcomes is unknown and will be reported after longer follow-up. Meta-analysis of available neoadjuvant trials is likely to be the only way to define subgroups of early breast cancer that would have clinically significant long-term benefit from bevacizumab treatment.

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Introduction

Survival rates from early breast cancer have improved substantially over the past 20 years. However, the incidence of breast cancer has increased and continues to represent a major health problem worldwide.1 Both the Early Breast Cancer Trialists Collaborative Group (EBCTCG) overview analyses,2,3 and individual trial data have shown benefit for adjuvant anthracycline4 and taxane-containing chemotherapy.5–9 Although considerable progress has been made in early breast cancer through large adjuvant randomised treatment trials, these advances have been relatively slow. Follow-up is prolonged in order to meet the prespecified event rate criteria for disease-free survival and overall survival analyses, as defined in the statistical analysis plans.10 Neoadjuvant trials in breast cancer have become increasingly common over the past 10 years, and the primary endpoint of pathological complete response can be reported more rapidly than in their adjuvant counterparts.

Neo-TAnGo was our previous randomised phase 3 neoadjuvant trial and looked at the addition of gemcitabine to an anthracycline-based chemotherapy followed by taxane sequential combination,2 and also the efficacy of giving paclitaxel first in the treatment sequence. Although the addition of gemcitabine did not prove effective, giving paclitaxel first in the treatment sequence resulted in greater proportions of patients achieving a pathological complete response. Therefore,
Research in context
Evidence before this study
When the ARTemis trial was developed, survival from early breast cancer had improved over the preceding 20 years, in part through large randomised adjuvant treatment trials. Individual trials and the Early Breast Cancer Trials Collaborative Group overviews had shown benefit for taxane and anthracycline chemotherapy, but intensification had failed to yield further rapid progress. Neoadjuvant trials in breast cancer have been increasingly pursued with the early primary endpoint of pathological complete response as a surrogate for activity and improvement in long-term outcomes. In 2008 there was great interest in the role of the anti-angiogenic monoclonal antibody bevacizumab because phase 3 trials of bevacizumab had reported benefit in the metastatic setting. Other neoadjuvant trials of bevacizumab in HER2-negative breast cancer were in progress or development including GeparQuinto, CALGB 40603, and NSABP B-40. We developed the ARTemis UK multicentre trial to test the hypothesis that the combination of bevacizumab with neoadjuvant taxane and anthracycline containing chemotherapy might improve the proportion of patients achieving a pathological complete response in HER2-negative breast cancer, with acceptable toxicity. The ARTemis trial also collected tumour and blood from participants for future translational studies seeking molecular characteristics predicting benefit from bevacizumab, a key to optimising cost-effectiveness in future.

Added value of this study
This report presents the primary endpoint of pathological complete response in ARTemis, showing a significant improvement in the proportion of patients achieving a pathological complete response with the addition of bevacizumab to neoadjuvant chemotherapy. Pathological complete response in oestrogen receptor (ER) strongly positive patients was achieved in fewer patients and did not appear to improve with bevacizumab, compared with ER negative and ER weakly positive patients.

Implications of all the available evidence
Our results extend those reported in GeparQuinto and CALGB 40603, but also offer an explanatory hypothesis for the otherwise apparently divergent results of NSABP B-40, in which ER positive patients appeared to benefit most. We postulate that known variations between the trials in ER status definition might have resulted in varying distribution of ER weakly positive patients across the dichotomised ER positive and negative categories, thus explaining the apparently divergent results. We also hypothesise that molecular characteristics of individual tumours, similar to those recently observed in ovarian cancer trials of bevacizumab, might correlate with ER status and underlie the variations in individual patient benefit observed. Meta-analysis of ARTemis, GeparQuinto, CALGB 40603, and NSABP B-40 with analysis of their linked translational collections could test these hypotheses with sufficient statistical power in the future.

Methods
Study design and participants
The ARTemis phase 3 randomised trial aimed to assess the benefit of the addition of neoadjuvant bevacizumab to chemotherapy in terms of short-term and long-term outcomes in women presenting with early breast cancer. We enrolled women aged 18 years or older with a histological diagnosis of early invasive breast cancer, and a radiological tumour size of more than 20 mm with or without axillary involvement. Patients were enrolled at 66 National Cancer Research Network sites in the UK. Patients with inflammatory cancer, T4 tumours with direct extension to the chest wall or skin, and ipsilateral supraclavicular lymph node involvement were eligible with any size of primary tumour. We regarded hormone oestrogen receptor (ER) status as negative when Allred score was 0–2/8; ER weakly positive was 3–5/8; and ER strongly positive was 6–8/8. These ER categories were based on those already used by our group in the tAnGo trial and subsequently confirmed by Petit and colleagues to be predictive of neoadjuvant chemotherapy response (pathological complete response) in ER-positive tumours. All patients were HER2 negative, defined as immunohistochemistry of 0/1+, or if 2+, fluorescence in situ hybridisation showed no evidence of amplification of the HER2 gene. Other eligibility criteria were adequate cardiac function (left ventricular ejection fraction within the normal institutional range, as assessed by muliple-gated acquisition scan or echocardiogram), adequate bone marrow, hepatic, and renal function, and appropriate Eastern Cooperative Oncology Group (ECOG) performance status (0–2). In view of potential side-effects from bevacizumab, patients had to have no previous diagnosis of ischaemic heart disease, cerebrovascular disease, peripheral vascular disease, arterial or venous thromboembolic disease, cardiac failure, gastroduodenal
ulcer, symptomatic diverticulitis, or inflammatory bowel disease. Additionally, no uncontrolled hypertension, defined by a systolic pressure greater than 150 mm Hg or diastolic pressure greater than 90 mm Hg, with or without antihypertensive medication was allowed. Patients with initial increases in blood pressure were eligible if initiation or adjustment of antihypertensive medication lowered pressure to meet entry criteria.

