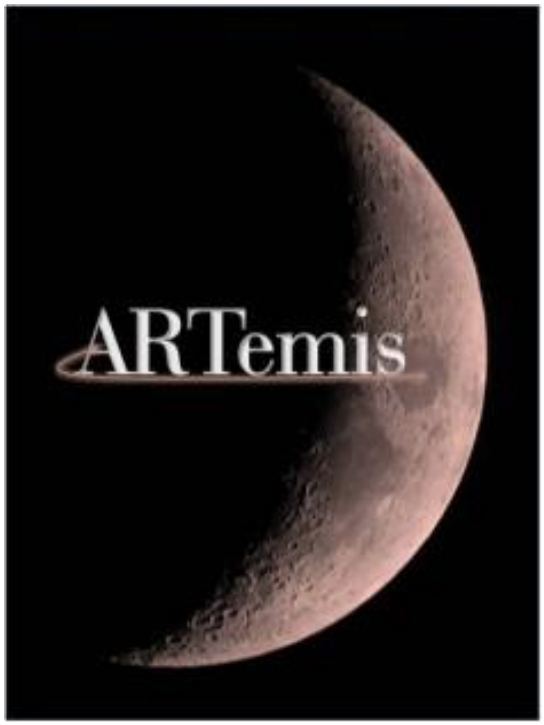

Avastin® Randomised Trial with neo-adjuvant chemotherapy for patients with early breast cancer

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Date:

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Date:

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List of Abbreviations Used

ABPI	Association of the British Pharmaceutical Industry
AC	adriamycin + cyclophosphamide
aCGH	array comparative genomic hybridisation
AC-T	adriamycin + cyclophosphamide followed by Taxol
AE	adverse event
ALT	alanine transaminase
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
aPTT	Partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	aspartate transaminase
BCIRG	Breast Cancer International Research Group
Bev	Bevacizumab (Avastin®)
BIG	Breast International Group
BSA	Body Surface Area
CAF	cyclophosphamide + doxorubicin + 5-fluorouracil
CALGB	Cancer and Leukemia Group B
(c)CMF	(classical) cyclophosphamide + methotrexate + 5-fluorouracil
COREC	Central Office for Research Ethics Committees
CTAAC	Clinical Trials Advisory & Awards Committee
CTCAE	Common Terminology Criteria for Adverse Events
CXR	chest X-ray
dFdCTP	deoxy-difluorocytidine triphosphate
(c)DNA	(complementary) deoxyribonucleic acid
D	docetaxel
DOP-PCR	degenerate oligonucleotide primed PCR
DOX	doxorubicin
DSMC	Data and Safety Monitoring Committee
EAM	energy-absorbing molecule
EC	epirubicin + cyclophosphamide
ECMF	epirubicin → CMF
ECOG	Eastern Co-operative Oncology Group
EGF(R)	epidermal growth factor (receptor)
EORTC	European Organisation for Research & Treatment of Cancer
ER	oestrogen receptor
FBC	full blood count
FEC	5-fluorouracil + epirubicin + cyclophosphamide
FISH	fluorescent *in situ* hybridisation
FNA	fine needle aspirate
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
GFR	glomerular filtration rate
Hb	haemoglobin
H&E	haematoxylin and eosin
HER2	human epidermal growth factor receptor 2
I.M.	intramuscular
I.V.	intravenous
ICAT	isotope coded affinity tag
IHC	immunohistochemistry
LCM	laser capture micro-dissection
LLN	lower limit of normal
LN	lymph node
LREC	Local Research Ethics Committee
LVEF	Left Ventricular Ejection Fraction
M/Z	mass/charge
MBC	Metastatic Breast Cancer
MDS	myelodysplastic syndrome
MHRA	Medicines & Healthcare products Regulatory Agency
MRC	Medical Research Council
MREC	Multi-centre Research Ethics Committee
MRI	magnetic resonance imaging
MS	mass spectrometry
N/saline	normal saline
NCI	National Cancer Institute (US)
NCRN	National Cancer Research Network
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NIH	National Institutes of Health (US)
NPI	Nottingham Prognostic Index
NSABP	National Surgical Adjuvant Breast & Bowel Project
p.o.	per oral
pathCR	complete pathological response
PCR	polymerase chain reaction
PEP-PCR	primer extension pre-amplification PCR
PR	progesterone receptor
prn	when required
PT	Prothrombin time
PTT	Partial thromboplastin
q3w	Every 3 weeks
R&D	research and development
RFS	relapse-free survival
RNA	ribonucleic acid
SAE	serious adverse event
SAR	serious adverse reaction
SBR	Scarff-Bloom-Richardson
SLNB	sentinel lymph node biopsy
SUSAR	suspected unexpected serious adverse reaction
TAC	docetaxel + doxorubicin + cyclophosphamide
TMA	tissue micro-array
TMG	Trial Management Group
ULN	upper limit of normal
UK	United Kingdom
WBC	white blood cell count
WHO	World Health Organisation
WMA	World Medical Association
WNL	within normal limits

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2. Trial Summary

Objective

A phase III, randomised trial to determine whether the addition to neo-adjuvant chemotherapy of an anti-angiogenic agent bevacizumab is more effective than standard chemotherapy alone in terms of short-term and long-term outcome in patients presenting with HER2-negative early breast cancer. Associated translational science will use prospective molecular profiling and candidate gene analysis on paraffin-embedded and fresh tissue to define molecular predictors of response/ resistance to bevacizumab and chemotherapy. A quality of life sub-study will collect data critical to any future health assessment of bevacizumab in this indication.

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Sponsoring Institutions:	Joint sponsorship: University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.
Trial Steering Committee:	All personnel detailed on the Trial Summary page.
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Phase III Coordinating Centre:	Warwick Clinical Trials Unit, University of Warwick

Translational Coordinating Centres: Cambridge Translational Cancer Research, University of Cambridge, Addenbrooke's Hospital.

Biomarkers and Companion Diagnostics Group, Edinburgh Cancer Research Centre, University of Edinburgh, Western General Hospital.

Number of patients enrolled: 800

Indication

Patients with early HER2-negative breast cancer eligible for neo-adjuvant chemotherapy, including inflammatory and locally advanced / inoperable disease.

Rationale

Worldwide breast cancer poses a major healthcare problem. Considerable improvements in treatment of the disease both in the adjuvant and in the metastatic setting have contributed to recent reductions in mortality. Although much of this progress has been made through large adjuvant randomised treatment trials, progress is slow because prolonged recruitment and follow up periods are required to measure treatment benefit in adjuvant trials. By contrast, clinical trials in the neo-adjuvant setting promise faster progress, because the endpoint of improved pathological complete response at the time of surgery has been shown to correlate well with later improvement in disease free survival for those in whom it is achieved. In addition neo-adjuvant chemotherapy offers additional advantages to the individual patient, improving the rates of conservative surgery, and has therefore become a standard of care for higher risk or larger tumours for which mastectomy might otherwise be indicated. Nevertheless pathological complete response rates with standard chemotherapy regimens remain relatively low and further improvements would be valuable.

Bevacizumab (Avastin®) is a new humanised monoclonal antibody which targets vascular endothelial growth factor (VEGF) and thereby the neo-angiogenic process in cancer. Bevacizumab has shown promising anti-tumour effect when given concurrently with taxane based chemotherapy in breast cancer. We propose this trial to address three central hypotheses:

(i) A *short course* of pre-operative bevacizumab in combination with chemotherapy will improve the pathological complete response to neo-adjuvant treatment for HER2-negative breast cancer patients, and thereby improve their chances of breast conservation, as well as improving disease-free and overall survival.

(ii) The existence of independent molecular signatures of response to chemotherapy and to bevacizumab with chemotherapy, which can be detected in paraffin embedded or fresh frozen tumour tissue. Cost effective utilisation of bevacizumab in combination with chemotherapy for NHS patients in future will require the use of these signatures to select optimal therapy.

(iii) The use of bevacizumab in combination with chemotherapy for NHS patients in future will require a demonstration of cost effectiveness, and data on the impact of bevacizumab on quality of life will be critical to any such future health assessment.

We propose therefore a prospective randomised clinical trial of standard anthracycline/taxane based chemotherapy with or without bevacizumab, together with a quality of life sub-study and prospective translational research to define molecular profiles predictive of tumour response and patient survival.

Primary endpoint

Complete pathological response (pathCR) rates (tumour and lymph nodes) after neo-adjuvant chemotherapy defined as no residual invasive carcinoma within the breast (DCIS permitted) AND no evidence of metastatic disease within the lymph nodes {Pinder, 2007 #86}

Secondary endpoints

- Disease-Free Survival
- Overall Survival
- pathCR rate in breast alone
- Radiological (ultrasound) response after 3 and after 6 cycles of chemotherapy.
- Rate of breast conservation
- Toxicities, including in particular cardiac safety and surgical complications (wound healing, bleeding, and

thrombosis).

Translational Science:

The primary hypothesis being tested within *ARTemis*-Science is that there are pharmacogenetic and pharmacogenomic markers that can be correlated with outcomes (pathCR and RFS) in patients randomised to receive bevacizumab versus those that do not. Secondary hypotheses being tested are markers for chemotherapy response / resistance which again can be correlated with outcome.

The overall aim is to identify molecular markers that predict for benefit (or lack thereof) of the addition of bevacizumab to conventional chemotherapy (in other words to discover the equivalent of the Trastuzumab/HER2 paradigm). To address this aim we propose to do both pharmacogenetic and pharmacogenomic studies in all patients accrued into the trial. The term pharmacogenetics is used here for the study of germline genetic variation and its influence in drug disposition and survival. Pharmacogenomics is the study of genomic/transcriptomic variation in tumours and how this somatic cancer genomic/transcriptomic composition influences drug activity and the natural history of the tumour.

Quality of life sub-study

- Open only to females (due to the nature of the questions asked).
- Standardised measures of quality of life (Instruments: FACT-B and EuroQoL questionnaires) will be collected pre-chemotherapy, after 3 cycles, after 6 cycles, after surgery and radiotherapy, and then annually for 2 years from completion of surgery, i.e. at follow-up visits 2 and 4 (see section 12.1 and Appendix 1 for time points).

Trial design

- Randomised (1:1), multi-centre, phase III, open-labelled trial.

Sample size determination

The power calculations assume a 70:30 split in the trial sample between ER positive and ER negative tumours respectively. PathCR rates with the standard treatment (D->FEC) are estimated as approximately 10% for ER positive tumours and 25% for ER negative tumours. On this basis, a trial randomising 400 patients into each of the two treatment groups will allow an absolute difference in the pathCR rates in excess of 10% to be detected at the 5% (2-sided) level of significance with an 85% power. The proposed total sample size for the trial is therefore 800 patients. All calculations take into account the expected nominal degree of non-compliance and loss to follow-up as well as variation in the trial sample ER status proportions and the pathCR rates on the standard treatment arm.

Analysis

The main analysis comparing the complete pathological response rates and the secondary analysis comparing breast conservation and radiological (ultrasound) response rates will utilise the chi-squared test and then logistic regression to allow for the adjustment of stratification variables. Regression methods will also be used to assess the correlations between marker levels and clinical outcomes. The secondary time-to-event outcomes will be assessed using Kaplan-Meier survival curves, and treatments will be compared using the Log-rank test. The effect of prognostic and predictive factors in addition to treatment will also be assessed using Cox-regression models. All analyses will be carried out on an ITT basis.

Duration of treatment

- The trial employs an 18-week neo-adjuvant chemotherapy regimen, in two phases, unless there is evidence of disease progression. If there is progression during the first phase (prior to the 3rd cycle of chemotherapy) patients may proceed either to the second phase of chemotherapy or to radiotherapy or surgery, according to the circumstance and the opinion of the responsible clinician. If there is progression during the second phase of chemotherapy (during the 4th to the 6th cycles) then premature termination of chemotherapy is also permitted and patients may proceed either to radiotherapy or surgery according to the circumstance and the opinion of the responsible clinician.
- Type and duration of post-operative adjuvant endocrine therapy will be selected by the responsible clinician.

Key inclusion criteria

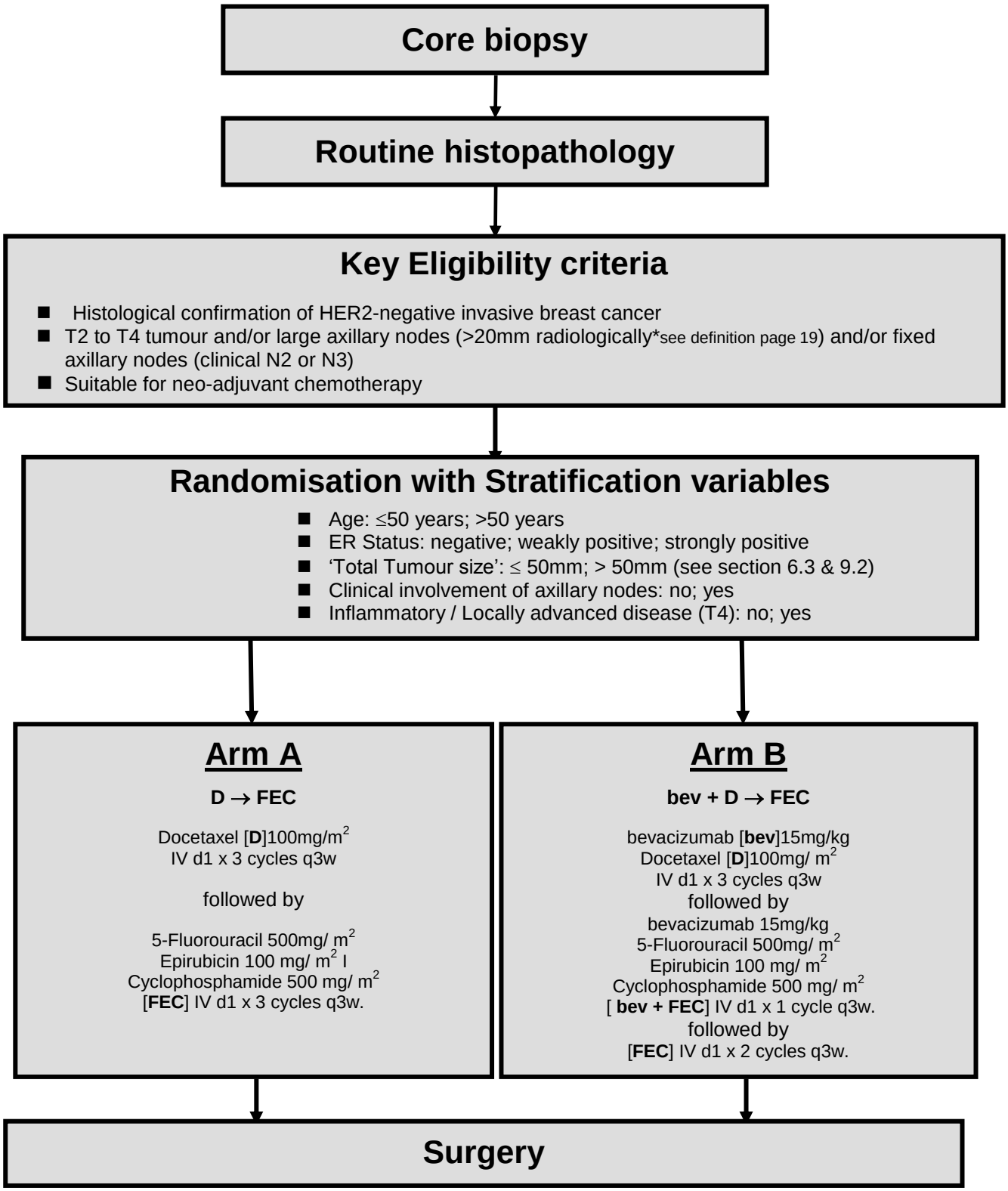
- Patient with histologically confirmed HER2-negative invasive breast cancer (either IHC 0/1 or IHC 2+ and FISH negative).
- T2 tumours and above (longest tumour diameter >20mm on ultrasound*) and T4 tumours (including inflammatory breast cancer).
- Any T stage with large axillary nodes (>20mm on ultrasound*) and/or fixed axillary nodes (clinical N2 or N3). For multi-focal tumours, the sum of each tumour's maximum diameter must be >20mm on ultrasound*, and will be designated 'total tumour size'.
- Suitable for neo-adjuvant chemotherapy in the opinion of the responsible clinician.