No previous exposure to chemotherapy, radiotherapy, or endocrine therapy as treatment for breast cancer was allowed. Full eligibility criteria can be found in the trial protocol.

ARTemis was an investigator designed and led trial, granted a Clinical Trials Authorisation (CTA) from the Medicines and Healthcare products Regulatory Agency (MHRA) on Feb 25, 2009, approved by the Multi-Centre Research Ethics Committee nationally on March 26, 2009, and subsequently by the local research ethics committees at all participating centres. All patients provided written informed consent.

Randomisation and masking
We randomly assigned participants (1:1) to two chemotherapy regimens using a central computerised minimisation procedure. Treatment allocations were made by telephone to the Warwick Clinical Trials Unit, where a central computerised minimisation procedure was used to generate the patients' random allocation. Stratification by minimisation was done by age (<50 years vs ≥50 years), ER status (negative vs weakly positive vs strongly positive), total tumour size (<3 cm vs ≥3 cm), clinical involvement of axillary lymph nodes (yes vs no), and disease type (inflammatory or locally advanced or both vs neither).

Randomisation was recommended within 4 weeks of the initial core biopsy and chemotherapy to start within 1 week of randomisation. The trial was open label.

Procedures
Chemotherapy regimens used were docetaxel 100 mg/m² once every 21 days for three cycles, followed by fluorouracil 500 mg/m², epirubicin 100 mg/m², with cyclophosphamide 500 mg/m² once every 21 days for three cycles (D-FEC). Bevacizumab 15 mg/kg (Genentech, South San Francisco and Vacaville, CA, USA) was given every 3 weeks with the first four cycles of chemotherapy in the experimental group (Bev+D-FEC).

We assessed adverse events for each chemotherapy cycle by grade according to Common Terminology Criteria for Adverse Events (CTCAE) version 3. We also recorded use of growth factor support (either granulocyte colony stimulating factor [GCSF] or pegylated [PEG] GCSF). The maximum permitted dose delay or interruption was 4 weeks to allow recovery from severe toxicity or for unscheduled procedures (eg, emergency surgery). If neutropenic fever or sepsis occurred after a cycle of chemotherapy, the next cycle was delayed until the absolute neutrophil count was at least 1·0 × 10⁹ cells \( \text{per L} \). After a delay, either dose reduction of all drugs to 80%, or GCSF support with 100% dose were allowed, and all remaining cycles of the same three-cycle block were given at those doses. For persistent thrombocytopenia, the next cycle was delayed until platelets had recovered to at least 100 × 10⁹ cells \( \text{per L} \) and chemotherapy doses were reduced to 80%, maintaining this dose reduction for subsequent cycles. Cycles from the next block of treatment were commenced at full protocol dose, and permitted delays and reductions were made as necessary. Primary prophylaxis with GCSF was allowed with docetaxel with or without bevacizumab, but not with FEC with or without bevacizumab. However, once started, prophylactic GCSF was usually continued into the second phase of chemotherapy at the discretion of the responsible physician.

If grade 2 neuropathy occurred during treatment with docetaxel, remaining doses were reduced to 75 mg/m². If grade 3 neuropathy occurred, docetaxel was stopped. If fewer than three cycles of docetaxel had been given, additional FEC cycles were allowed up to a maximum of six cycles in total, at the discretion of the treating consultant.

Cardiac toxicity was checked by left ventricular ejection fraction before treatment started and after four cycles of chemotherapy with or without bevacizumab. If congestive cardiac failure developed, patients were investigated and
treated as appropriate, bevacizumab and epirubicin were discontinued, and other chemotherapy was given at the discretion of the treating clinician.

In the event of an allergic reaction to docetaxel, the infusion was stopped if mild symptoms of skin rash, flushing, and localised pruritus occurred. Intravenous steroids and antihistamines were given and immediate slow rechallenge of chemotherapy was used on recovery. Docetaxel infusion was stopped if moderate symptoms of generalised pruritus or rash, mild dyspnoea, or mild hypotension occurred, and intravenous steroids and antihistamines were given. Steroids were then advised for 48 hours before cautious docetaxel rechallenge. If severe allergic symptoms occurred, including bronchospasm, generalised urticaria, angio-oedema, hypotension (systolic blood pressure <100 mm Hg), or life-threatening anaphylaxis, docetaxel infusion was stopped and treatment was given with intramuscular epinephrine (1 mL, 1:1000), intravenous steroids, and intravenous antihistamines; rechallenge was contraindicated.

Surgery (breast and axillary), radiotherapy, and adjuvant endocrine treatment were done according to local protocols. Clinical surveillance will continue for 5 years at the clinical centres, and after 5 years with the Office for National Statistics. Patients will continue to receive follow-up through the National Cancer Intelligence Network (NCIN) with monitoring of disease-free survival and overall survival.

The reporting of pathological complete response and minimal residual disease was by an independent, central, two-reader masked review of all anonymised local surgical histopathology reports undertaken by the co-chief investigators (HME and LHa). When designing the trial, the primary endpoint pathological complete response was based on local report review and this is consistent with the study protocol. This manuscript adheres to our protocol and provides a measure comparable to other recent and similar trials (GeparQuinto, NSABP B-40, and CALGB 40603). When agreement was not reached, further review by the two study histopathologists (JT and EP) was done. In each case, a consensus on pathological complete response, and pathological complete response or minimal residual disease in the breast alone was agreed. Additionally, a detailed anonymised and masked central pathological review of all biopsies and surgical haematoxylin and eosin slides for pathological complete response is in progress. A quality assurance comparison (using a κ statistic) has been done in cases reviewed to date, between central pathological review of the haematoxylin and eosin slides (all cases done by JT) and the local histopathology reports for the primary endpoint of pathological complete response.

**Outcomes**

Our primary endpoint was pathological complete response, defined as absence of invasive breast cancer in the breast and axillary lymph nodes, after neoadjuvant chemotherapy. Residual non-invasive ductal carcinoma in situ was allowed. Secondary endpoints included pathological complete response or minimal residual disease in the breast alone, which was defined as 10% or
less of original tumour burden remaining at surgery, and has previously been reported as a potentially useful secondary endpoint in neoadjuvant studies.16 Other protocol-defined secondary endpoints—that are not reported here—include disease-free survival and overall survival. These will be analysed in early 2016 when it is anticipated that median follow-up will be at least 36 months or 120 disease-free survival events will have occurred.

**Statistical analysis**

The power calculations assume a 70 to 30 split in the trial sample between ER-positive and ER-negative tumours, respectively. The proportion of patients achieving a pathological complete response with the standard treatment (D-FEC) was estimated as about 10% for ER-positive tumours and 25% for ER-negative tumours. On this basis, a trial randomly assigning 400 patients into each of the two treatment groups would allow an absolute difference in the proportion of patients achieving a pathological complete response in excess of 10% to be detected at the 5% (two-sided) level of significance with 85% power. A 10% difference in the proportion of patients who achieve a pathological complete response is the most widely accepted in neoadjuvant chemotherapy trials and was used in this group’s previously reported Neo-tAnGo trial.11

The proportion of patients achieving pathological complete response was calculated for all patients known to have had surgery. The \( \chi^2 \) test with continuity corrections was used to compare treatment groups. Multivariate logistic regression provided \( p \) values for the treatment effect after adjustment for stratification factors.

As a secondary analysis, the proportion of patients achieving pathological complete response in the breast alone was assessed along with those achieving pathological complete response or minimal residual disease, and comparisons across treatment groups were made using multivariate logistic regression with adjustment for prognostic factors.

The sample size of our study is too small to permit multiple subgroup analyses, and so we chose not to do tests for statistical significance in each ER subgroup separately.

The methods for dose intensity calculations have been described previously.17 Chemotherapy course delivered dose intensities were compared across treatment groups using Wilcoxon rank-sum tests and Fisher’s exact tests. Common Toxicity Criteria (CTC) grades were recorded for each chemotherapy cycle and growth factor support (usually GCSF) was also recorded. The reports of grade 3 and 4 toxicities were examined in detail.

Statistical analysis was done on an intention-to-treat basis and therefore all patients who were ineligible, or whose treatment violated the trial protocol, were analysed within their randomised treatment groups. All reported \( p \) values are two-sided. Analysis was undertaken by Warwick Clinical Trials Unit, using SAS statistical software.