Key exclusion criteria

- HER2 positive invasive cancer (IHC 3+ or FISH positive)
- Unifocal T0 and T1 tumours with no fixed axillary node or no node >20mm on ultrasound*. (Multifocal tumours where the total tumour size [sum of longest diameter of each breast lesion] is >20mm on ultrasound* can be included, see above).
- Patient not suitable for neo-adjuvant chemotherapy in opinion of responsible clinician.
- Evidence of metastatic disease.
- Prior diagnosis of ischaemic heart disease, cerebrovascular disease, peripheral vascular disease, arterial or venous thrombo-embolic disease, cardiac failure, inflammatory bowel disease, gastro-duodenal ulcer, symptomatic diverticulitis, or bleeding diathesis.
- Uncontrolled hypertension.

**Ultrasound measurements are mandatory for completion of trial CRFs however, if a more accurate radiological test (MRI, spiral CT) is performed at baseline and shows >20mm measurement where the US is ≤20mm, please contact the ARTemis trial office for confirmation of eligibility.*

3. Trial schema



4. Introduction

The incidence of breast cancer in the US and Western Europe continues to rise and breast cancer remains a major healthcare problem. Stepwise improvements in treatment of the disease both in the adjuvant and in the metastatic setting have contributed to some recent reductions in mortality (Peto 2000). However, worldwide considerable numbers of women still die of the disease, many at a young age. Although progress has been made through large adjuvant randomised treatment trials (EBCTCG 2005) this progress is by necessity slow because prolonged recruitment and follow up is required to measure adjuvant effects. By contrast, clinical trials in the neo-adjuvant setting promise faster progress, because the early endpoint of improved pathological complete response at the time of surgery has been shown to correlate well with later improvement in disease free survival for those in whom it is achieved. In addition neo-adjuvant chemotherapy offers advantages to the individual patient, improving the rates of conservative surgery, and has therefore become a standard of care for higher risk or larger tumours for which mastectomy might otherwise be indicated (Mamounas and Fisher 2001; Wolmark 2001; Kaufmann 2003; Hanrahan 2005; Mauri 2005; Jones and Smith 2006; Mieog 2007; Rastogi 2008; Bonnefoi 2007). Nevertheless pathological complete response rates with standard chemotherapy regimes remain relatively low and further improvements would be valuable.

ARTemis is a neo-adjuvant trial using an anti-angiogenic agent bevacizumab in combination with standard neo-adjuvant chemotherapy, with prospective candidate biomarker and molecular profiling analysis. There are three central hypotheses:

(i) The first hypothesis is that a short course of preoperative bevacizumab in combination with chemotherapy will improve the pathological complete response to neo-adjuvant treatment for HER2-negative breast cancer patients, and thereby improve their chances of breast conservation, disease free and overall survival.

(ii) The second hypothesis is that there exist independent molecular signatures of response to chemotherapy and to bevacizumab with chemotherapy, which can be detected in fresh frozen or in paraffin embedded tumour tissue. Cost effective utilisation of bevacizumab in combination with chemotherapy for NHS patients in future will require the use of these signatures to select optimal therapy.

(iii) The final hypothesis is that the use of bevacizumab in combination with chemotherapy for NHS patients in future will require a demonstration of cost effectiveness. Data on the impact of bevacizumab on quality of life will be critical to any such future health assessment.

ARTemis is therefore a prospective randomised clinical trial of standard anthracycline and taxane based chemotherapy with or without bevacizumab, with prospective translational research to define molecular profiles predictive of tumour response and patient survival.

Crucially, ***ARTemis*** will provide data on the efficacy of a ***short course*** of four cycles of bevacizumab combined with chemotherapy in breast cancer. This data will complement results from ongoing phase III trials elsewhere exploring adjuvant or neo-adjuvant bevacizumab in breast cancer (e.g. Beatrice, NSABP-B40 see below), which involve concurrent neo-adjuvant and/or sequential adjuvant bevacizumab, but are of ***prolonged*** duration (1 year minimum). When viewed in this context, ***ARTemis*** data will provide data to support and motivate a future generation of head to head studies of duration or sequencing of bevacizumab in adjuvant and neo-adjuvant regimens. In the absence of data from ***ARTemis***, and if all available phase III data uses prolonged therapy only in future, it may prove difficult to motivate patients and the clinical community to address important questions of duration. Indeed we note that the short duration studies of adjuvant Herceptin, which were performed in the same era as the longer duration adjuvant Herceptin studies, have now provided the motivation for head to head studies of duration and sequencing for Herceptin. This new generation of studies (e.g. **SOLD**, ***Persephone***) may well yield significant improvements in the optimal cost effective use of Herceptin, an issue of critical importance to the National Health Service. Herceptin and bevacizumab are similarly expensive, and it is important therefore to plan ahead for future studies optimising the duration and sequencing of this drug. ***ARTemis***, with its proposed ***short course*** along with other adjuvant or neo-adjuvant studies of longer duration bevacizumab, will provide critical data for the design of this future generation of studies.

ARTemis is designed as a clinical and translational research trial, which combines a randomised design to answer a clinical question, together with prospective molecular tumour profiling using both a whole genome and a candidate-gene (directed) approach. The trial will have clinical and pathological endpoints, and it will be possible to rapidly accrue molecular and biological data, and analyse this in the light of clinical and pathological outcomes, seeking prognostic or predictive molecular signatures relevant to the future selection of optimal treatment.

5. Background and rationale

5.1 Neo-adjuvant chemotherapy in breast cancer

Combination chemotherapy regimens administered as post-operative adjuvant therapy in appropriately selected patients result in a significant reduction in risk of recurrence and death from breast cancer. Anthracycline-containing regimens are superior to non-anthracycline combinations (EBCTCG 2005) and recent trials and meta-analyses indicate that the addition of adjuvant taxanes (docetaxel, paclitaxel) to anthracycline based regimes improves survival further (Henderson 2003; Mamounas 2005; Martin, Pienkowski et al. 2005; Roche, Fumoleau et al. 2006; Ferguson, Wilcken et al. 2007; De Laurentiis, Cancello et al. 2008) . In the light of these results with adjuvant treatment, and with the additional aim of improving breast conservation rates and cosmetic outcomes for larger tumours, pre-operative chemotherapy or neo-adjuvant chemotherapy has progressively been incorporated into the multidisciplinary treatment of early breast cancer. First demonstrated effective in rendering locally advanced disease operable, it has progressively been extended to stage 2 breast cancer not amenable to breast conserving therapy, or even to smaller tumours (1-3cms) when the cosmetic outcome of immediate surgery is expected to be poor. Neo-adjuvant chemotherapy has been evaluated in a number of prospective randomised studies and meta-analyses, and the following general principles can be derived:

- Chemotherapy used in the neo-adjuvant setting, with subsequent surgery, achieves the same results as adjuvant chemotherapy in terms of local recurrence rates, disease free survival and overall survival (Fisher, Brown et al. 1997; Fisher, Bryant et al. 1998; Wolmark, Wang et al. 2001; Jones and Smith 2006; Mieog, van der Hage et al. 2007)
- Pathological Complete Response (pathCR - absence of residual invasive carcinoma), is a good surrogate for prolonged disease free survival (DFS), so that it is now used as an endpoint to compare the efficacy of different regimens (Kaufmann, von Minckwitz et al. 2003; Mauri, Pavlidis et al. 2005; Jones and Smith 2006; Kaufmann, Hortobagyi et al. 2006; Mieog, van der Hage et al. 2007; Pinder, Provenzano et al. 2007; Rouzier, Pusztai et al. 2005; Peintinger, Kuerer et al. 2006; Symmans, Peintinger et al. 2007; Gralow, Burstein et al. 2008)
- Longer (≥4 cycles) neo-adjuvant chemotherapy regimens achieve better pathCR rates than shorter (<4 cycles) regimens (Reitsamer, Peintinger et al. 2005; Steger, Galid et al. 2007)
- In HER2-negative disease best results in terms of pathCR (20-30%) have been achieved with anthracycline-based regimens and taxanes given sequentially (Hanrahan, Hennessy et al. 2005; Mazouni, Kau et al. 2007; Cuppone, Bria et al. 2008) including docetaxel (Bear, Anderson et al. 2003; Bear, Anderson et al. 2006) while simultaneous administration of anthracyclines and taxanes does not improve pathCR rates (Miller, McCaskill-Stevens et al. 1999; Dieras, Fumoleau et al. 2004; Evans, Yellowlees et al. 2005).
- While a clinical response is observed in 50-70% of patients, the breast conservation rate reported can be as low as 34% among patients initially assessed as Breast Conservation Therapy (BCT) ineligible (Newman, Buzdar et al. 2002).
- Improved clinical or pathological response rates remain desirable endpoints, but the incorporation of additional conventional chemotherapy agents into standard anthracycline / taxane containing regimens has not yet provided additional benefit (Von Minckwitz 2007)

This last point indicates that we may be nearing the limit of what can be achieved with conventional chemotherapy agents, and that significant further improvements in neo-adjuvant treatment outcomes may require incorporation of new targeted therapies (Burstein, Harris et al. 2003; Buzdar, Ibrahim et al. 2005), or optimisation of conventional treatment selection on the basis of molecular characteristics of the individual patients tumour (Bonnefoi, Potti et al. 2007).

In Europe and the United Kingdom (UK) a widely used sequential anthracycline / taxane adjuvant regimen for high risk early breast cancer is based on the superior experimental arm of the influential PACS-01 adjuvant trial (Roche, Fumoleau et al. 2006), consisting of 3 three-weekly cycles of FEC (5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2, cyclophosphamide 500mg/m^2) and 3 three-weekly cycles of docetaxel (100mg/m^2). Direct evidence from formal phase II or III trials for the use of this particular regimen in the neo-adjuvant setting is lacking, and its widespread use in the neo-adjuvant setting in the UK is based on extrapolation from the adjuvant data, using the general principles of neo-adjuvant treatment described above. Nevertheless in practical terms this regimen has become an established standard of care for neo-adjuvant treatment in the United Kingdom. Similar regimens, though in some instances of slightly longer duration, are widely used throughout the world.

Some biological predictors of pathCR following conventional neo-adjuvant chemotherapy have been identified. In tumours with lack of Estrogen receptor expression/null phenotype, a higher rate of pathCR is reported (Colleoni, Viale et al. 2004), especially among triple receptor null/basal type breast cancer defined by gene expression profiling (Chang, Wooten et al. 2003; Ayers, Symmans et al. 2004; Gianni, Zambetti et al. 2005; Liedtke, Mazouni et al. 2008). The prognostic and predictive value of p53 has also been evaluated in a large trial, full results of which are awaited (Bonnefoi, Potti et al. 2007). Gene expression profiling might also improve selection of specific chemotherapy drugs on the basis of properties of the individual patient's tumour, with potential for further major improvement in pathCR rates even with established conventional chemotherapy regimens (Bonnefoi, Potti et al. 2007). A complementary approach involves incorporation of new specifically targeted agents which lack the toxicity profile of conventional chemotherapy and can therefore be combined with conventional regimens without compromising dose. For example the incorporation of herceptin into anthracycline / taxane based neo-adjuvant regimens for HER2-positive breast cancer dramatically improved pathCR rates (Burstein, Harris et al. 2003; Buzdar, Ibrahim et al. 2005). For HER2-negative disease no such tumour specific target has yet been identified, but the incorporation into standard chemotherapy regimens of anti-angiogenic agents with non-overlapping toxicity profiles offers an opportunity to improve results. Trials of this approach are underway in the United States (see below) but involve prolonged and therefore costly post-operative anti-angiogenic therapy in the adjuvant setting too. Anti-angiogenic agents are presently expensive, and their future use within the UK National Health Service will rely on demonstration of cost effectiveness. Combination of conventional neo-adjuvant regimens with anti-angiogenic therapy of short duration therefore merits exploration, especially if molecular signatures of tumour sensitivity to anti-angiogenic therapy can be identified by accompanying translational science, with the aim of future optimal selection of patients most likely to benefit from treatment. Appropriate patient selection can markedly improve the cost effectiveness of expensive therapeutic agents.

Bevacizumab (Avastin®) is a new humanised monoclonal antibody which targets VEGF (vascular endothelial growth factor) and thereby targets the neo-angiogenic process in cancer. Bevacizumab has shown promising anti-tumour effect when given concurrently with taxane based chemotherapy.

5.2 Bevacizumab in the treatment of breast cancer

Vascular Endothelial Growth Factor (VEGF)

Angiogenesis is regulated by local pro- and anti-angiogenic factors. VEGF is the most potent and specific promoter of angiogenesis known and is a key regulator of pathological angiogenesis such as that associated with tumour growth. Newly formed tumour vessels are markedly dependent on VEGF and VEGF mRNA is upregulated in many tumours (Ferrara and Davis-Smyth 1997). Increased levels of VEGF have been found in most tumours examined to date, including those of lung, breast, colon, kidney and ovary (Yoshiji, Gomez et al. 1996), where over expression is associated with a poorer prognosis (Clark, Sledge et al. 1987; Gasparini, Toi et al. 1997; Obermair, Kucera et al. 1997; Linderholm, Tavelin et al. 1998; Heffelfinger, Miller et al. 1999; Salven, Perhoniemi et al. 1999; Linderholm, Lindh et al. 2000; Pronk, Vasey et al. 2000). These findings suggested that inhibiting the VEGF pathway would be useful in treatment of these cancers.

Bevacizumab (rhuMAb VEGF, Avastin®)

The murine monoclonal antibody muMAb VEGF A.4.6.1 was found to consistently and potently neutralize the biologic activity of human VEGF (Kim, Li et al. 1992; Kim, Li et al. 1993). A variety of tumour cell lines were inhibited in vivo by treatment with muMAb VEGF A.4.6.1 and several independent laboratories showed that anti-VEGF antibodies inhibit the growth of many tumour types in animal models (Kim, Li et al. 1993; Warren, Yuan et al. 1995; Borgstrom, Hillan et al. 1996; Chung and Carlson 2003). Bevacizumab is a humanized (Presta, Chen et al. 1997) monoclonal antibody to VEGF (rhuMAb VEGF) composed of human IgG1 framework regions and antigen-binding complementary determining regions from the murine monoclonal antibody (muMAb VEGF A.4.6.1) that blocks the binding of human VEGF to its receptors. Bevacizumab recognizes all isoforms of VEGF with a Kd of around 8 x 10-10 M. It may exert a direct anti-angiogenic effect by binding to and clearing VEGF from the tumour environment. Additional anti-tumour activity may be obtained via the effects of bevacizumab on tumour vasculature, providing for enhanced chemotherapy delivery to tumour cells (Jain 2001). Anti-VEGF antibodies have shown benefit when combined with chemotherapy in preclinical models of different tumour types. For example, it has been shown that (Borgstrom, Gold et al. 1999) combination treatment with muMAb VEGF A.4.6.1 and doxorubicin results in significantly increased efficacy relative to either agent alone and in some cases leads to complete regression of tumours derived from MCF-7 breast carcinoma cells in nude mice. In addition, bevacizumab showed synergistic anti-angiogenic activity with docetaxel in vitro (Sweeney, Miller et al. 2001).

Pharmacokinetics

Bevacizumab's pharmacokinetics are characterized by dose linearity within the dose range 1-10 mg/kg (Study AVF0737g) in a 2-compartment model, a low clearance and a volume of distribution consistent with limited extravascular distribution. A comprehensive population PK analysis was conducted using pooled data from eight clinical trials (Phase I, Phase II, and Phase III), in which several dosing regimens, patient populations, and concomitant anti-neoplastic regimens were used (Report 03-0324-1751) [Study No 96-375-1751-40]. The analysis included a total of 4629 bevacizumab samples from 491 patients who received intravenous (IV) doses of bevacizumab ranging from 1 to 20mg/kg at a dosing frequency ranging from every 1 to 3 weeks. The population PK analysis indicated an initial half-life of 1.4 days (both genders) and a terminal half-life of 19 - 20 days. Due to its long half-life, bevacizumab can be administered on a 2 or 3 week schedule, depending on the chemotherapy regimen with which it is combined. This is supported by simulations based on the results of the population PK analysis. The potential for pharmacokinetic interaction between bevacizumab and anti-neoplastic agents has been investigated in two studies in cynomolgus monkeys (study No 96-375-1751 and 00-376-1756 [Study No 00-376-1756-41]). These two studies suggested no interaction between bevacizumab and the anti-neoplastic agents, cisplatin, paclitaxel, 5-fluorouracil or irinotecan. In human beings, no formal drug-drug interaction studies have been conducted with bevacizumab and other agents. However, the pharmacokinetics of bevacizumab when co-administered with other chemotherapy agents have been evaluated in one Phase I study (AVF0761g), two Phase II studies (AVF0757g, AVF0780g) and two Phase III studies (AVF2119g and AVF2107g). Pharmacokinetic parameters were similar for bevacizumab across all doses and studies and whether administered as a single agent or in combination, suggesting that the pharmacokinetics of bevacizumab are not affected by dosing of concomitant chemotherapy drugs including doxorubicin, carboplatin/paclitaxel, 5-FU/leucovorin, capecitabine, and 5-FU/leucovorin/irinotecan (IFL).