### Table 2: Pathological complete response and minimal residual disease in D-FEC and Bev+D-FEC groups

<table>
<thead>
<tr>
<th></th>
<th>D-FEC group</th>
<th>Bev+D-FEC group</th>
<th>( p ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>% (95% CI)</td>
<td>n/N</td>
</tr>
<tr>
<td>pCR in all breast tumours and absence of disease in all removed auxiliary lymph nodes (ypT0/Tis ypN0)†</td>
<td>66/393</td>
<td>17% (13–21)</td>
<td>87/388</td>
</tr>
<tr>
<td>ER negative (Allred 0–2) (n=241)</td>
<td>38/122</td>
<td>31% (23–40)</td>
<td>54/119</td>
</tr>
<tr>
<td>ER weakly positive (Allred 3–5) (n=74)</td>
<td>11/32</td>
<td>30% (16–47)</td>
<td>19/37</td>
</tr>
<tr>
<td>ER strongly positive (Allred 6–8) (n=466)</td>
<td>17/234</td>
<td>7% (4–11)</td>
<td>14/232</td>
</tr>
<tr>
<td>Grade 1/2 (n=293)</td>
<td>13/354</td>
<td>8% (5–14)</td>
<td>8/139</td>
</tr>
<tr>
<td>Grade 3 (n=403)</td>
<td>45/298</td>
<td>23% (17–29)</td>
<td>73/205</td>
</tr>
<tr>
<td>pCR in all breast tumours (ypT0/Tis)</td>
<td>76/394</td>
<td>19% (16–24)</td>
<td>99/388</td>
</tr>
<tr>
<td>ER negative (Allred 0–2) (n=241)</td>
<td>41/122</td>
<td>34% (25–43)</td>
<td>58/119</td>
</tr>
<tr>
<td>ER weakly positive (Allred 3–5) (n=75)</td>
<td>15/38</td>
<td>39% (24–57)</td>
<td>22/37</td>
</tr>
<tr>
<td>ER strongly positive (Allred 6–8) (n=466)</td>
<td>20/234</td>
<td>9% (5–13)</td>
<td>19/232</td>
</tr>
<tr>
<td>Grade 1/2 (n=293)</td>
<td>14/354</td>
<td>9% (5–15)</td>
<td>13/139</td>
</tr>
<tr>
<td>Grade 3 (n=403)</td>
<td>51/199</td>
<td>26% (20–32)</td>
<td>79/205</td>
</tr>
<tr>
<td>pCR or MRD in all breast tumours</td>
<td>114/394</td>
<td>29% (25–34)</td>
<td>138/388</td>
</tr>
<tr>
<td>ER negative (Allred 0–2) (n=241)</td>
<td>54/122</td>
<td>44% (35–54)</td>
<td>69/119</td>
</tr>
<tr>
<td>ER weakly positive (Allred 3–5) (n=75)</td>
<td>19/38</td>
<td>50% (33–67)</td>
<td>26/37</td>
</tr>
<tr>
<td>ER strongly positive (Allred 6–8) (n=466)</td>
<td>41/234</td>
<td>18% (13–23)</td>
<td>43/232</td>
</tr>
<tr>
<td>Grade 1/2 (n=293)</td>
<td>25/354</td>
<td>16% (11–23)</td>
<td>22/139</td>
</tr>
<tr>
<td>Grade 3 (n=404)</td>
<td>76/199</td>
<td>38% (31–45)</td>
<td>103/205</td>
</tr>
</tbody>
</table>

pCR=pathological complete response. MRD=minimal residual disease. *Adjusted for the five stratification variables (age ≤50 years, >50 years), ER status (negative, weakly positive, strongly positive), tumour size ≤50 mm, >50 mm, clinical involvement of axillary nodes (no, yes), and inflammatory or locally advanced disease (no, yes). †Primary endpoint for the ARTemis trial. ‡Tumour grade of each patient’s largest breast tumour at baseline.
software (version 9.3). ARTemis is registered with EudraCT (2008-002322-11), ISRCTN (68502941), and ClinicalTrials.gov (NCT01093235).

Role of the funding source
The funders of the study (Cancer Research UK, Roche, and Sanofi-Aventis) had no role in study design, data collection, data analysis, data interpretation, or writing of the report. HME, LHi, and LHa had full access to all of the data and had final responsibility for the decision to submit for publication with the agreement of all the authors and the data monitoring and safety committee.

Results
800 patients were recruited at 66 UK centres (between May 7, 2009, and Jan 9, 2013) and randomly assigned to either D-FEC (401 patients) or Bev+D-FEC regimens (399 patients; figure 1). Patient and tumour characteristics were balanced across treatment groups (table 1). We subsequently identified seven patients as ineligible; six in the D-FEC group (four with tumours 20 mm or less, one with a second breast tumour that was HER2-positive, and one with liver metastases); and one in the Bev+D-FEC group (with bone metastases). Additionally, 14 patients (eight in the D-FEC group and six in the Bev+D-FEC group) had baseline blood pressure measurements outside of the protocol stated limits. All patients were included in analyses on an intention-to-treat basis.

782 patients had post-chemotherapy pathology reports from surgery available for review, 781 of which could be used for assessment of the primary endpoint of absence of invasive breast cancer in the breast and axillary lymph nodes. 153 (20%) of all 781 patients achieved a pathological complete response: 66 (17%, 95% CI 13–21) in the D-FEC group and 87 (22%, 18–27) in the Bev+D-FEC group (difference 6%, 0·1–11·2; p=0·03 after adjustment for stratification factors; table 2 and figure 2). The proportion of patients overall who achieved a pathological complete response differed significantly across both ER status (ER negative 38% [95% CI 32–45], weakly positive 41% [29–53], strongly positive 7% [5–9]; p<0·0001), and tumour grade (grade 1/2 7% [4–11], grade 3 29% [25–34]; p<0·0001). ER negative, ER weakly positive, and grade 3 patients appeared to benefit from the addition of bevacizumab whereas ER strongly positive and grade 1/2 patients did not appear to benefit from the addition of bevacizumab (table 2). An analysis of benefit for bevacizumab for each level of Allred score (ER status), confirmed that our categories of ER weakly positive (Allred 3–5/8) and ER strongly positive (Allred 6–8/8) were the correct cut points both for pathological complete response rate and benefit for bevacizumab (appendix). Complete central pathology specimen review for the trial is in progress and will be reported later. Preliminary results show very good agreement between central pathology review and local pathology report review for pathological complete response in the breast and axilla (agreement in 95% of cases, κ 0–83 [95% CI 0·76–0·90]).

The proportion of patients achieving a pathological complete response in the breast alone was significantly higher in the Bev+D-FEC group than in the D-FEC group (difference 6% [95% CI 0·1–12·1]; p=0·02 after adjustment for stratification factors; table 2). Pathological complete response differed significantly across both ER status (negative 51% [95% CI 35–48], weakly positive 49% [38–61], strongly positive 8% [6–11]; p<0·0001) and tumour grade (grade 1/2 9% [6–13], grade 3 32% [28–37]; p<0·0001). Similarly, the proportion of patients who obtained a pathological complete response or minimal residual disease was significantly better in the Bev+D-FEC group than in the D-FEC group (difference 7% [95% CI 0·1–13·2]; p=0·03 after adjustment for stratification factors; table 2). The proportion of patients who obtained a pathological complete response or minimal residual disease differed significantly across both ER status (negative 51% [95% CI 45–58], weakly positive 60% [48–71], strongly positive 18% [15–22]; p<0·0001) and tumour grade (grade 1/2 16% [12–21], grade 3 44% [39–49]; p<0·0001). An exploratory analysis of inflammatory breast cancers was done and the results

![Table 3: Reasons for 53 patients not having bevacizumab administered for four cycles](image-url)

![Figure 2: Effect of bevacizumab on pathological complete response according to oestrogen receptor (ER) status](image-url)
showed no significant advantage for bevacizumab, although the sample sizes are too small to draw any conclusions (data not shown).

779 (97%) patients had full treatment details available (391 [98%] of 401 patients in the D-FEC group and 388 [97%] of 399 in the Bev+D-FEC group). Four patients in the Bev+D-FEC group received no treatment cycles for the following reasons: diagnosis of colitis and patient consent to withdraw from trial before any treatment was given; allergic reaction and no chemotherapy given; diagnosis of metastatic disease during the trial screening phase.

695 (90%) of 775 patients (352 [90%] of 391 patients in the D-FEC group) who started treatment received six cycles of chemotherapy; the main reason for switching early was allergy to docetaxel (19 [83%] of 23 patients; 11 [79%] of 14 D-FEC patients, eight [89%] of nine Bev+D-D-FEC patients).

331 (86%) of 384 Bev+D-FEC patients who started treatment received four cycles of bevacizumab. 53 patients stopped bevacizumab early because of allergic reaction to docetaxel (so switching to FEC early), wound complications, and hypertension (table 3). The median bevacizumab course delivered dose intensity for the 388 Bev+D-FEC patients was 99% (IQR 95–101). 85% (95% CI 81–88%) of Bev+D-FEC patients received a bevacizumab course delivered dose intensity of at least 85%.

The individual cycle dose intensities both for chemotherapy and bevacizumab did not show substantial attrition over time (appendix). There were also no substantial differences between the docetaxel and the FEC blocks of chemotherapy (data not shown). Of the 386 (9%) reports of chemotherapy dose reductions from 4418 chemotherapy cycles, dose reductions were most commonly reported in cycles 2 and 3 and again in cycles 5 and 6, in both treatment groups (table 4). A slight increase in reports of treatment delays was also noted in cycles 2, 5, and 6 across both treatment groups (table 4). Reports of modifications to bevacizumab administration were infrequent throughout the four cycles (table 5).