Clinical activity of bevacizumab

Bevacizumab has been tested in phase I, II and III studies in a variety of solid tumours in combination with chemotherapy. Bevacizumab is registered in over 40 countries world-wide (including the member states of the European Union, and the United States) for the first line treatment of metastatic colorectal cancer in combination with intravenous 5-FU based chemotherapy (Cohen, Gootenberg et al. 2007; Scott 2007). Bevacizumab has also been approved by the U.S. FDA in combination with carboplatin and paclitaxel for treatment of stage IIIB or IV non-small cell lung cancer based on a significant improvement in response rate and overall survival in a randomised, open label, multi-centre clinical trial, conducted by the Eastern Cooperative Oncology Group (ECOG), in chemotherapy-naïve patients, evaluating bevacizumab plus carboplatin and paclitaxel (BV/CP, n = 434) versus carboplatin and paclitaxel alone (CP, n = 444). The improvement in overall survival was achieved despite a ~3% excess of treatment related deaths in the BV/CP arm, due to a ~1% excess of fatal neutropenic sepsis, and to a ~1% excess of fatal pulmonary haemorrhage (Sandler, Gray et al. 2006; Cohen, Gootenberg et al. 2007). Indeed in the therapy of advanced malignant disease of hollow organs (colon, lung) a similar risk of perforation or of major bleeding has been consistently observed. These serious adverse events seem related in part to the underlying nature of the disease, and have proved less significant in trials of combination chemotherapy with bevacizumab in breast cancer. A number of single arm phase II studies explored the use of neo-adjuvant bevacizumab with chemotherapy in colorectal or breast cancer. Whilst these small studies have in general reported encouraging safety data, a number have reported wound complication rates in excess of those expected from historical data. Careful analysis of these events has indicated that the risk is greatest if surgery proceeds within 4 weeks of last bevacizumab dose, and wound healing complications are uncommon when this interval is greater than 4 weeks. To allow a margin of safety, and considering the biological variability of the tissue half life of bevacizumab, a recommendation has been made that surgery should not be planned to proceed routinely within 8 weeks of last bevacizumab dose. We have followed this recommendation in the design of ARTemis, and therefore bevacizumab will only be administered during the first four of the six planned chemotherapy cycles (each of 3 weeks duration). Surgery will not be planned to proceed before a minimum of 3 weeks have elapsed after the sixth and final chemotherapy cycle, and thus a minimum interval of 9 weeks between last bevacizumab dose and planned surgery is ensured.

Bevacizumab in Breast Cancer

In study AVF0776g, 75 women with relapsed metastatic breast cancer received 3, 10 or 20mg/kg bevacizumab as a single agent every other week over a 168-day (24-week) treatment period or until disease progression (Cobleigh, Langmuir et al. 2003). The safety profile was acceptable, with medically manageable hypertension and mild proteinuria being the most significant adverse events. Thrombotic events were uncommon, no embolic events occurred, and all bleeding events were mild in severity. There was modest evidence of anti-tumour activity - seven patients achieved a confirmed objective response and a significant number of patients had prolonged disease stabilization. Bevacizumab has also been evaluated in patients with metastatic breast cancer in a phase III study (AVF2119g), conducted in the USA in patients who had received prior anthracycline and taxane therapy, either in the adjuvant or metastatic setting (Miller, Chap et al. 2005). Four hundred and sixty two patients received treatment with capecitabine (2500 mg/m^2/day for 14 days followed by 7 days rest, repeated

every 3 weeks) plus bevacizumab (15 mg/kg IV on day 1, repeated every 3 weeks), or capecitabine alone. Treatment in both arms was continued for 35 cycles (105 weeks), or until toxicity or progressive disease. Overall, the addition of bevacizumab to capecitabine resulted in a near doubling of response rate (19.8% versus 9.1%, p=0.001, after independent review of responses). However, this did not translate into an improvement in progression-free survival (PFS) or survival. The addition of bevacizumab to capecitabine did not alter the time to deterioration of quality of life compared to capecitabine alone, nor was there any excess of infective, thromboembolic or serious bleeding events. Minor bleeding, hypertension and proteinuria were more common in the combination arm. There was an ~2% excess of grade 3 or 4 congestive cardiac failure in the combination arm, but of course all patients had previously received anthracyclines.

A phase III trial was initiated in patients with chemotherapy naive MBC (Metastatic Breast Cancer). This trial, Eastern Oncology Co-operative Group or ECOG Study E2100 compared bevacizumab (10 mg /kg/q2w) combined with a weekly paclitaxel regimen compared to weekly paclitaxel alone. E2100 enrolled patients who presented with either de novo MBC, or who relapsed following adjuvant chemotherapy for early disease. This study closed to recruitment in May 2004 and randomised 715 patients. Results were announced after the first interim analysis revealed that the investigational arm had crossed the boundary for statistical significance in terms of progression free survival. The interim analysis after 355 events was presented at the 41st ASCO Congress in June 2005 and demonstrated an improvement in response rate: 28.2% vs 14.2%, progression free survival: 10.97 months vs 6.11 months and overall survival: HR 0.674 for the combination of paclitaxel and bevacizumab compared to paclitaxel alone. Both treatment arms were well tolerated and in keeping with the known side effect profile of both drugs, with hypertension requiring treatment and proteinuria more common in the combination arm, but no significant excess of infective, thromboembolic or bleeding events. Updated results essentially confirmed these findings (Miller, Wang et al. 2007).

Ramswany et al have reported results of a single arm phase II trial of bevacizumab (10 mg/kg on day 1 and day 15 - every 28 days) combined with docetaxel (30 mg/m^2 on day 1, 8 and 15 - every 28 days) in patients with MBC, who had received up to but no more than, one prior chemotherapy regimen for metastatic disease. At the time of the report 27 patients had been treated (Ramaswamy and Shapiro 2003). The overall response rate was 52%, four patients experienced Grade 4 toxicity (2 pulmonary embolisms and 1 each febrile neutropenia and infection) and Grade 3 toxicities were also within the known safety profile of docetaxel and bevacizumab. This schedule of docetaxel and bevacizumab is not that we propose.

The 'AVADO' study (BO17708) used our proposed combination of docetaxel 100mg/m^2 on D1 (q3w) ± bevacizumab (15mg/kg IV, q3w). A report of efficacy and safety was provided at ASCO in June 2008 by Miles et al, affirming that the primary endpoint of statistically significant prolongation of progression free survival for the combination treatment relative to docetaxel alone has been met, and that no new safety issues have been encountered (Miles 2008). Furthermore results of a series of phase II studies have recently been presented at the San Antonio Symposium in December 2007 demonstrating encouraging efficacy and safety data for bevacizumab at our proposed dose in combination with a variety of taxane and anthracycline containing combination chemotherapy regimens. In particular, abstracts from the SABCS meeting show high rates of pathological complete response (pathCR) rates (22% - 52%), in neo-adjuvant chemotherapy studies with bevacizumab (Ferrari B 2007; Greil R 2007; Makhoul I 2007; Mehta RS 2007). All of these studies included ER positive and ER negative patients. No new safety issues were identified, and in addition several abstracts included reassuring cardiac safety data in combination with anthracyclines and/or taxanes (those above and three adjuvant studies, (McArthur HL 2007; Miller KD 2007; Yardley DA 2007). The data therefore already exists to support a phase III neo-adjuvant trial as proposed here. Of interest is the recently announced NSABP-B40 study, of similar design to ARTemis, testing bevacizumab in combination with AC-docetaxel +/- capecitabine or gemcitabine in a 3x2 factorial design in the neo-adjuvant setting in 1200 HER2-ve patients in North America (ER+ or ER-) [NSABP-B40] http://clinicaltrials.gov/ct/show/NCT00408408. The statistical assumptions underlying this NSABP study are similar to those underlying our ***ARTemis*** design. The two studies should ultimately be amenable to a combined meta-analysis in respect of our primary endpoints by virtue of the marked similarities of inclusion and exclusion criteria, endpoints and chemotherapy regimens.

In summary there are extensive data available which suggest that the combination of bevacizumab with neo-adjuvant anthracycline and taxane based chemotherapy might improve the pathological complete response in breast cancer rate with acceptable toxicity, and we therefore propose a randomised trial to test this hypothesis, and to define molecular predictors of response to better select therapy. In addition we will collect quality of life data critical for any future health assessment of this approach.

5.3 ARTemis Translational Research (ARTemis-science)

Breast cancer remains a public health problem with over 40,000 new cases diagnosed every year in the UK. The molecular classification of breast cancers obtained over the past 5 years using modern genomics technology has highlighted the great heterogeneity of breast cancer, and this has profound clinical and biological implications. In the clinic a watershed moment has been reached where the clinical utility of molecular classifiers needs to be addressed. To improve this state of affairs we need to recognise the molecular heterogeneity of breast cancer (and to producing a more clinically relevant and applicable classification) to facilitate the development of prognostic and predictive markers with clinical utility, and to stratify rationally patients into trials of novel agents such as bevacizumab. To date no markers that predict benefit from bevacizumab in addition to conventional chemotherapy have been identified.
The work by the Oslo/Stanford groups has showed that using expression analysis breast cancers can be consistently classified into five main groups (luminal A, luminal B, HER2-type, normal-like and basal), and more somatic cancer genomic/transcriptomic composition influences drug activity and the natural history of the tumour.
Recently using array-CGH different genomic subtypes have also been described (Chin 2006 and 2007*).* Overwhelming evidence suggests that some subtypes might have some predictive value for therapy response (for example luminal B tumours benefit more from aromatase inhibitors than luminal A tumours). Using tumours from a population-based collection and new clustering algorithms we have discovered further complexity in the taxonomy of breast cancer (for example by defining within basal-like tumours four subtypes), with both clinical and biological implications (Teschendorff 2007).

We have also shown that small subsets of genes from larger signatures can be used for outcome prediction (for example 29 poor prognosis gene signature (Naderi 2006, Teschendorff 2006) and 7 good-prognosis genes in ER- disease (Teschendorff 2007). These observations have also highlighted that to fully characterize this complexity much larger studies, that include ER and lymph node stratification, will be required to establish a more definitive classification. Importantly ARTemis includes an element of prospective molecular stratification in that eligibility requires tumours to be HER2 negative and hence one confounding variable (HER2 amplification) is prospectively removed.

ARTemis-*science* will benefit from our current approach to derive more robust candidate signatures using larger legacy sample sets (see METABRIC below) and applying different and complementary profiling methodologies (expression, copy number, miRNA) and to validate these signatures in completed clinical trials so that prognostic and predictive markers with clinical utility will be available as soon as a result of our research programme (within 18 months). The value of such multi-modality profiling approaches to identify predictive markers has just been reported (Salter 2008). Furthermore work of many laboratories throughout the world will eventually define bevacizumab predictive signatures that ARTemis-*science* will then test. This data will be available by the time accrual into ARTemis is completed and will give us a unique competitive hedge in robustly characterizing both chemotherapy and bevacizumab signatures (both prognostic and predictive). The pharmacogenetics of chemotherapy will also be much more advanced by the time ARTemis closes for recruitment and again we will have data from PG-SNPs which will have bearing on ARTemis-*science*.

Predicting drug response in the neo-adjuvant setting
In neo-adjuvant therapy pathological complete response (pathCR) is usually predictive of long-term survival, constituting a useful surrogate clinical endpoint. The molecular profiling of tumour biopsies obtained before neo-adjuvant therapy has resulted in the identification of signatures (by expression microarrays) that correlate with the pathological trial endpoint (Potti 2006; Bonnefoi 2007). We hypothesize that even more robust signatures can be derived using a combined approach (aCGH/mRNA/miRNA).

METABRIC project- producing a catalogue of breast cancer subtypes
The METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) project is funded by Cancer Research UK and the British Columbia Cancer Agency (~£1.8million) and aims at the generation of a robust molecular taxonomy of clinically annotated breast cancers. The project is based at the CRI (Caldas laboratory) and the BCCA in Vancouver and is currently profiling 2000 frozen breast tumours (high resolution array-CGH, mRNA expression profiling, sequencing of TP53 and PIK3CA) and analysing histology-based markers on TMAs from the same tumours. METABRIC will therefore generate a 'definitive' molecular taxonomy of breast cancer, identify robust prognostic markers and establish the correlation with histology-based markers. METABRIC will represent the largest and most definitive effort into profiling legacy tumour banks to unravel the molecular heterogeneity of breast cancer. The dataset will represent an invaluable resource for the studies planned within ARTemis.

Testing the clinical utility of breast cancer subtypes
The molecular heterogeneity of breast cancer, which METABRIC will unravel, means that profiling large numbers of samples from patients treated in clinical trials will be required to validate molecular markers that are highly correlated with outcome (disease-free and overall survival) independently of treatment received

(prognostic markers) or markers that can be used to determine which of the two treatment arms is better for a given group (predictive markers). By the time ARTemis finishes recruiting we will have data in Cambridge from a unique resource of clinically annotated tumours (in total more than 10,000 paraffin-embedded samples) from a population-based cohort (SEARCH) and from four randomised clinical trials (BR9601/NEAT, tAnGo, Neo-tAnGo, Persephone) to robustly address these questions. Since at least 5000 of these tumours will be HER2-negative the validated markers will be relevant to ARTemis-Science. The Bartlett group in Edinburgh will have data from 3 randomised trials TEAM, TACT, TACT2.

Prognostic and predictive signatures in the adjuvant setting

The results of validation of the signatures developed by the Caldas, Bartlett and other programmes (see above), and those derived by METABRIC, as well as putative predictive markers (e.g. TGFBI, ERBB-1/-2/-3, Topo IIa, Bcl-2, TP53, EP300, MDR1, MDR3, Aurora A, Survivin, BubR1, p21, p34Cdc2 and phospho-p34Cdc2, Tau, BIM, BRCA1, PTEN, AKT, VEGF, VEGFR) will be available by the time ARTemis finishes recruiting.

miRNAs, breast cancer subtypes, drug response and regulation of angiogenesis

The Caldas group was first to demonstrate that miRNAs define molecular subclasses in human breast cancer that are related to those defined by mRNA expression (Blenkiron 2007). More recently integrated miRNA/mRNA signatures have been shown to be predictive of chemotherapy response in human breast cancer (Salter 2008) *a*nd to modulate chemosensitivity of tumour cell lines (Blower 2007 and 2008: Kovalchuk 2008). miRNAs have also been shown to augment tumour angiogenesis, and to modulate angiogenic properties of human endothelial cells in part by down-regulation of homeobox genes (Dews 2006; Poliseno 2006; Chen 2008). Given i) the potential for miRNAs to replace mRNA signatures, ii) the relation of miRNAs with chemosensitivity and iii) the fact that miRNAs have a central role in controlling tumour angiogenesis, we hypothesize that miRNAs could be robust predictive markers for bevacizumab. Moreover miRNAs are extremely stable and can be easily profiled from paraffin-embedded material and so would be ideal markers to validate within ARTemis.

Pharmacogenetics and germline polymorphisms in breast cancer

Significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents is observed within cancer populations. Pharmacogenetics (PGx) is the study of inheritance in inter-individual variation in drug disposition. A combination of different approaches (candidate gene, genome-wide and pathway driven) can be used to identify variants that are associated with outcomes (Abraham 2006). Germline polymorphisms can also be associated with survival, either by altering pharmacodynamics (Fagerholm 2008) or by other less clear or unknown mechanisms (Udler 2007; Azzato 2008). We have collected to date germline DNA samples from around 2,000 breast cancer patients (target of 3,000) treated with FEC-like chemotherapy or a taxane (paclitaxel) as part of the CRUK TRICC-funded PG-SNPs study. Results will be available by the time ARTemis is finished. PGx of bevacizumab is not well studied but there is preliminary evidence that germline polymorphisms might affect response (Brantley 2007).