GCSF was used in 2744 (62%) of 4418 cycles with slightly higher rates in the Bev+D-FEC group in all treatment cycles (appendix). GCSF was used in 626 (81%) of 775 patients: 301 (77% [95% CI 73–81]) in the D-FEC group and 325 (85% [95% CI 81–88]) in the Bev+D-FEC group (p=0.009). No patients died during chemotherapy, or within 4 months of chemotherapy completion. No patient deaths after this time were attributed to their randomly assigned treatment. Full treatment details were available for 4418 cycles delivered to 775 patients (2234 cycles in 391 D-FEC patients and 2184 cycles in 384 Bev+D-FEC patients). Reported severe toxicities by patient showed no unexpected findings (table 6). More patients receiving bevacizumab with D-FEC had grade 3 infections than those receiving D-FEC alone (68 [18%] vs 40 [10%]). Allergies were reported separately: 43 (5%) of 808 patients (26 D-FEC patients, 17 Bev+D-FEC patients) had grade 3 and 4 allergic reactions attributable to docetaxel and only one patient had grade 3 allergy attributable to bevacizumab. 153 (20%) patients had grade 4 neutropenia, 13 (2%) had grade 4 infection, and six (<1%) had grade 4 fatigue (table 6). More patients in the Bev+D-FEC group had grade 4 neutropenia than those in the D-FEC group (85 [22%] vs 68 [17%]). No adverse drug reactions were both serious and unexpected.

In total, 461 serious adverse events were reported, 196 by D-FEC patients and 265 by Bev+D-FEC patients. Both regimens appear tolerable and deliverable.

Full surgery details were available for 764 (98%) of 781 patients (378 D-FEC patients, 386 Bev+D-FEC patients) analysed for pathological complete response by local report review. The Bev+D-FEC group did not have
significantly lower proportions of patients having mastectomy compared with the D-FEC group (48% vs 51%; p=0.47; appendix). However, overall, significantly fewer patients who achieved a pathological complete response had a mastectomy compared with those who did not achieve a pathological complete response (42/146 [29%] vs 335/618 [54%]; p<0.0001; appendix). Having a pathological complete response or minimal residual disease in the breast also significantly lowered the frequency of mastectomy compared with those patients who achieved neither (90/243 [37%] vs 287/522 [55%]; p=0.0001; appendix).

Discussion

In the ARTemis study, the addition of short course bevacizumab to standard neoadjuvant anthracycline and taxane-based chemotherapy in HER2-negative, early breast cancer, was associated with a significant improvement in the proportion of patients achieving a pathological complete response. A greater proportion of ER-negative (Allred 0–2) and ER weakly positive (Allred 3–5) patients achieved pathological complete response compared with the ER strongly positive (Allred 6–8) patients. Additionally, bevacizumab combined with chemotherapy appeared to provide more significant benefit to ER-negative and ER weakly positive patients than to other types. The benefit of bevacizumab for ER-negative and weakly positive patients is in marked contrast to the ER strongly positive group in which low proportions of patients achieved pathological complete response and there was also an apparent lack of benefit from bevacizumab. The ARTemis treatment protocol was deliverable for both chemotherapy and bevacizumab, and toxicity from chemotherapy was as expected for D-FEC, although there was an increase in grade 4 neutropenia in patients given Bev+D-FEC, as reported previously. The observed differences in pathological complete response were statistically significant in the group as a whole. However, the clinical significance of the addition of bevacizumab appears to be most compelling within the ER-negative subgroup.

Three other randomised studies have reported on the addition of bevacizumab to neoadjuvant chemotherapy in HER2-negative breast cancer5–9 (appendix). Pathological complete response was the primary endpoint in all four studies, albeit with differing definitions. In GeparQuinto, pathological complete response was defined as lack of invasive or in situ cancer in both breast and axilla (ypT0 ypN0). Using this stringent definition, bevacizumab significantly increased the proportion of patients achieving pathological complete response in the bevacizumab-containing group compared with the chemotherapy alone group (18.4% vs 14.9%; p=0.04) in the overall study population. Bevacizumab significantly increased the proportion of patients achieving a pathological complete response in the 633 patients with triple-negative disease (27.9% increased to 39.3%, p=0.003) but not in the 1262 patients with hormone-receptor-positive tumours (7.7% increased to 7.8%, p=1.0). The GeparQuinto results are supported by those of CALGB 40603, which enrolled triple-negative patients only.16 In this smaller study, the addition of bevacizumab significantly increased the proportion of patients achieving a pathological complete response (defined as absence of invasive disease in breast only [ypT0/Tis]) in the bevacizumab group compared with the chemotherapy alone group (59% vs 48%; p=0.009). The results of these two studies are very similar to our study, in which a greater proportion of patients treated with bevacizumab achieved a pathological complete response (defined as no invasive disease in breast or axilla [ypT0/Tis ypN0]) than those treated with chemotherapy alone (22% vs 17% in the overall study population and 44% vs 32% in ER/HER2-negative patients). Contrary to these three studies, in NSABP B-40, the addition of bevacizumab increased the proportion of patients achieving a pathological complete response (defined as ypT0/Tis) in hormone-receptor-positive breast cancers when compared with the chemotherapy alone group (23.2% vs 15.1%, p=0.007) but not in triple-negative patients (51.5% vs 47.1%, p=0.34).17

Several points can be made if one is to try to reconcile the differences in outcomes between these four studies. First, since the tests for interaction between hormone receptor status and treatment group were non-significant in both NSABP B-40 and GeparQuinto, a statistical differential effect of bevacizumab according to hormone receptor has not yet been proven. Second, all four studies used different chemotherapy backbones and both NSABP B-40 and CALGB 40603 had factorial designs where modifications of the chemotherapy regimens were tested in parallel with the addition of bevacizumab, rendering cross-trial comparisons even more difficult. As an example, in CALGB 40603, the