Circulating angiogenic markers and response to bevacizumab

Bevacizumab is a humanized monoclonal antibody that neutralises circulating human VEGFs. However, circulating levels of VEGF rise significantly following bevacizumab treatment and total VEGF assays have failed to demonstrate a link between circulating levels of total VEGF and bevacizumab response. Bevacizumab has a long half-life and binds VEGF with high affinity. It is therefore likely that the VEGF concentrations measured in bevacizumab treated patients may not be bioavailable. If this is correct the observed rise in VEGF levels suggests that a physiological feedback loop has been engaged to restore physiological circulating levels of VEGF. Recent data would support this hypothesis (Loupakis 2007). Measurement of free VEGF levels shows a marked decrease in bevacizumab treated patients (Brostjan 2008) and a corresponding reduction in the anti-vascularisation marker thrombospondin. This further supports the hypothesis that bevacizumab acts as a “sink” for circulating VEGF, and that levels of free VEGF and TSP1 may more accurately reflect the physiological impact of bevacizumab treatment on angiogenic pathways in treated patients. We aim to test the hypothesis that circulating levels of pro-angiogenic markers (free VEGF, bFGF, sFLlt1 etc) and anti-angiogenic markers such as thrombospondin provide an index of bevacizumab efficacy. Diurnal variations in circulating VEGF levels have been reported and samples will be collected before 10am to combat this. We propose to collect serial serum samples from a minimum of 200 patients before treatment, and after the 1st, 2nd, 4th and 6th cycles to assess changes in these and other markers and the relationship between these changes and clinical effect in patients in both arms of the trial. Samples will be collected as for ongoing sample collections in TRANS-SUPREMO and will be focused on sites with sufficient infrastructure.

ARTemis-*science* represents a collaborative effort between the ARTemis Trial Management Group, the trial clinical/surgical co-ordinators, Professors Carlos Caldas (Cambridge), John Bartlett (Edinburgh), Roche products ltd, and the patients and clinicians supporting the trial. The ARTemis-*science* tissue bank will be housed at two sites; Carlos Caldas’ laboratory in Cambridge and John Bartlett’s laboratory in Edinburgh.

Custodianship of this resource will reside with the Trial Management Group. Research projects, including those described above will be ratified by the Trial Management Group that will authorise release of tissue/RNA/DNA etc to the appropriate researchers. Due to the rapidly changing fields in which these projects are framed, changes to protocols/platforms are expected between the time of writing and the implementing of these projects, and these will form part of the review carried out by the TMG.

6. ARTemis: trial design

This is a neo-adjuvant phase III trial evaluating the efficacy of adding bevacizumab (bev) to a standard **neo-adjuvant** chemotherapy regimen of docetaxel (D) and 5-fluorouracil, epirubicin, cyclophosphamide (FEC), in patients presenting with high risk Her-2 negative early breast cancer. 800 patients will randomly be assigned to one of the two treatments arms. In both treatment arms, definitive surgery is planned after cycle 6 of chemotherapy.

6.1. Trial Committees

Trial Management Group

Members are identified in the trial summary. The Trial Management Group will meet regularly to formally discuss the conduct of the trial and to receive reports, and agree all publications. The Chief Investigators will discuss all matters of importance with regard to the conduct and running of the trial, as and when this may prove necessary.

Translational Science Group

Members are identified in the trial summary. The Translational Science Group will meet regularly to discuss the translational aspects of the trial and to receive reports, and agree all publications. The Chief Investigators will discuss all matters of importance with regard to the translational aspects of the trial, as and when this may prove necessary.

Trial Steering Committee

Members are identified in the trial summary. This group formally supervises the conduct of the trial, monitoring progress including recruitment, data completeness, losses to follow-up, and deviations from the Protocol.

Data and Safety Monitoring Committee (DSMC)

An independent Data and Safety Monitoring Committee (DSMC) will be instituted and will include members of international repute chosen from the following disciplines: medical oncology, cardiology, breast cancer surgery, breast cancer histopathology, and biomedical statistics. The DSMC members will be independent of the trial and familiar with the methodology of oncology trials. The main responsibility of the DSMC will be to ensure the ethical conduct of the trial and to protect the safety interests of patients in this trial. The DSMC will be responsible for the ongoing review of safety data collected throughout the course of the trial, as well as the review of efficacy data at the time of the interim analyses defined as per the Statistical Analysis Plan. The first DSMC review of the safety data from the trial will take place when the first 200 patients have completed trial treatment. The exact frequency of further DSMC meetings will depend on accrual as well as observed event rates, although annual meetings are anticipated.

The DSMC will adhere to written operating procedures that will be specified in a DSMC charter, clearly defining their roles and responsibilities as well as having a confidentiality agreement. The DSMC will also contribute to the Statistical Analysis Plan which will state the frequency and timing of all analyses.

After each meeting, the DSMC will provide the Trial Management Group with a written recommendation to continue the trial without any changes, or to modify the trial (including the need to stop the trial or halt the recruitment into the trial for safety reasons), or to make the interim trial results of the trial public (e.g. based on the results of the interim analyses). In a situation where the DSMC recommends a change to the trial conduct, the final decision to accept the recommendation (e.g. to amend the protocol or to discontinue the trial) will be taken by the Trial Management Group.

6.2 Early interim safety analysis

During the clinical development of bevacizumab, several potential safety issues specific to the drug were identified. These included: a risk of wound complications during subsequent surgery if undertaken within four weeks of bevacizumab dosing; a risk of haemorrhage when used to treat advanced malignant disease in colon or lung; a possible risk of thrombotic events; and a potential impact on left ventricular function in patients previously exposed to anthracyclines, perhaps by virtue of a recognised incidence of hypertension associated with bevacizumab. In contrast, subsequent studies in early breast cancer, and specifically in the neo-adjuvant setting, have indicated a favourable safety profile in all these respects, as discussed at greater length above. Nevertheless, and in addition to the usual systems for pharmacovigilance, the TMG will ran an early interim analysis after 200 patients had been accrued and completed treatment (i.e. 100 to the bevacizumab arm) seeking any excess in the experimental arm of pre-specified and prospectively sought safety endpoints, namely: wound healing complication rates, thromboembolic events; haemorrhagic events; or declines in left ventricular ejection fraction (LVEF). These data are collected "in real time", analysed by the ***ARTemis*** Trial Offices, discussed by the independent Data and Safety Monitoring Committee (DSMC) and the DSMC recommendations reported to Roche. Any concern on behalf of the DSMC (with or without agreement from Roche) about the safety of patients will prompt a suspension of recruitment pending a protocol amendment to address the issue. Any concern on behalf of Roche regarding the safety of patients will need to be agreed by the DSMC, with Roche providing the DSMC with additional relevant safety information as necessary.
The result of the first 200 patient safety analysis conducted by the DSMC in June 2011 reported no safety concerns outside those expected from the current product information.

6.3 Eligibility criteria

INCLUSION CRITERIA

- Patient with histological diagnosis of HER2 negative invasive breast cancer.
- Unifocal tumour:
  - T2 or T3 tumours (>20mm diameter on ultrasound*; see Appendix 3).
  - T4 tumour of any size with direct extension to (a) chest wall or (b) skin.

OR

Inflammatory carcinoma with tumour of any size.

OR

Other Locally Advanced disease:

- Involvement of ipsilateral large or fixed axillary lymph nodes, or infra or supraclavicular nodes (>20mm diameter on ultrasound*,or clinical N2 or N3, see appendix 3) and primary breast tumour of any diameter.
- Involvement of ipsilateral large or fixed axillary lymph nodes, or infra or supraclavicular nodes (>20mm diameter on ultrasound*, or clinical N2 or N3, see appendix 3), without a primary breast tumour identified, the presence of breast cancer in a Lymph Node (LN) must be histopathologically confirmed by LN biopsy (trucut or whole LN).

OR

Multifocal tumour:

- The sum of each tumours' maximum diameter must be >20mm on ultrasound* (total radiological tumour size >20mm in the breast only, i.e. excluding axilla).

- Patients with bilateral disease are eligible to enter the trial provided that one of the breast diseases meets the above criteria.
- Any hormone receptor status.
- Patient fit to receive the trial chemotherapy regimen in the opinion of the responsible clinician:
  - Patient must have adequate bone marrow, hepatic, and renal function^.
- Patient must not have clinically significant cardiac abnormalities and must not have had a previous myocardial infarction during the 6 months prior to recruitment. Cardiac function should be assessed by physical examination and baseline measurement MUST be made of LVEF by MUGA scan or ECHOcardiogram. LVEF must be within the normal range as defined locally by the treating centre (usually at least 50%).
- ECOG performance status of 0, 1, or 2 (see Appendix 2).

- No previous or concomitant chemotherapy or biological agents.
- No previous diagnosis of other malignancy unless:
  - managed by surgical treatment with or without radiotherapy or endocrine therapy, and disease-free for 5 years

  OR
  - previous basal cell carcinoma
  - previous cervical carcinoma in situ, or other in situ malignancy without invasion
  - contralateral ductal carcinoma in situ of the breast treated by surgery with or without radiotherapy
  - ipsilateral ductal carcinoma in situ of the breast treated by surgery only
- Non-pregnant and non-lactating, with no intention of pregnancy during chemotherapy, and prepared to adopt adequate barrier contraceptive measures if there is any possibility of pregnancy (males and females).
- No concomitant medical or psychiatric problems that might prevent completion of treatment or follow-up.
- 18 years or older
- Male or female
- Written informed consent for the trial.
- Randomisation is recommended within 4 weeks of diagnostic biopsy and chemotherapy should start within 1 week after randomisation. Authorisation must be sought from the Trial Coordinators before randomisation, if there is any delay outside of these time frames.
- Availability of slides and paraffin embedded tumour blocks from pre-chemotherapy biopsy and from surgical specimen is required.

^Recommendations:
Hepatic function:
- AST/ALT ≤1.5 x ULN (If <2 x ULN, contact TMG for randomisation authorisation)
- Alkaline phosphatase ≤2 x ULN
- Bilirubin within normal range. If AST/ALT and Alkaline phosphatase are within normal limits then isolated elevation of bilirubin to ≤3 ULN and a presumptive diagnosis of Gilbert's syndrome is permitted.
Renal function: *Creatinine ≤1.5 x ULN (see Appendix 4 for Cockroft and Gault formula)*
Bone marrow function*:* *Hb >10g/dL; WBC >3 × 10^9/L; platelets >100 x 10^9/L*
Prothrombin time (PT) and Partial thromboplastin time (PTT/ aPTT): *≤1.5 x ULN.*

**Ultrasound measurements are mandatory for completion of trial CRFs however, if a more accurate radiological test (MRI, spiral CT) is performed at baseline and shows >20mm measurement where the US is ≤20mm, please contact the ARTemis trial office for confirmation of eligibility.*

EXCLUSION CRITERIA

- HER2 positive invasive breast cancer (IHC 3+ or FISH positive).
- T0 and T1 tumours in absence of large (total tumour size >20mm) or fixed axillary nodes (see Appendix 3).
- Evidence of metastatic disease.
- Prior diagnosis of ischaemic heart disease, cerebrovascular disease, peripheral vascular disease, arterial or venous thromboembolic disease, cardiac failure.
- Prior diagnosis of gastroduodenal ulcer, symptomatic diverticulitis, inflammatory bowel disease.
- Bleeding diathesis.
- On full therapeutic dose of anti-coagulants, or aspirin >325mg/day, clopidogrel >75mg/day or corticosteroids
- Uncontrolled hypertension defined by a systolic pressure >150mmHg or diastolic pressure >90mmHg, with or without anti-hypertensive medication. Patients with initial blood pressure elevations are eligible if initiation or adjustment of antihypertensive medication lowers pressure to meet entry criteria.
- Presence of active uncontrolled infection.
- History of nephritic or nephrotic syndrome.
- Major surgical procedure or traumatic injury within 28 days prior to randomisation.
- Any evidence of other disease which in the opinion of the investigator places the patient at high risk of treatment related complication.
- Any concomitant medical or psychiatric problems which in the opinion of the investigator would prevent completion of treatment or follow-up.
- Non-healing wound, or peptic ulcer, or bone fracture.
- On LHRH-agonists for ER strongly positive disease

6.4 Pre-randomisation screening investigations

(See also section 7.3 for investigations to conduct for all subsequent cycles.)

- HER2 and ER status
- Full blood count recommended within 7 days prior to cycle 1
- Biochemical screen recommended within 7 days prior to cycle 1
- Blood pressure within 7 days prior to cycle 1,
- Pregnancy test: for females with child-bearing potential
- Urinalysis: Proteinuria by dipstick unless proteinuria has been determined by 24-hour urine. Collect 24-hour urine in the event of proteinuria ≥2+ on dipstick.
- Coagulation test: A baseline PT and PTT/aPTT.
- LVEF measured by ECHO or MUGA within 6 weeks prior to randomisation
- It is advised to assess clinically or radiologically enlarged / abnormal axillary nodes by fine needle aspiration or core biopsy to define involvement (not mandated).
- Standard practice ultrasound. It is strongly advised to repeat as close to cycle 1 as possible, any baseline radiological (ultrasound) measurements that have been performed more than 6 weeks prior to randomisation.
- Staging to exclude metastatic disease is mandated in accordance with standard early breast cancer practice for patients with an identifiable risk of metastatic disease: (i) locally advanced ([†]see below) or inflammatory breast cancer (defined on clinical presentation and/or histopathological confirmation); (ii) clinically or histologically proven involved lymph nodes. This should include:
  - CXR or cross sectional imaging of the thorax by CT.
  - Liver ultrasound or cross sectional imaging of the upper abdomen by CT or MRI
  - Whole body bone scintigraphy or CT of axial skeleton.
- It is also recommended that patients with abnormal liver or bone biochemistry (*AST/ALT >1.5 x ULN, Alkaline phosphatase >2 x ULN)* are staged in accordance with local practices.

For those patients with abnormal liver or bone biochemistry: the results of the relevant tests must be made available prior to randomisation. In these circumstances, if staging investigations show metastatic disease, the patient is not eligible for the trial, and must proceed with standard management at the discretion of the responsible clinician.

For patients with locally advanced or inflammatory disease, or clinically involved axillary nodes, patients can be randomised before results are available, but the results of these tests must be made available as soon as possible. If these patients are diagnosed with metastases at this stage, they will be withdrawn from the trial and should discontinue any trial medication. However, follow-up data will be collected on these patients (see sections 7.8 and 11).

[†]Definition of Locally Advanced Disease for the purposes of the ARTemis trial:

- *T4 tumour with direct extension to chest wall or skin (T4 a, b, c, see Appendix 3)*
- *Involvement of ipsilateral fixed or matted axillary nodes (N2), or ipsilateral infra or supraclavicular nodes (N3), see Appendix 3.*
- *Inflammatory breast cancer (T4d, see Appendix 3)*

6.5 Timing of neo-adjuvant chemotherapy and surgery

It is a UK Cancer Standard that definitive treatment commences within 31 days of a 'decision to treat' which would in general be regarded as the date of diagnosis. Best practice would be to start in a significantly shorter interval. In ***ARTemis***, investigators will be encouraged to start neo-adjuvant chemotherapy as soon as possible after core biopsy, preferably within 4 weeks, and certainly within 8 weeks. Similarly investigators will be encouraged to proceed to definitive surgery promptly after the completion of neo-adjuvant chemotherapy, within 3 weeks after the final day (day 21) of the last cycle of chemotherapy (i.e. between 3 and 6 weeks after the final dose of chemotherapy). In addition, in keeping with the recommendation to plan surgery more than 8 weeks after the last dose of bevacizumab in order to avoid wound complications, bevacizumab will be delivered with the first four cycles of chemotherapy only, and will NOT be delivered with the final two cycles of chemotherapy. The design thereby allows a washout period from the final bevacizumab dose to the date of surgery of 9 weeks minimum.

6.6 Translational blood and tissue sample collection

The primary hypothesis being tested within ARTemis-Science is that there are pharmacogenetic, pharmacogenomic and proteomic markers that can be correlated with outcomes (pathCR and RFS) in patients randomised to receive bevacizumab versus those that do not.

The **overall aim** is to identify molecular markers that predict for benefit (or lack thereof) of addition of bevacizumab to conventional chemotherapy (in other words to discover the equivalent of the trastuzumab/HER2 paradigm). To address this aim we propose to do both pharmacogenetic and pharmacogenomic studies in all patients accrued into the trial. The term pharmacogenetics is used here for the study of germline genetic variation and its influence in drug disposition and survival. Pharmacogenomics is the study of genomic/transcriptomic variation in tumours and how this somatic cancer genomic/transcriptomic composition influences drug activity and the natural history of the tumour.

Mandatory tissue and blood samples to be collected for analyses

1. Formalin-fixed, paraffin-embedded diagnostic tumour block, and tumour and normal tissue blocks from surgery, with corresponding diagnostic and surgery slides, from all 800 patients (for DNA/RNA/miRNA isolation and for TMA construction). See section 6.6.1 below for details.

2. One blood sample from each of the 800 patients for DNA isolation. See section 6.6.2 below for details.

Optional tissue and blood samples to be collected for analyses (also see section 12)

1. Fresh tissue biopsies prior to start of treatment, and when possible after 3 cycles of chemotherapy and from surgical resection, in 200-300 patients (for DNA/RNA/miRNA isolation). See section 12.2 for further details.