<table>
<thead>
<tr>
<th>D-FEC group (n=391)</th>
<th>Bev+D-FEC group (n=384)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1/2</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>62 (16%)</td>
</tr>
<tr>
<td>Infection</td>
<td>141 (36%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>359 (92%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>203 (52%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>306 (78%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>129 (33%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36 (9%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>243 (62%)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>199 (51%)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>10 (3%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>365 (93%)</td>
</tr>
</tbody>
</table>

Data are n (%). Alopecia does not have a Common Terminology Criteria for Adverse Events (CTCAE) grade of 3 or 4. Nausea does not have a CTCAE grade of 4.

Table 6: Adverse events
A point of convergence can be found if pathological complete response is defined as we have in our study (ie, absence of invasive disease in breast and axilla but allowing residual ductal carcinoma in situ in the breast [ypT0/Tis ypN0]). This is reported as the primary outcome in our study and as a secondary endpoint in the other three studies. Using this definition, pathological complete response in our study and as a secondary endpoint in the GeparQuinto trial had double the risk of relapse of those in the chemotherapy-only group (HR 2.02, 95% CI 0.965–4.22). Additionally, precisely because bevacizumab is an anti-angiogenic agent, once the treatment course is completed, there might be a rebound of tumour cell growth, as has been reported in advanced ovarian cancer after completion of bevacizumab therapy. Similar mechanisms could explain the lack of benefit from adjuvant bevacizumab added to standard chemotherapy, reported in both the BEATRICE trial and the ECOG Study E5103.

At the 2014 San Antonio Breast Cancer Symposium, the NSABP B-40 group presented disease-free survival, distant recurrence-free interval, and overall survival results with a median follow-up of 4-7 years. These results were surprisingly different from those of the recently published GeparQuinto, and showed benefit for bevacizumab given in the neoadjuvant setting followed by adjuvant treatment. For ER-positive patients the HRs for disease-free survival, distant recurrence-free interval, and overall survival were 0.73 (95% CI 0.53–1.00; p=0.05), 0.68 (0.47–0.97; p=0.03), and 0.63 (0.42–0.96; p=0.03), respectively. The inclusion of adjuvant bevacizumab in the NSABP B-40 study is the major difference between these four neoadjuvant bevacizumab studies and might be an important factor in explaining the benefit of bevacizumab on longer term outcomes in this trial, although this was not confirmed in the adjuvant studies.

Another explanation is that since bevacizumab is a targeted anti-angiogenic agent, it is potentially less likely to eradicate micrometastatic disease in the bone marrow. We hypothesise that there is unlikely to be synergism between chemotherapy and bevacizumab in the bone marrow because this disease is not dependent on angiogenesis for its survival. Whereas an increased incidence of pathological complete response occurs in the well-developed primary tumour, which is angiogenesis-dependent, there might be no such advantage in terms of increased eradication of eventually lethal distant micrometastatic bone marrow disease. An indication that this mechanism might be operating is provided by the observation that patients who achieved pathological complete response in the bevacizumab group in the GeparQuinto trial had double the risk of relapse of those in the chemotherapy-only group (HR 2.02, 95% CI 0.965–4.22). Additionally, precisely because bevacizumab is an anti-angiogenic agent, once the treatment course is completed, there might be a rebound of tumour cell growth, as has been reported in advanced ovarian cancer after completion of bevacizumab therapy. Similar mechanisms could explain the lack of benefit from adjuvant bevacizumab added to standard chemotherapy, reported in both the BEATRICE trial and the ECOG Study E5103.

The use of pathological complete response as a trial endpoint is to some extent dependent on its role as a surrogate for meaningful clinical outcomes such as disease-free survival, distant relapse-free interval, breast cancer specific survival, and overall survival. Some of these have very recently been reported for the GeparQuinto study and show no difference for the addition of bevacizumab at 3 years for either disease-free survival (HR 1.03, 95% CI 0.84–1.25) or overall survival (0.97, 0.75–1.26). Results might be confounded because no bevacizumab was given after surgery and patients who were not responding on ultrasound assessment, at the midpoint of their neoadjuvant treatment, were offered randomisation into a novel treatment group with everolimus. Another explanation is that since bevacizumab is a targeted anti-angiogenic agent, it is potentially less likely to eradicate micrometastatic disease in the bone marrow. We hypothesise that there is unlikely to be synergism between chemotherapy and bevacizumab in the bone marrow because this disease is not dependent on angiogenesis for its survival. Whereas an increased incidence of pathological complete response occurs in the well-developed primary tumour, which is angiogenesis-dependent, there might be no such advantage in terms of increased eradication of eventually lethal distant micrometastatic bone marrow disease. An indication that this mechanism might be operating is provided by the observation that patients who achieved pathological complete response in the bevacizumab group in the GeparQuinto trial had double the risk of relapse of those in the chemotherapy-only group (HR 2.02, 95% CI 0.965–4.22). Additionally, precisely because bevacizumab is an anti-angiogenic agent, once the treatment course is completed, there might be a rebound of tumour cell growth, as has been reported in advanced ovarian cancer after completion of bevacizumab therapy. Similar mechanisms could explain the lack of benefit from adjuvant bevacizumab added to standard chemotherapy, reported in both the BEATRICE trial and the ECOG Study E5103.