2. Sequential blood samples: one prior to treatment, one pre cycle 2, one pre cycle 3, one pre cycle 5, and one after cycle 6 / pre surgery, from 200-400 patients for circulating angiogenic markers. See section 12.3 for further details.

The profiling of the above samples has the following specific aims:

1. To derive molecular signatures (from combined DNA/RNA/miRNA profiling) predictive of bevacizumab response in fresh frozen biopsies and to validate these signatures using the nucleic acids and TMAs from paraffin-embedded samples.
2. To validate anthracycline and taxane 'resistance' signatures derived by profiling tumours from NEAT, Neo-tAnGo and tAnGo in the Caldas laboratory (CRUK TRICC and Caldas Programme funded) and to determine if tumours with such chemotherapy resistance signatures derive benefit from bevacizumab.
3. To determine whether molecular subtypes identified and validated in METABRIC have predictive value for bevacizumab response (in other words is there a subtype of breast cancer particularly 'sensitive' to bevacizumab).
4. To test the hypothesis that miRNAs that regulate angiogenesis are robust predictive markers of bevacizumab response.
5. To identify and/or validate candidate pharmacogenetics markers that predict benefit (or lack thereof) from addition of bevacizumab.
6. To validate candidate chemotherapy pharmacogenetics markers identified in Cambridge by PG-SNPs (CRUK TRICC-funded).
7. To determine if circulating angiogenic or other proteomic markers could be used to predict bevacizumab benefit.

The project outlined here builds on the extensive portfolio of CRUK-funded translational research in the Caldas laboratory (TRICC grants and Programme Grant), and the core research infrastructure at the Cambridge Research Institute (CRI: Core Genomics, Core Molecular Pathology, Core Bioinformatics) and at the NIHR Cambridge Biomedical Research Centre (and its embedded Breast Cancer Research Unit); and translational research in the Bartlett laboratory in Edinburgh.

6.6.1 Mandatory core biopsies and tumour block collection

Please be aware that it will be the responsibility of the local research team to obtain their patient's pathology material if the material is stored at a separate site to either the randomising hospital or the hospital of the named pathology contact.

Tumour block pre-treatment

Core biopsies will be performed on all patients to make the diagnosis of breast cancer. These will normally be done with 14-gauge under ultrasound control.

The patient's diagnostic paraffin embedded tumour block will be requested from the relevant Pathology department, and the remaining diagnostic core material will be returned to this original pathology department. If the tissue is stored in a pathology department not registered with the trial office (other hospital or private hospital) the local research team will be in charge of obtaining the tissue for the trial.
If diagnostic material is urgently needed, please contact the Cambridge Trial Office who will immediately retrieve the material and send it back to the Pathology department from whom it was requested. Permission to remove the specified material will be sought from the nominated pathologist at each centre. It is important to highlight that cores will only be taken if there is enough material available, and that a sufficient amount of diagnostic material will remain for a representative sample of the disease (at least three 0.6mm cores), because we understand that diagnostic material is the priority over research material. The diagnostic blocks will be returned to the original pathology department after cores have been removed for research purposes.
Positional marking of the tumour is recommended to be performed on all patients. In previous neo-adjuvant studies it has sometimes been difficult to locate tumours at the time of definitive surgery, particularly if there has been complete radiological response. Best practice, where possible, is to place a clip in the centre of the tumour, perhaps at the time of an extra biopsy (either pre-treatment or at the time of a second ultrasound / biopsy where this is being performed), so that definitive surgery (if this is not mastectomy) will remove the site of the original tumour. If the definitive surgical intention is mastectomy because of multi-focal disease, disease beneath the nipple, inflammatory or locally advanced breast cancer, then marking clips are still necessary to assist the pathologist in identifying the tumour site in case of significant pathological response (Dash, Chafin et al. 1999).

A copy of the associated diagnostic biopsy report will be requested for each patient, this should be anonymised to contain only the patient's unique ARTemis trial number.

Tumour blocks at surgery

In the same way that tumour material is requested at diagnosis, tumour blocks (tumour tissue and normal tissue) obtained from surgical material will also be requested for the ***ARTemis*** trial. If the tissue is stored in a pathology department not registered with the trial office (other hospital or private hospital) the research team will be in charge of obtaining the tissue for the trial.

At surgery, pathologists should take several tumour samples for embedded paraffin tumour blocks.
Where a complete clinical or pathological response is observed, it is still requested that one block of the tumour/original site of the tumour and one of adjacent normal tissue, from the same quadrant as the carcinoma, is taken and supplied to the Cambridge ARTemis Trial Office on request.

A copy of the associated surgery pathology reports will be requested for each patient, this should be anonymised to contain only the patient's unique ARTemis trial number.

The surgical blocks will be returned to the original pathology department after cores have been removed for research purposes.

Central review of histopathology reports from the local pathology department

A copy of the associated surgery pathology reports will also be requested for each patient. All patient identifiable data items will be removed by the originating hospital and the patients will only be identified by their unique ***ARTemis*** trial number. The histopathology reports will be used to assess the response to treatment and yield a pathCR primary endpoint which is robust and quality assured. The reports will be independently reviewed by two readers blinded to the treatment arm. When the original reviews do <u>not</u> reach a consensus then there is a further review with the two readers together, and a conclusion is reached for each discordant case.

Central review of diagnostic and surgery H&E (Haematoxylin and Eosin) slides

H&E slides from all blocks taken at diagnosis and surgery (tumour and lymph nodes where applicable) will be requested retrospectively from the nominated pathologist for all patients participating in the trial. Where slides are unavailable, blocks from surgery may be sent to the Cambridge Trial Office, and slides will be made. There will be central pathological review of all patients for the primary endpoint of pathological complete response. Our trial pathologists will review all cases comparing the diagnostic slides with the surgical slides provided. All grades of response (see section 8.3 for grade 1-5 criteria) will be defined for each patient by this central review. Cellularity will be compared with the original diagnostic slides prior to chemotherapy.

Definition of primary endpoint: pathological complete response will therefore be defined by central pathological review of the diagnostic and surgical slides, and by central review of histopathology reports.

Pathology departments will receive a per-patient payment for supplying these requested surgical slides, together with the tissues taken for research with patient consent (pre-treatment core biopsy, and paraffin-embedded blocks from the surgical specimen – tumour and normal tissue).

6.6.2 Mandatory blood sample collection

One mandatory blood sample, consisting of 2x 9ml EDTA bottles, should be obtained once only from each patient randomised into the trial. It is recommended that the blood sample is obtained at the same time as routine bloods samples are taken, to minimise the impact on the patient.
Blood sample kits and instructions should be requested from the Cambridge ARTemis trial office.

7. ARTemis treatment plan

7.1 Neo-adjuvant chemotherapy

The trial regimens are based on the PACS-01 regimen of FEC x 3 cycles and docetaxel x 3 cycles (Roche, Fumoleau et al. 2006). At the present time there is more safety and efficacy data on the combination of bevacizumab and docetaxel, than for bevacizumab with anthracycline-based chemotherapy. Bevacizumab therapy should stop at least 8 weeks prior to elective surgery. Therefore the trial management group favour reversing the sequence of chemotherapy, to give docetaxel prior to FEC, with or without bevacizumab during the first 4 chemotherapy cycles only.
FEC and docetaxel are to be administered as per local hospital policy: the use of pre-filled syringes of chemotherapy and the practice of dose banding is acceptable if this is your standard site practice.
All pre-medications, anti-emetics, G-CSF, and medications prescribed for or information on chemotherapy side-effects and treatment cautions/contra-indications and drug/food interactions, are given as per local hospital policy.

Arm A (D → FEC)

Docetaxel [**D**]100mg/m^2 IV x 3 cycles every 3 weeks [q3w]; followed by **FEC** [5-Fluorouracil 500mg/m^2 IV; Epirubicin 100 mg/m^2 IV and Cyclophosphamide 500 mg/m^2] day1 x 3 cycles q3w.

Arm B (bev + D → FEC)

Bevacizumab 15mg/kg q3w x 3 cycles plus Docetaxel [**D**]100mg/m^2 IV x 3 cycles every 3 weeks [q3w] ; followed by bevacizumab 15mg/kg plus **FEC** x 1 cycle, and 3 w later **FEC** x 2 cycles q3w.

7.2 Treatment compliance

Doses of all trial drugs will be recorded on the Treatment Case Report Form (CRF) together with dates administered. Details and reasons for dose reductions and delays will also be requested.

7.3 Investigations to conduct (Also see section 6.4 pre-randomisation investigations and Appendix 1)

Prior to each cycle of chemotherapy (+/- bevacizumab):

- Full blood count within 7 days prior to treatment cycle 1, and within 3 days prior to treatment cycles 2 – 6. If local practice is outside this time window, authorisation must be gained from the Trial Coordinators prior to randomisation of patients.
- Biochemical screen within 7 days prior to treatment cycle 1, and within 3 days prior to treatment cycles 2 – 6. If local practice is outside this time window, authorisation must be gained from the Trial Coordinators prior to randomisation of patients.
- Heart rate and blood pressure.
- Temperature.
- Weight.

Prior to each cycle of bevacizumab (cycles 1 – 4):

- Urinalysis: Proteinuria by dipstick unless proteinuria has been determined by 24-hour urine. Collect 24-hour urine in the event of proteinuria ≥2+ on dipstick.
- Coagulation test

After each treatment of chemotherapy (+/- bevacizumab), before the patient leaves the hospital:

- Heart rate and blood pressure.

After cycles 3 and 6 of chemotherapy (+/- bevacizumab):

- Radiological measurement of tumour(s) with ultrasound scans (see section 7.9).

After cycle 4 of chemotherapy (+/- bevacizumab):

- LVEF measurement by ECHO or MUGA.

7.4 Supportive therapies

As shown by a number of audits of FEC-taxotere therapy in standard care published by UK centres, febrile neutropenia is common. We recommend consideration of primary and certainly secondary prophylaxis with Granulocyte-Colony Stimulating Factors (G-CSFs) to minimise this risk. The use of supportive therapies, G-CSFs, antiemetics, and / or prophylactic antibiotics should be prescribed at the responsible clinician's discretion, following the standard hospital practice.

7.5 Management in response to toxicity

Toxicities should be recorded on the Treatment CRF, according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 for each cycle of chemotherapy. The guidelines shown in Table 1 below are recommended for bevacizumab toxicities. Any planned deviations from these guidelines is to be discussed on a case-by-case basis with the ***ARTemis*** Trial Office, and the Chief Investigators or members of the TMG. Chemotherapy-related toxicities and subsequent dose reductions will be managed as per local hospital policy.

Extravasation

If extravasation of bevacizumab occurs during an infusion, it is recommended to take the following actions:
- Discontinue the bevacizumab infusion.
- Treat the extravasation according to institutional guidelines for extravasation of a non-caustic agent.
- If a significant volume of bevacizumab remains in the infusion bag, restart the infusion at a more proximal site ipsilaterally, or on the contralateral limb.

If extravasation of chemotherapy occurs during an infusion, local hospital policies must be followed.

Bevacizumab related toxicities

The overall safety profile of bevacizumab is based on the experience of several thousands of patients with various cancers who received bevacizumab either as single agent or in combination with chemotherapy in clinical trials. The most frequently observed adverse events are: asthenia, diarrhoea, nausea, epistaxis, hypertension, proteinuria and pain-not specified. Analysis of safety data suggests that the occurrence of hypertension and proteinuria with bevacizumab therapy are likely to be dose-dependent. The most Serious Adverse Events (SAEs) were gastrointestinal perforations, wound healing complications, tumour associated haemorrhage (primarily in NSCLC) and thromboembolism (in colorectal, breast, and NSCLC). In addition, Congestive Heart Failure (CHF) was observed rarely and predominantly in patients with metastatic breast cancer who had received prior anthracycline based chemotherapy and prior chest wall radiation.
There is a possibility of increased risk of neutropenia in those patients receiving bevacizumab with chemotherapy.

No bevacizumab dose reductions are planned in this trial. However, bevacizumab treatment might be delayed or discontinued if certain toxicities occur.

Table 1: Bevacizumab Related Toxicities Management Guidelines

Toxicity	Severity CTCAE (V3.0) grade	Action
Hypertension	Grade 2 or 3 **Grade 4** or *encephalopathy grade 2*	Delay the administration of bevacizumab. Bevacizumab may be restarted with standard anti-hypertensive medication if the BP is well-controlled (see below). Stop bevacizumab if hypertension is not controlled with medication within 3 weeks. Stop bevacizumab
Left Ventricular Systolic		

Dysfunction Asymptomatic decline in LVEF Symptomatic decline in LVEF	 If LVEF < institution's normal limit **Grade 2, 3 or 4** (including symptomatic CHF)	 Delay and repeat MUGA/ECHO in 3 weeks. Stop bevacizumab
Haemorrhage Pulmonary or CNS Non pulmonary or Non-CNS	 **Grade 2 / 3 / 4** **Grade 3 / 4**	 Stop bevacizumab Stop bevacizumab
Wound complications (non infectious)	Grade 1 **Grade 2 / 3 / 4**	Delay bevacizumab until a substantial healing has taken place. Stop bevacizumab
Proteinuria	Grade 2 *2+ or 3+ on urine dipstick* *>3.5g/24 hrs* Grade 3 *4+ on urine dipstick* *>3.5g/24 hrs* **Grade 4** *Nephrotic syndrome*	Continue Delay until proteinuria improved to ≤ grade 2. Stop bevacizumab
Allergy	GRADE 1 DURING INFUSION: Grade 2 during infusion Grade 3 **Grade 4**	SUPERVISE THE PATIENT AND COMPLETE INFUSION. Stop the infusion. After recovery, resume infusion at half the previous rate for 15 minutes. If no further symptoms occur, complete the infusion at this rate. If infusion related AE occurs, all the subsequent infusions should be administered over the shortest period that was well tolerated with pre-medication. The bevacizumab infusion should be stopped and not –restarted the same day. Discontinue or re-challenge with pre-medication and close monitoring at responsible clinicians discretion. Stop bevacizumab
Gastrointestinal (GI) perforation	**Any grade**	Stop bevacizumab
Fistula or intra-abdominal abscess formation	**Any grade**	Stop bevacizumab
Development of Angina or Myocardial Infarction	**Any grade**	Stop bevacizumab
Arterial Thrombosis / embolism	**Any grade**	Stop bevacizumab
Deep Venous Thrombosis / embolism	**Grade 3 or 4**	Stop bevacizumab.
Reversible Leukoencephalopathy	**Any grade**	Stop bevacizumab
Other clinically significant AE	Grade 3	Delay until the AE has resolved to ≤ grade 1

	Grade 4	Stop bevacizumab

Management of Hypertension

A referral to a cardiologist is suggested for patients who develop hypertension during the trial – see Appendix 5 for guidelines. Ideally, cardiovascular assessments and management of hypertension should be supervised by a cardiologist. Treatment for emergent hypertension should be the treating clinician's decision, taking into account standard cardiology practice.

The management plan provided below should also be incorporated in the clinical decision making:

- If the blood pressure is at any time *>140mmHg systolic or >90mmHg diastolic,* a second reading should be taken after a 'resting' period (patient seated comfortably, not lying down) of 15mins. If the second reading is over the limits stated above, delay the administration of bevacizumab and initiate / intensify anti-hypertensive therapy. Bevacizumab may be restarted in conjunction with standard anti-hypertensive medication if the BP is controlled (i.e. BP ≤ *140mmHg systolic or ≤90 mmHg diastolic).*
- If the BP is not controlled with medication within 3 weeks, permanently discontinue bevacizumab.
- If Grade 4 life-threatening toxicities arise (e.g. hypertensive crisis / hypertensive encephalopathy) then permanently discontinue bevacizumab.
- For the purpose of bevacizumab treatment, grade 2 encephalopathy possibly related to hypertension will require permanent discontinuation of bevacizumab.

For discussion on recommendations for BP measurement, the following article by the American Heart Association is helpful: http://hyper.ahajournals.org/cgi/content/full/45/1/142.

For guidance on the management of hypertension, see Appendix 5: Guidelines for the management of bevacizumab-induced Hypertension.

Chemotherapy related toxicities

For chemotherapy dose modifications, standard national / international dose reductions should be used, at the discretion of the responsible clinician. Dose reductions for haematological toxicity and its consequences (neutropenic fever / sepsis) should be avoided if possible by the the use of G-CSF, or pegfilgrastim.

When patients have required a dose reduction of docetaxel during the first phase of their treatment (i.e. D ± bevacizumab depending on treatment allocation), we recommend that they should not be routinely dose-reduced for their second phase of treatment (FEC ± bevacizumab).

Where a trial participant has had a significant allergic reaction attributed to docetaxel, they may, according to the severity of the reaction, and at the discretion of the responsible treating clinician, be re-challenged with docetaxel according to a locally determined desensitisation procedure, or withdrawn from the trial to receive alternative therapies / switched to a FEC chemotherapy regime early, depending on the opinion of the responsible clinician. If any information is required on using a desensitisation procedure for the administration of docetaxel, please contact the Cambridge ARTemis Trial Office for further information.

For further information on patients switching to FEC early, see section 7.8.

Concomitant Medication / Treatment

At trial entry, given that it is not an exclusion criterion, patients may continue with previous concomitant treatments (i.e. prescription, non-prescription or alternative therapies) at the same doses and schedule as prior to the start of trial treatment if judged clinically appropriate. Institutions should follow their local and or national / international administration guidelines for the selected chemotherapy regimen.

Anti-emetic Therapy

Anti-emetics should be prescribed at the responsible clinician's discretion and according to local policy and established national and international guidelines.

Granulocyte–Colony Stimulating Factors (G–CSFs)

As shown by a number of audits of FEC-taxotere therapy in standard care published by UK centres, febrile neutropenia is common. We recommend consideration of primary and certainly secondary prophylaxis with Granulocyte-Colony Stimulating Factors (G-CSFs) to minimise this risk, and the use of G-CSFs should be prescribed at the responsible clinician's discretion, following the standard hospital practice.

Prophylactic Antibiotics

At the investigator's discretion, primary oral prophylaxis with fluoroquinilones may be prescribed as an alternative to G-CSFs, e.g., levofloxacin 500mg daily or ciprofloxacin 500mg given twice daily.

7.6 Treatment Delays

The decision whether to continue with trial medication should be based on the individual circumstance and the responsible clinician's judgment that continuation is in the patient's best interest.

Bevacizumab specific toxicity

If a treatment delay of bevacizumab is required the chemotherapy administration should not be affected and should remain on schedule. If 1 week delay of bevacizumab is required to allow resolution of the relevant toxicity then give 10mg/kg one week after chemotherapy. If 2 week delay of bevacizumab is required then give 5 mg/kg two weeks after chemotherapy. If a longer delay of bevacizumab is required then reassess when the next cycle of chemotherapy is due. If, when the next cycle of treatment is due, the reason for the bevacizumab delay has resolved / clinically improved to grade 1 or less, treatment with bevacizumab can restart at 15mg/kg as normal. If the reason for the delay has not improved sufficiently to allow treatment by the time the next chemotherapy cycle is due, the bevacizumab is discontinued indefinitely. When a patient randomised to bevacizumab has to discontinue bevacizumab early because of bevacizumab-related toxicity, they should complete their chemotherapy regimen without being withdrawn from the trial.

Chemotherapy specific toxicity

When the treatment delay is due to the chemotherapy, in order to maintain adequate bevacizumab levels single agent bevacizumab should be given at a dose of 5mg/kg for each week that chemotherapy is delayed. When the chemotherapy is re-commenced bevacizumab should be given at a dose of 15mg/kg per cycle as normal.

Maximum dose-delay

The maximum permitted dose-delay for the chemotherapy (+/- bevacizumab) is 4 weeks for recovery of severe toxicity or for unscheduled procedures (e.g. appendectomy). If longer delays are required, then the patient will be withdrawn from trial, and alternative therapy considered by the responsible clinician.

7.7 Disease progression

If there is evidence of disease progression at any stage during the neo-adjuvant treatment this should be confirmed radiologically, and if confirmed the patient should be withdrawn from trial treatment (but remain on trial for follow-up and sampling purposes – see section 7.8).
It is recommended that if progression occurs during the first phase of neo-adjuvant chemotherapy (Docetaxel +/- bevacizumab); patients should proceed to the second phase of treatment (FEC as non-trial treatment), or to definitive surgery (if that is planned). This decision is at the discretion of the responsible clinician. Since there is some evidence for non-cross resistance between the taxanes and the anthracycline-based treatment, proceeding with the second phase of treatment is acceptable, but depends on the specific clinical circumstances. If progression occurs during the second phase of treatment (FEC +/- bevacizumab) then immediate surgery, radiotherapy or endocrine therapy can be considered at the decision of the responsible clinician. However, if progression occurs during either phase, surgeons need to be alerted to the possibility of early surgery for the patient. If the patient is receiving bevacizumab attention should be paid to the desirability of a wash-out period before surgery as discussed above. For this reason, patients in arm B will have bevacizumab discontinued at the time of premature change of chemotherapy (i.e. withdrawal from trial treatment).

Progression of any patient should be communicated to the ***ARTemis*** Trial Office in Warwick immediately by telephone. Progression must be recorded on the Relapse / Death Case Report Form, and a Withdrawal form must also be completed and sent to Warwick CTU as soon as possible.

7.8 Patient withdrawal

Withdrawal for the purposes of ARTemis is defined as:

- **Patient request for complete withdrawal from the trial**, i.e. the patient wants no further treatment or samples taken.
- **Withdrawal from trial treatment**: where the patient is not able to continue with their randomised trial chemotherapy regimen, but remains on-trial for all other aspects i.e. data collection, follow-up, blood and tissue samples, etc.

Withdrawal of patients for any reason should be communicated to the ***ARTemis*** Trial Office in Warwick as soon as possible by telephone. Reasons for withdrawal should be recorded on the Withdrawal Form. Patients electing to withdraw voluntarily need not provide any explanation.
Patients should **discontinue trial treatment** in the following circumstances:

- If the patient is inadvertently enrolled without meeting the eligibility criteria.
- The investigator decides that the patient should be withdrawn from the trial at any point due to toxicity.
- If a patient within 4 weeks has not recovered from toxicity to an extent that allows further treatment.
- The patient opts to withdraw from the trial or chooses not to comply with trial procedures.
- The patient becomes pregnant or fails to use adequate birth control (for those patients who are able to conceive).
- Confirmed disease progression (according to WHO criteria).

Please note: Patients who are switched to FEC earlier than cycle 4 (for example due to an allergic reaction to docetaxel), should have a Withdrawal Form and all the usual CRFs completed (e.g. Treatment Forms, Radiology Forms, Surgery Form) along with all sub-study samples or questionnaires that the patient has consented to, e.g. fresh tissue and/or blood samples, and/or Quality of Life Questionnaires.

Follow-up data will be collected on all withdrawn patients (this also applies to patients who opt to withdraw consent for the trial unless they explicitly forbid further data to be collected).

7.9 Clinical and radiological monitoring during neo-adjuvant chemotherapy

In the neo-adjuvant setting, radiology has a role in estimating chemotherapy effect both in terms of tumour size and activity. Mammography in this situation shows poor correlation, as many of the tumours are irregular and lie in dense breasts. Candidates for neo-adjuvant chemotherapy are more commonly younger women, and mammographic density is associated with both age and high tumour grade (more common in candidates for neo-adjuvant chemotherapy). Ultrasound also has limitations again due to the often irregular and multi-focal nature of these tumours and is operator dependent, and the record is subjective. On the other hand, MRI has the potential to view tumours in three dimensions, and gives a better estimate of volume. Moreover, the behaviour of the contrast gives some information of tumour activity. However, our experience from Neo-tAnGo suggests that ultrasound is a robust radiological assessment which fulfils the RECIST criteria.

In order not to compromise the analysis, and as Ultrasound is the most common measurement tool available across the country, for consistency of radiological measurement comparisons, ultrasound measurements will be used for ARTemis. Other scan techniques can be performed, and Investigators at centres already using MRI in the neo-adjuvant setting are encouraged to continue in light of the discussion above, however **Ultrasound scans are mandatory for all ARTemis patients before treatment, after cycle 3 and after cycle 6 (before surgery).**

Variation in timing of ultrasound scans across tumours or patients can compromise the analysis. Therefore, the time points below must be followed as closely as possible:

- Before the first cycle of chemotherapy: ultrasound (US) must be obtained as close as possible to the first cycle of treatment.
- After completion of the first 3 cycles of neo-adjuvant chemotherapy: US will be repeated to assess radiological response. The date of measurement must be as close to day 21 of cycle 3 as possible.
- After completion of 6 cycles of neo-adjuvant chemotherapy: US will be repeated to assess radiological response. The date of measurement must be as close to day 21 of cycle 6 as possible, and prior to surgery.

Radiological response

At each radiological (ultrasound) measurement the sum of the longest single diameter of all measurable tumours (including axillary LNs if enlarged) must be recorded on first section of each of the Radiology Forms in the ARTemis CRF.
In the second section of the Radiology Forms a response must be categorised, this must be calculated from the 'total tumour size' defined as the **sum of the longest single diameter of each measurable tumour located in the breast only.**
Ultrasound measurements taken at both the midway and end of treatment scans must be compared to the pre-treatment (Baseline) ultrasound measurements in order to assess the radiological response.

Radiological response criteria (for ultrasound measurements) are as follows:

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): ≥30% decrease in the sum of the longest diameter of all measureable breast lesions

Stable Disease (SD): Between 30% decrease and 20% increase in the sum of the longest diameter of all measureable breast lesions

Progressive Disease (PD): ≥20% increase in the sum of the longest diameter of all measureable breast lesions OR the appearance of at least 1 new lesion.

The three 'Radiology' forms in the CRF must be signed by the responsible radiologist or PI at the individual sites.

7.10 Surgery

Axillary surgery and sentinel node biopsy

Pre-chemotherapy sentinel node biopsy procedures are allowed and indeed encouraged in accordance with standard local practice. The Cambridge Breast Unit (in the Neo-tAnGo trial) has adopted a policy of axillary lymph node (LN) biopsy when axillary lymph nodes are suspicious on US (Britton et al 2009). If US showed normal axillary LNs or if axillary LN biopsy was negative, then pre-chemotherapy sentinel node biopsy was carried out. This policy results in accurate pre-chemotherapy pathological staging of the axillary LNs, which in our view provides important standard prognostic information and optimises axillary management after neo-adjuvant treatment. Our experience, showing no significant delay in time to commencing chemotherapy, has been presented at San Antonio Breast Cancer Symposium 2008 (Wishart 2008). Trial treatment is recommended to start within 5 weeks of the initial core biopsy. Unless sentinel node biopsy has been performed before chemotherapy, and is negative, axillary surgery is required at the time of definitive breast surgery, in accordance with local standard practice. This may include sentinel node biopsy (Mamounas, Brown et al. 2005) or axillary sample (for example if clinically and radiologically the axilla was thought to be free of disease prior to chemotherapy) or formal axillary dissection. There is a lack of data on the accuracy of sentinel node assessment after neo-adjuvant chemotherapy.

Timing of surgery

The high rates of local recurrence which have been reported in other neo-adjuvant studies when surgery is not part of primary treatment, leads us to encourage all investigators to perform some form of surgery after chemotherapy. Surgery must be planned to occur at completion of the protocol stated six cycles of chemotherapy, at any time following the last day (day 21) of the final cycle of chemotherapy, and certainly within 3 weeks of that date (i.e. between 3 and 6 weeks after the last dose of chemotherapy).

Surgery type – Breast and Axilla

At the time of diagnosis, the surgeon will decide on the intended surgery to be carried out after chemotherapy. For smaller lesions in any quadrant, breast-conserving surgery with wide local excision is the standard. For larger but peripheral lesions breast conserving surgery may be possible pending a good clinical and radiological response to neo-adjuvant treatment.

Patients with clinically inflammatory or large locally advanced cancers at diagnosis, or where the primary tumour is under or close to the nipple, will not be offered breast-conserving surgery but will be offered mastectomy, with or without immediate reconstruction. Patients with smaller, peripheral, locally advanced tumours who respond well to chemotherapy may be offered breast-conserving surgery. Patients with multiple lesions will be offered mastectomy, with or without immediate reconstruction.

After neo-adjuvant chemotherapy definitive axillary surgery remains a matter of debate at the present time (see **Axillary LN surgery** section above), and post treatment axillary SLNB, axillary LN sampling, and axillary lymph node clearance are all acceptable in the trial, depending on local practice. Experience from the Cambridge Breast Cancer Group, recommends pathological axillary LN assessment prior to neo-adjuvant treatment, and if

positive, recommends an axillary lymph node clearance at the time of definitive breast surgery. When the axilla is pathologically negative before treatment then no further axillary surgery is required after treatment. When axillary pathological LN staging is positive, by any procedure (LN biopsy, SLNB, or axillary LN sampling), or at any point (before or after neo-adjuvant treatment), then definitive axillary node clearance is recommended, either with or following definitive breast surgery.

7.11 Endocrine therapy

LHRH agonists during chemotherapy for ER-ve pre-menopausal women

The prescription of LHRH-agonists to ER negative pre-menopausal women during the chemotherapy treatment (in an attempt to preserve fertility) remains at the discretion of the responsible clinician following standard local therapy protocols however, patients who are ER strongly positive and prescribed LHRH-agonists should not be randomised into ARTemis due to the potential detrimental effect of these drugs on the chemotherapy regimen.

Adjuvant Hormonal Treatment after Surgery

Following completion of neo-adjuvant chemotherapy and definitive surgery, women with ER-positive disease would expect to be offered adjuvant hormonal therapy for a minimum of five years. Choice of endocrine therapy is at the discretion of the responsible clinician in accordance with standard local therapy protocols; the following are guidelines only:

- For pre-menopausal women, acceptable hormonal therapy options are tamoxifen alone or ovarian suppression and tamoxifen.
- For these high risk postmenopausal women, aromatase inhibitors should be given, either for five years alone or as a tamoxifen / aromatase inhibitor 'switch' for a minimum of 5 years.

7.12 Adjuvant chemotherapy

Additional adjuvant chemotherapy following surgery in patients who have involved axillary nodes after neo-adjuvant treatment in the ARTemis trial may be considered at the discretion of the responsible clinician. Standard therapy in this situation is currently discussed on an individual patient basis. At present there is no randomised evidence to assist decision-making in this context.

7.13 Herceptin® (trastuzumab)

Patients taking part in the ARTemis trial have HER2-negative breast cancer by definition and are not eligible to receive Herceptin®. However if a patient on study with HER2-ve disease on core biopsy, changes to HER2+ve on the definitive resection specimen, the patient would have to be withdrawn from the trial at that point and could receive Herceptin® as per local practice. At this stage the patient would by definition be withdrawn from the trial, although all data would continue to be collected for analysis.

7.14 Radiotherapy

Radiotherapy will be given after definitive surgery according to local protocols. It should be initiated ideally within 4 weeks of surgery and no later than 8 weeks.

Following breast conserving surgery radiotherapy is mandated. The irradiated area should be the breast and surgical bed.

A radiotherapy boost may be used taking into account criteria of local recurrence in keeping with local treatment policies:
- inadequate surgical margins after breast conserving surgery
- age less than 50

Following mastectomy the indications for chest wall irradiation are according to local protocols. Decisions on the role of post-mastectomy radiotherapy should take into account pre-chemotherapy tumour characteristics, to avoid compromising potential gains achieved by neo-adjuvant treatment. Standard indications for post mastectomy radiotherapy include:

- tumour size ≥ 50 mm (T3)
- any T4 tumour
- 4 or more involved axillary nodes
- inadequate tumour excision
- Skin involvement

8. Histopathology and assessment of histopathological response

8.1 Histopathological examination

Histopathological assessment will be carried out at diagnosis and repeated on the definitive surgical specimens; either wide local excision or mastectomy, and axillary clearance. These specimens will be handled and reported according to national guidelines for non-operative procedures and for pathology specimen handling (NHS BSP Publication No 50. 2001 and NHS BSP Publication No 58, 2005). It should be noted that the macroscopic search for tumours which have undergone significant pathological response will be aided by clear communication of the original sites of the tumour, on a copy of each of the three Radiology CRFs, and histology request form or by marking the sites with clip or suture. Pathologists should sample this area thoroughly.

8.2 Receptor assays

Oestrogen receptor (ER) and HER2 receptor assessment will be carried out routinely on all tumours at diagnosis. Assessment of ER status should, if possible, be repeated on the definitive surgical specimen if adequate numbers of tumour cells remain. Where progesterone receptor (PR) assays are carried out routinely this information will also be collected at diagnosis and again at definitive surgery, however PR testing will be part of the translational research carried out in the ***ARTemis-Science*** study. ER, PR and HER2 will be reported as per UK National Guidelines (NHS BSP Publication No 58, 2005).

8.3 Grading of pathological response

Ideally, a comment as to the degree of chemotherapy effect should be included in the pathology report. However histopathological response will also be assessed centrally by the trial pathological advisors using H&E slides from blocks taken at diagnosis and surgery (tumour +/- lymph nodes), see section 6.