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Another explanation is that with a median follow-up of 4-7 years, it is too early to draw conclusions about overall survival and distant recurrence-free interval in the ER-positive group in this trial. The relation between the primary endpoint of pathological complete response in therapeutic neoadjuvant trials and longer term outcomes is complex. Hatzis and colleagues have explored this in depth, and have...
modelled data from neoadjuvant trials and made two conclusions. First, that in high-risk breast cancer the design of neoadjuvant trials could increase the numbers of patients to a level that would make a positive correlation between the primary endpoint (proportion of patients achieving pathological complete response) and longer term outcomes more likely. Second, that including lesser degrees of pathological response could improve the correlation between a (thereby newly defined) primary endpoint of pathological response. Pathological complete response has been convincingly proven to be a strong positive predictive factor for improved event-free survival and overall survival in individual patients by the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) Consortium. In their analysis, pathological complete response, defined as ypT0/Tis ypN0, resulted in improved event-free survival (HR 0.48, 95% CI 0.43–0.54) and overall survival (0.36, 0.31–0.42). This relation, however, breaks down at the trial level. In the CTNeoBC analysis of 12 trials and 11955 patients, the coefficient of determination ($R^2$) was only 0.03 for event-free survival and 0.24 for overall survival, meaning that only 3% and 24% of the variability in event-free survival and overall survival, respectively, between trial groups can be explained by differing proportions of patients achieving a pathological complete response. These results have been corroborated in a further trial-level analysis of 29 trials and 14641 patients that reported $R^2$ values of 0.08 and 0.09 for disease-free survival and overall survival, respectively. In light of this, the Food and Drug Administration accelerated licensing approval for new compounds doing well in neoadjuvant breast cancer studies in early breast cancer subtypes with high risk of recurrence should be treated with caution, and depending on the results from trials of newer targeted agents, might need modification in the future.

Are there potential independent tumour biomarkers of response to bevacizumab available? The recent report at the American Society for Clinical Oncology 2014 annual meeting of an expression signature for angiogenesis in ovarian cancer is an exciting development. This signature might prove to be a poor prognosis biomarker in epithelial ovarian cancer, and might be predictive for benefit from bevacizumab. Absence of this signature in epithelial ovarian cancer possibly denotes good prognosis disease and paradoxically detrimental effect from the addition of bevacizumab to standard chemotherapy. These results need to be tested prospectively in other datasets. The translational ARTemis group plan to explore, using samples from the ARTemis trial biobank, whether the same angiogenic signature in breast cancer could provide a predictive biomarker of response to bevacizumab.

Contributors
HME and LH were responsible for the conception, design, recruitment, interpretation of the data, and drafting of the manuscript. LH was responsible for the acquisition of data, analysis and interpretation of the data, and drafting of the manuscript. JAD was responsible for the design and analysis. A-LV was responsible for the design and acquisition of data. CB and LG were responsible for the acquisition of data. JA, LH-D, KM, RA, SC, TH, SH, and DR were responsible for the patient recruitment. IG was responsible for the recruitment and drafting of the manuscript. JT was responsible for the design, acquisition of data, analysis, and interpretation of results. EP was responsible for the acquisition of data, analysis, and interpretation. JB was responsible for the conception, design, and interpretation. CC was responsible for the conception and design. DAC was responsible for the conception, design, acquisition, and interpretation of data. All authors revised the manuscript, approved the final manuscript, and agreed on all aspects of the work.

Declaration of interests
HME received funding from Cancer Research UK for trial coordination, Roche for trial coordination and free bevacizumab, and Sanofi-Aventis for trial coordination. LH was received funding through the University of Cambridge (as sponsors of the trial) from Cancer Research UK, Roche, and Sanofi-Aventis, and disbursed the proportion due to NHS Lotian for work on the trial; and personal fees from Roche for attendance at advisory board and international meetings. LH, JAD, and CB received grants from Cancer Research UK and Cambridge University Hospitals NHS Foundation Trust. KG reports grant and non-financial support from Roche, and grants from Sanofi-Aventis and Cancer Research UK. KM reports personal fees from Roche. DR reports personal fees from Roche, outside the submitted work. JB reports personal fees from Insight Genetics and BioNTech GmbH, and three patients pending. DC reports grant and consultancy fees from Roche. A-LV, JA, LH-D, RA, IG, SC, TH, JT, EP, CC, and SH declare no competing interests.

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References