The primary endpoint for the trial is complete pathological response in the tumour following neo-adjuvant treatment, and is defined below:

> **Complete pathological response (pathCR) rates (tumour and lymph nodes) after neo-adjuvant chemotherapy defined as no residual invasive carcinoma within the breast (DCIS permitted) AND no evidence of metastatic disease within the lymph nodes (Pinder, Provenzano et al. 2007)**

One recommended system for evaluating histopathological tumour response following neo-adjuvant chemotherapy in early breast cancer which is used in clinical trials, is shown below:

Grade 1: some alteration to individual malignant cells but no reduction in overall numbers compared with the pre-treatment core biopsy.

Grade 2: a mild loss of invasive tumour cells but overall cellularity still high

Grade 3: a considerable reduction in tumour cells up to an estimated 90% loss

Grade 4: a marked disappearance of invasive tumour cells such that only small clusters of widely dispersed cells could be detected

Grade 5: no invasive tumour cells identifiable in the sections from the site of the previous tumour, i.e. only *in situ* disease or tumour stroma remain.

9. Trial organisation

9.1 Conduct of trial

ARTemis will be conducted in compliance with ICH Good Clinical Practice (GCP) and the UK legislation.

The Principle Investigator (PI) at each participating site will be required to sign a Participating Site Agreement and supply the Warwick Trial Office with a current curriculum vitae with evidence of up-to-date GCP training. PI's are also required to complete a signature page for each new version of the ARTemis protocol issued by the ARTemis trial office.

All site personnel involved in the conduct of the trial (at a minimum the Principle Investigator, Pharmacist and a lead nurse or coordinator or data manager for the trial) will be asked to complete Registration Forms, staff Signature and Delegation Logs, and attend an initiation meeting which will cover trial rationale, protocol procedures, and collection and reporting of data. These meetings will be conducted by the ARTemis Trial Offices personnel either in person or by telephone as appropriate. Following this, all sites will be provided with an Investigator Site File and Pharmacy Site File containing instructional materials and documentation required for the conduct of the trial. The ***ARTemis*** Trial Offices will offer continued support for sites via telephone, , email, and mail. New site staff who did not complete initiation training will be offered initiation training by the trial offices, otherwise site staff present at the initiation training are able to train their new staff.

9.2 Stratification variables.

Stratification variables are defined as follows:

- **Age:** ≤50 years; >50 years
- **ER Status:** negative (Allred score 0-2); weakly positive (Allred score 3-5) and strongly positive (Allred score 6-8).
- **Tumour size:** ≤ 50mm; > 50mm. (In cases with multi-focal disease in one breast, or bilateral disease, the size to be used for the stratification is the sum of the largest single diameter of all measurable tumours).
- **Clinical involvement of axillary nodes:** no; yes. (Clinical involvement is defined as palpable nodes.)

NB: Whenever possible, the TMG strongly recommend diagnosing the involvement of the axillary nodes by FNA or core biopsy. Also, if possible, when nodes are negative, the TMG strongly encourages a SLN biopsy pre-chemotherapy. For more details see section 7.10.

- **Inflammatory / Locally advanced disease:** no, yes.

Definition of Locally Advanced Disease for the purposes of ARTemis:

- Inflammatory cancer (T4d, see Appendix 3)
- T4 tumour with direct extension to chest wall or skin (T4 a, b, c, see Appendix 3)
- Involvement of ipsilateral fixed or matted axillary nodes (N2) or ipsilateral infra or supraclavicular nodes (N3), see Appendix 3.

9.3 Randomisation of patients

Randomisation is recommended within 4 weeks of the initial core biopsy and chemotherapy should start within one week after randomisation; please contact the ARTemis trial office if timelines are outside these recommendations.

Eligibility and randomisation forms must be completed prior to randomisation.

Details should then be phoned or emailed through to Warwick Clinical Trials Unit, **between 9am and 5pm, Monday to Friday** (excluding Bank Holidays and specific dates as notified by the Warwick CTU).

Tel: 02476 150600 or Email: artemis@warwick.ac.uk

***Note:* The name of the investigator directly responsible for the patient's care will be requested at randomisation. Investigators must be pre-registered with the trials unit before they are permitted to enrol patients into the trial.**

9.4 Data collection

Case Report Forms will be designed by Cambridge Trial Office in collaboration with Warwick Clinical Trials Unit and will comprise the following:

Table 4: Standard forms and summary of data collected

Form	Summary of data recorded	Schedule for submission
Eligibility	Confirmation of eligibility and satisfactory staging investigations where necessary;	Emailed at point of randomisation
Randomisation	Patient details; details of stratification variables; optional consent issues	As soon as possible after randomisation
On-study	Details of biopsy histology; (with copy of diagnostic biopsy report); details of planned surgery	Within 1 month of randomisation.
Treatment	One form for each cycle of chemotherapy recording actual chemotherapy doses and dates given; details and reasons for dose reductions and delays; details of supportive treatment; details of reported toxicity	Within 1 month of completion of relevant chemotherapy cycle.
Radiological assessment by ultrasound(pre-treatment, mid-treatment, and end of treatment)	Results of radiological assessment of tumour and radiological response (from ultrasound) when applicable	Within 1 month of assessment taking place.

Treatment summary	Summary of chemotherapy treatment and reasons for any non-compliance; details of any further adjuvant therapy to be given.	Within 1 month of completion of neo-adjuvant chemotherapy or patient withdrawal.
Surgery	Full details of tumour histology, including a copy of the histology report (from National Minimum Dataset, if applicable).	Within 1 month of completion of all surgery.
Follow-up	Annually for 10 years. NB: Visits should be every 6 months the first 2 years.	Completed annually, and within 2 months of anniversary of surgery.

***Ad hoc* forms**

- Serious Adverse Event Form to be submitted to Cambridge trial office within 24 hours of first knowledge of the event (refer to section 10.2).
- Relapse/Death Form (refer to section 11.2). Relapse to be reported within 1 week of confirmation. Death (except death due to disease progression) to be reported as an SAE immediately – see section 10.
- Transfer Form to be submitted within 1 week of transfer of care (please notify the Warwick Trial Office of the transfer of patient care to another centre or GP).
- Additional Information Form – when information is known.
- Withdrawal form to be submitted within 1 week of decision to withdraw.
- PI Declaration Form to be submitted after all primary treatment completion.
- Query forms to be submitted within 1 month.

Data collection will be the responsibility of Warwick Trial Office. Pharmacovigilance, and sample collection and anonymisation will be the responsibility of Cambridge Trial Office. All data will be handled and stored in accordance with the 2018 Data Protection Act, which includes General Data Protection Regulations (GDPR).

9.5 Data quality assurance & monitoring

Case report forms must be submitted to the ***ARTemis*** Warwick Trial Office in a timely manner according to the schedule outlined in Table 4 above. On receipt, all forms will be checked for completeness and congruity. Forms containing empty data fields or data anomalies will be queried and returned to site for resolution.

Queries should be answered within a month, and within a week if related to an SAE.

10. Safety reporting

The collection and reporting of data on Adverse Events (AEs) and Serious Adverse Events (SAEs) will be in accordance with the EU Directive 2001/20/EC and UK legislation.

10.1 Adverse Events (AE)

An AE is defined as any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

The incidence and nature of AEs is an important endpoint for this trial and all AEs should be documented on the Treatment forms in the toxicity section completed for each cycle of treatment. However, the following should NOT be reported as an AE:

- A pre-existing condition, unless the condition worsens or episodes increase in frequency during the reporting period and the investigator deems this related to use of the trial drugs
- Symptoms relating to, or the treatment of, disease progression unless the investigator deems them related to the use of trial drugs
- Death due to disease progression

10.2 Serious Adverse Events (SAEs)

Definitions for the purposes of the ARTemis trial

A SAE is any untoward medical occurrence that at any dose results in:

- death
- initial or prolonged inpatient hospitalisation (excluding hospitalisation for trial drug administration) *
- a life-threatening event (i.e. immediate risk of dying)
- persistent or significant disability/incapacity
- congenital anomaly or birth defect

Important medical events that may not be immediately life-threatening or result in death or hospitalisation, but which may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

***** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure, for continued observation. Hospitalisation for a pre-existing condition, including elective procedures, which has not worsened, does not constitute a serious adverse event.

Progression alone should not be reported as an SAE, except in circumstances where a patient progresses and experiences any event which constitutes an SAE as defined above. Progression (see 7.7) should be communicated immediately by telephone to ARTemis Trial Office in Warwick.

Reporting SAEs

SAEs must be reported to the Cambridge Trial Office, within 24 hrs of becoming aware of the event. A Serious Adverse Event Form should be completed and emailed to the *ARTemis* Cambridge Trial Office at:

cctuc@addenbrookes.nhs.uk

If required, Investigators should also report SAEs to their Trust Research & Development Department in accordance with their local institutional policy.

Documenting SAEs

The responsible clinician must determine the severity (grade) of an event (according to the NCI CTCAE Version 3.0), and the relatedness of the event to each of the trial drugs. Relatedness will also be independently assessed by the Chief Investigator (or designee) as well as Expectedness. A Serious Adverse Event judged by the Investigator or Chief Investigator to have a reasonable causal relationship with the trial medication will be regarded as a serious adverse reaction (SAR). If the event meets the definition of a serious adverse reaction that is unexpected in nature it will be classified as a suspected unexpected serious adverse reaction (SUSAR).

Reporting period for SAEs

If a patient experiences an SAE after the informed consent document is signed, but before receiving the first dose of trial drug, the event will be reported only if the Investigator believes that the event may have been caused by a protocol procedure.

Details of all SAEs will be documented from the commencement of treatment until 30 days post-treatment (i.e. 30 days from last administration of a trial drug).

SAEs occurring after a patient's 30-day follow-up assessment should be reported only if the Investigator believes that a trial drug or a protocol procedure may have caused the event.

Follow-up of SAEs

In the case of an SAE, the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the disease has stabilised. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information will be noted on the Serious Adverse Event Form by ticking the box marked 'follow-up' and sending to the ***ARTemis*** Cambridge Trial Office as information becomes available. Extra annotated information and/or copies of test results should also be provided where available.

Reporting of SARs to regulatory authorities

The Cambridge Trial Office will report all fatal or life threatening SUSARs to the Medicines and Healthcare products Regulatory Agency (MHRA) and the Multi-Centre Research Ethics Committee (MREC) within 7 days of receiving initial notification from the trial site. Any follow-up information will be provided within an additional 8

days. Non-fatal and non-life threatening SUSARs will be reported within 15 days. The Cambridge Trial Office will submit an annual report to the MHRA and MREC summarising all SAEs.

The Cambridge Trial Office will forward details of SUSARs to all Investigators in the form of a safety report produced every 6 months.

SAEs (including SUSARs) will be reported to Roche Limited, and SUSARs will be reported to Sanofi-Aventis Limited as appropriate.

11. Patient follow-up

11.1 Follow-up schedule

Patients will be followed-up with a physical examination and medical review routinely every 6 months for the first two years following completion of surgery, then annually (with mammography if as per standard practice and applicable) for a total of ten years after surgery (see Appendix 1 for summary of schedule). Once a patient has been discharged from clinical review, physical examinations do not need to be performed for the purpose of the trial. Telephone follow-up is permitted for patients who have been discharged from clinical review. Follow-up by email is permitted subject to local information governance policies. Follow-up data will be submitted via the 'Follow-up' CRF annually for 10 years based on the surgery date anniversary. Site staff should attempt to contact patients at least annually until the 10 year follow-up period is complete for that patient.
Where necessary, follow-up data will be obtained from patients' GPs or through the Office for National Statistics (ONS) using the patients' NHS number.

11.2 Relapse and death

Long-term follow-up will include assessment of cardiac failure, vital signs (until discharged from clinical review), dates and sites of first relapse (see table 5), both local and systemic treatment at relapse, and date and cause of death. This is necessary for the trial's secondary endpoints of disease-free and overall survival. As soon as definite confirmation has been obtained, a Relapse/Death form should be completed and returned to the trials unit. Patients who relapse should remain on follow-up.

***Table 5*: Definition of relapse and death**

Loco-regional	Ipsilateral breast/ chest wall, ipsilateral axilla nodes, ipsilateral supraclavicular nodal relapse and contralateral breast disease.
Distant	Distant relapse (excluding ipsilateral supraclavicular nodes)
2nd primary	Including contralateral malignant breast disease
Death	Death from any cause

12. Optional Sub-studies

12.1 Quality of life sub-study

The quality of life sub-study will be carried out using the FACT-B and EuroQoL questionnaires and will be offered to all randomised female patients until the accrual target is met. Investigators inviting patients to take part in the Quality of Life sub-study will need to obtain consent (ARTemis Patient Information Sheet / Informed Consent Form 2). Participating patients will be provided with all questionnaires by trial site staff at the relevant timepoints as follows:

- Prior to commencement of chemotherapy (baseline)
- Following completion of first three cycles of chemotherapy (9 weeks post-randomisation)
- Following completion of six cycles of chemotherapy (5 months post-randomisation)
- Following completion of surgery and radiotherapy (8 months post-randomisation)
- Then annually for 2 years from completion of surgery (at follow-up visits 12 and 24 months post surgery) to document long-term effects on quality of life.

Details of treatment related toxicities will be collected at each cycle of treatment using the NCI CTCAE Version 3.0 scoring system. Data will also be collected on hospital admissions and use of G-CSF.

12.2 CTCR-BR05: Fresh Tissue sub-study

Investigators inviting patients to take part in the ***CTCR-BR05*** Fresh Tissue sub-study will need to obtain consent (ARTemis Patient Information Sheet / Informed Consent Form 3) from patients for an extra research biopsy to obtain fresh tissue prior to start of treatment and after 3 cycles of treatment. A third fresh tissue sample (both tumour and normal tissue) will be taken at the time of definitive surgery for research purposes. In the event that taking fresh tissue at surgery is not possible for logistical collection reasons, the patients in this optional study may also be consented for a third fresh tissue biopsy after completion of treatment and before definitive surgery.

For procedures and guidelines regarding this sub-study, please contact the Cambridge ARTemis Trial Office for further information on site-specific logistics, specimen handling, storage and postage.

12.3 Sequential blood samples sub-study

Investigators inviting patients to take part in the sequential blood samples sub-study will need to obtain consent (ARTemis Patient Information Sheet / Informed Consent Form 4) from patients for 1 extra blood sample (20ml) to be taken for research purposes at each of the following time points: prior to treatment cycle 1, before cycle 2, before cycle 3, before cycle 5, and after cycle 6 (pre-surgery). Ideally the blood samples should be taken before 10am due to diurnal variations in the molecules under observation, and before any food is eaten that day.

Sites will require the availability of a centrifuge, and a -80°C freezer for sample storage.

For further information, or to request a Sequential Blood Sample Lab Manual or supplies, please contact the Biomarkers and Companion Diagnostics Group in Edinburgh.

12.4 SCARF sub-study: *(Samples Collection At Relapse and Follow-up)*

Investigators inviting patients to take part in the SCARF sub-study will need to obtain consent from patients using ARTemis Patient Information Sheet / Informed Consent Forms 6a and/or 6b.

PIS/ICF 6a is to be used for patients who have not relapsed and are on follow-up: sequential blood samples (plasma, serum, and buffy coat) should be collected at each follow-up visit up to and including year 5.

PIS/ICF 6b is to be used for patients who have already relapsed, or who do relapse during follow-up. Any or all of the following samples should be obtained: a blood sample (plasma, serum, and buffy coat) collected at the point of relapse; an additional tissue sample (which can be 'fresh' or fixed, or both) collected from the metastatic or relapsed site(s) at the time of relapse.

Please see the SCARF sub-study protocol and accompanying Lab Manual for full details, procedures and guidelines for this sub-study.

13. Concurrent studies

Compatible studies would be those of imaging, supportive treatment and of radiotherapy, or hormonal therapy after completion of primary chemotherapy and surgery.

The eligibility of ***ARTemis*** patients for these studies should be cleared with the ARTemis Trial Office.

14. Statistical considerations

14.1 Sample size determination

The power calculations assume a 70:30 split in the trial sample of ER positive and ER negative tumours respectively. PathCR rates with the standard treatment (D->FEC) are estimated as approximately 10% for ER positive patients and 25% for ER negative patients. On this basis, a trial randomising 400 patients into each of the two treatment groups will allow an absolute difference in the pathCR rates in excess of 10% to be detected at the 5% (2-sided) level of significance with an 85% power. The proposed total sample size for the trial is therefore 800 patients. All calculations take into account the expected nominal degree of non-compliance and loss to follow-up as well as variation in the trial sample ER status proportions and the pathCR rates on the standard treatment arm.

14.2 Analysis

The main analysis comparing the complete pathological response rates and the secondary analysis comparing radiological (ultrasound) response and breast conservation rates will utilise the chi-squared test and then logistic regression to allow for the adjustment of stratification variables. Regression methods will also be used to assess the correlations between marker levels and clinical outcomes. The secondary time to event outcomes will be assessed using Kaplan-Meier survival curves, and treatments will be compared using the Log-rank test. The effect of prognostic factors in addition to treatment will also be assessed using Cox-regression models. All analyses will be carried out on an ITT basis.

14.3 Milestones

ARTemis will randomise 800 patients, from an estimated 80 centres in the UK. The aim is to complete accrual within 3 years if possible by maximising the number of UK centres and the speed with which they are activated.

It is anticipated that the main body of the trial will be launched mid-2009. The following milestones assume projected event rates, which may alter depending on the final patient population recruited.

Second quarter 2009:	Start randomisation
First quarter 2013:	Finish recruitment of 800 patients.
First quarter 2014:	Anticipated planned analysis of primary outcome data when all patients have completed randomised treatment and surgery, for path response determination. Presentation of results will include toxicity and dose-intensity as well as available secondary outcomes.
First quarter 2016:	Anticipated interim analyses of disease-free and overall survival assuming 120 events have occurred or that median follow up is at least 3 years.
First quarter 2018:	Anticipated 5-year analysis of disease-free and overall survival assuming 280 events have occurred or that median follow up is at least 5 years.

15. Pharmacy and Drug Supply

Detailed guidelines on trial medications for site pharmacies will be provided in the Pharmacy Site File.

The IMPs for the purpose of this trial are docetaxel and bevacizumab.

The FEC chemotherapies are not IMPs for the purposes of this trial.

15.1 Bevacizumab

Bevacizumab (Avastin®) is manufactured by F.Hoffman La Roche Ltd. It will be provided free of charge to the investigational centres for patients randomised to receive it, throughout the duration of the trial.

Preparation and Administration

Preparation and administration of bevacizumab should be in accordance with the Summary of Product Characteristics, at a dose of 15mg/kg.

Bevacizumab must be prepared by a healthcare professional using aseptic technique. Withdraw the necessary amount of bevacizumab and dilute to the required administration volume with 0.9% sodium chloride solution for injection. The concentration of the final bevacizumab solution is to be kept within the range of 1.4-16.5 mg/ml. There is no dose capping for bevacizumab. Any unused portion left in a vial is to be discarded, as the product contains no preservatives.

Parenteral drug products must be inspected visually for particulate matter and discoloration prior to administration.

Bevacizumab should be given before any chemotherapy drugs. No routine pre-medications, including anti-emetics are required for bevacizumab.

Bevacizumab is to be administered as a continuous intravenous infusion, NOT as an IV push or bolus.

Bevacizumab is to be administered initially over 90 minute period; if the first infusion is well tolerated the second infusion may be delivered over a 60 minute period. If the 60 minute infusion is well tolerated, all subsequent infusions may be delivered over a 30 minute period. It is important to flush the lines with saline solution after the bevacizumab has been infused.

Storage

Bevacizumab will be provided as single use 400mg and 100mg vials containing a 25mg/ml concentrate for solution for IV infusion.
Vials are to be stored in a temperature-monitored refrigerator at 2 - 8°C and are to be kept in the outer carton due to light sensitivity.

DO NOT FREEZE. DO NOT SHAKE.

Bevacizumab does not contain any antimicrobial preservative: therefore, care must be taken to ensure the sterility of the prepared solution.

Chemical and physical in-use stability has been demonstrated for 48 hours at 2°C to 30°C in sodium chloride 9 mg/ml (0.9%) solution for injection. From a microbiological point of view, the product should be used immediately.

Calculated dose and rounding
The bevacizumab dose will be calculated for each patient based on actual weight at the baseline visit. Patients will then receive the same dose at each treatment, unless the patients' body weight changes by more than 10% from baseline, in which case the dose should be recalculated. There are no dose caps for bevacizumab. Rounding of the dose is optional and if the investigator decides to round the dose it must only be rounded to the nearest ml.

Incompatibilities
No incompatibilities between bevacizumab and polyvinyl chloride or polyolefin bags have been observed. Concentration-dependent changes in the ion exchange chromatography profile were observed when bevacizumab was diluted with dextrose solutions (5%). Therefore, bevacizumab should not be administered or mixed with dextrose or glucose solutions.

Accountability
All investigational sites will maintain drug accountability documentation which includes information on bevacizumab dispensing, destruction, drug supply, inventory: all logs will be provided in the Pharmacy Site File.

15.2 Chemotherapy

Supply, storage, preparation and administration of the chemotherapy (docetaxel, fluorouracil, epirubicin and cyclophosphamide) must be as per local policies and procedures, along with reference to the current applicable SmPC for the products used.
Chemotherapy should be prescribed and dispensed from the hospital pharmacy as per routine clinical supply throughout the duration of the trial. None of the chemotherapy treatment is subsidised for the purpose of ***ARTemis.*** These drugs should be ordered and purchased directly from the usual supplier.

Accountability
Accountability must be maintained as per pharmacy guidelines.

16. Sponsorship and indemnity

Cambridge Universiy Hospitals NHS Foundation Trust and the University of Cambridge are joint Sponsors of ARTemis.

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical trial caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the trial, but where no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent harm caused as a result of protocol design and for non-negligent harm arising thorough participation in the clinical trial.

17. Ethical and regulatory issues

17.1 Ethical considerations

The trial will be conducted in full conformance with the principals of the 1996 Declaration of Helsinki and subsequent amendments, and in accordance with UK legislation. The trial will also adhere to the principles of ICH Good Clinical Practice (GCP) taken from the European Community Guideline CPMP/ICH/135/95.

Copies of the World Medical Association Declaration of Helsinki and ICH GCP Guidelines can be obtained from Warwick Clinical Trials Unit or from the World Medical Association

(http://www.wma.net/e/ethicsunit/helsinki.htm) or European Agency for the Evaluation of Medicinal Products website: (http://www.emea.eu.int/pdfs/human/ich/013595en.pdf) respectively.

The protocol will be submitted for National Research Ethics Service's approval prior to circulation in accordance with the new guidance in force from March 1st 2004. Before enrolling patients into the trial, each trial site must ensure that the local conduct of the trial has the approval of the relevant Trust Research & Development (R&D) department. Sites will not be permitted to enrol patients onto the trial until written confirmation of R&D approval is received by the Clinical Trials Unit, Warwick, and fully signed original Participating Site Agreements (including Pharmacy Appendix 1) are received by Cambridge Trial Office.

17.2 Informed consent

The local Investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the trial using the current approved patient information sheet, answering any questions the patient may have throughout the trial, and sharing any new information that may be relevant to the patient's willingness to continue their participation in the trial in a timely manner.

It is the responsibility of the local investigator to obtain written informed consent from each patient prior to performance of any protocol procedures and prior to the administration of trial drug in order to document that the patient is satisfied with their understanding of the risks and benefits of participating in the trial and desires to participate.

17.3 Patient confidentiality

The personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the 2018 Data Protection Act, which includes General Data Protection Regulations (GDPR). Patients will be identified using only their unique trial number, initials and date of birth oncase report forms (with the exception of eligibility and randomisation). Any correspondence between the ***ARTemis*** Trial Offices and the participating sites will only include initials and trial number.

The investigator must maintain documents not for submission to the trials unit (e.g. patients' written consent forms) in strict confidence. In the case of special problems and/or governmental queries, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The Clinical Trials Unit, Warwick will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party, other than those directly involved in the treatment of the patient's breast cancer.

17.4 End of trial and Archiving

The end of the intervention period of the trial will be 1 year after the last patient completes trial treatment (expected to be approximately second quarter 2013). The non-interventional observation period of the trial will continue for at least 10 years following the completion of surgery of the last patient to enter the trial (per the follow-up protocol mentioned above); projected to be 2023.

The archiving period will begin immediately after this date or following the processing of all the biological material collected for research, whichever is the later. All essential trial documents (including patient notes) must be retained for at least 5 years following the end of the observational period of the trial. The trial Sponsor will notify the centres when documents may be destroyed.

18. Financial matters

ARTemis is an investigator-designed and -led trial, which is funded through a project grant from Cancer Research UK, and educational grants from Roche and Sanofi-Aventis. The trial has been independently peer reviewed and endorsed by CTAAC, and is part of the NCRN portfolio.

There are no funds available for investigator payments or pharmacy fees in this trial. However, free bevacizumab (Avastin®) will be made available throughout the trial, for all patients randomised to receive it.

Pathology departments will receive a per-patient payment for supplying the requested pathology material.

The clinical trial data, including quality of life information and pathological material collected as part of the biological studies, will remain the property of the Trial Management Group.

19. Publication policy

The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the collaborators. The trials unit and all participating centres and investigators will be acknowledged in this publication. All presentations and publications relating to the trial, including scientific publications which used specimens collected for the trial must be authorised by the Trial Management Group.

Appendix 1: Timetable of events and investigations for ARTemis

Schedule of investigations during on-study phase

Event	Prior to randomisation	Prior to start of treatment	Day 1 Prior each cycle	Day 1 After each cycle	End of third cycle of chemo[a]	End of fourth cycle of chemo	End of sixth cycle chemo/ Rx [b]/surgery
Informed consent for trial	X						
Pregnancy test (if applicable)	X						
Mandatory diagnosis tumour block and slides		X					
Full blood count, Biochemical screen	X		X				
ER and HER2 status	X						
Urinalysis[d] and Coagulation test[d]	X[d]		X[d]				
Pulse rate and blood pressure	X		X	X			
Medical history, ECOG status and temperature	X		X				
Liver ultrasound (or abdominal CT scan), and Chest X-ray (or Chest CT), and Whole body scintigraphy	X [e]						
LVEF measurement	X [e]					X	
Quality of Life Questionnaire[g]		X[g]			X[g]		X[i]
Weight		X	X				
Height		X					
Radiological measurement of tumour using ultrasound (2D or 3D)	X				X		X
Toxicity review of previous cycle			X				
Surgery							X[f]
Mandatory surgery blocks with set of slides							X
Fresh tissue [c]		X[c]			X[c]		X[c]
Sequential Blood samples[h]	Pre-treatment, pre-cycle 2, pre-cycle 3, pre-cycle 5, and pre-surgery						
Mandatory Blood sample	Can be taken anytime on-trial						

a Cycles 1-3 = Docetaxel. Investigations to be carried out whether or not all 3 cycles of chemotherapy given.

b Cycles 4-6 = FEC. Investigations to be carried out whether or not all 3 cycles of chemotherapy given.

c Only patients enrolled on *CTCR-BR05* ***(fresh tissue)*** sub-study.

d Coagulation test and Urinalysis are mandatory only prior to cycles of bevacizumab

e Only after consent for main trial obtained, if not part of local routine clinical practice ([1] where applicable, see section 6.3).

f Within 3 weeks of last day of final cycle of neo-adjuvant chemotherapy.

g Only patients enrolled in the QoL questionnaire sub-study

h Only patients enrolled in the Sequential Blood sub-study. Please refer to the current Sequential Bloods Lab Manual for collection timepoints

Schedule for follow-up visits

FU visit #	Time from surgery	Approx. time from randomisation*	Annual Follow-up CRF to be completed
1	6 months	11 months	**x**
2	12 months (1 year)	17 months	✔
3	18 months	23 months	**x**
4	24 months (2 years)	29 months	✔
5	36 months (3 years)	41 months	✔
6	48 months (4 years)	53 months	✔
7	60 months (5 years)	65 months	✔
8	72 months (6 years)	77 months	✔
9	84 months (7 years)	89 months	✔
10	96 months (8 years)	101 months	✔
11	108 months (9 years)	113 months	✔
12	120 months (10 years)	125 months	✔

***Based on 18 weeks of neo-adjuvant chemotherapy and allowance of up to 3 weeks for surgery following completion of chemotherapy.

Appendix 2: ECOG performance status

Grade	Description
0	Asymptomatic: normal activity
1	Symptomatic: fully ambulatory
2	Symptomatic: in bed < 50% of time
3	Symptomatic: in bed > 50% of time - not bedridden
4	100% bedridden
5	Death

Appendix 3 : TNM Staging System for Breast Cancer

The original and primary source for this information is the AJCC Cancer Staging Manual, Sixth Edition (2002) published by Springer-Verlag New York. (For more information, visit www.cancerstaging.net.) Any citation or quotation of this material must be credited to the AJCC as its primary source. The inclusion of this information herein does not authorize any reuse or further distribution without the expressed, written permission of Springer-Verlag New York, Inc., on behalf of the AJCC.

Definitions of TNM

Primary Tumour (T)

TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma *in situ*: intraductal carcinoma, lobular carcinoma *in situ*, or Paget's disease of the nipple with no tumour
T1 Tumour ≤2 cm in greatest dimension
- T1mic Micro-invasion ≤0.1cm in greatest dimension
- T1a Tumour >0.1 but ≤0.5 cm or less in greatest dimension
- T1b Tumour >0.5 cm but ≤1 cm in greatest dimension
- T1c Tumour >1 cm but ≤2 cm in greatest dimension

T2 Tumour >2 cm but ≤5 cm in greatest dimension

T3 Tumour >5 cm in greatest dimension
T4 Tumour of any size with direct extension to (a) chest wall or (b) skin, only as described below.
- T4a Extension to chest wall, not including pectoralis muscle
- T4b Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
- T4c Both (T4a and T4b)
- T4d Inflammatory carcinoma

Note: Paget's disease associated with a tumour is classified according to the size of the tumour.

Regional Lymph Nodes (N)

NX Regional lymph nodes cannot be assessed (for example, previously removed)
N0 No regional lymph node metastasis
N1 Metastasis to movable ipsilateral axillary lymph node(s)
N2 Metastasis to ipsilateral axillary lymph node(s) fixed or matted, or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident* axillary lymph node metastasis
- N2a Metastasis in ipsilateral axillary lymph nodes fixed (or matted) to one another or to other structures
- N2b Metastasis only in clinically apparent* ipsilateral internal mammary nodes *and* in the absence of clinically evident axillary lymph node metastasis

N3 Metastasis to ipsilateral infraclavicular lymph node(s) with or without axillary lymph node involvement, or in clinically apparent* ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary or internal mammary lymph node involvement; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
- N3a Metastasis in ipsilateral infraclavicular lymph node(s)
- N3b Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
- N3c Metastasis in ipsilateral supraclavicular lymph node(s)

**** Clinically apparent is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination or grossly visible pathologically.***

Distant Metastasis (M)

MX Presence of distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Stage grouping and eligibility for *ARTemis*:

STAGE GROUPING	T	N	M
Stage 0	*Tis*	*N0*	*M0*
Stage I	*T1*	*N0*	*M0*
Stage IIA	*T0*	N1*	*M0*
	T1	N1**	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2*	M0
	T1	N2**	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

White italics indicate ineligible for ARTemis trial.

**** /** TO and T1 tumours are eligible in presence of axillary node >20mm***

****TO N1,N2 tumours must be histopathologically confirmed by LN biopsy (trucut or whole LN)***

Appendix 4: Formulae, Calculations, and Abbreviations:

Cockroft-Gault formula for calculating Creatinine Clearance:

The estimated GFR is given by:

$$\text{Males: } \frac{1.25 \times (140 - \text{age}) \times \text{weight (kg)}}{\text{serum creatinine (umol/l)}}$$

$$\text{Females: } \frac{1.05 \times (140 - \text{age}) \times \text{weight (kg)}}{\text{serum creatinine (umol/l)}}$$

Dubois & Dubois formula for calculation Body Surface Area:

$$\text{Body Surface Area (m)} = 0.007184 \times (\text{patient height (cm)})^{0.725} \times (\text{patient weight (kg)})^{0.425}$$

Appendix 5: Guidelines for the management of Bevacizumab-induced hypertension

SBP>140 or DBP>90

↓

Calcium channel antagonists (Amlodipine 2.5mg or Felodipine 2.5mg)**

↓

If not controlled, add:
Increase dose to 5mg or 10mg **

OR

Add Angiotensin Converting Enzyme inhibitors (ACE inhibitors)**
Ramipril 2.5mg or Enalapril 2.5mg

↓

Cardiology opinion should be considered
If not controlled,
Increase dose to 5mg or 10mg**

OR

ADD
Beta –blockers (Atenolol 25mg or Metoprolol 25mg)**
Angiotensin receptor blockers (Losartan 25mg or Candesartan 4mg)

**** Monitor the renal function and Urine dipstick to monitor for renal cause of hypertension.**

(Consider adding a new anti-hypertensive agent as an alternative to increasing the dose early in management.)

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