EPHOS-B

Effect of Perioperative AntiHER-2 therapy on Early Breast Cancer Study – Biological phase

Chief Investigator: Professor Nigel Bundred

Co-Sponsors: University of Manchester/ Manchester University NHS Foundation Trust
The Institute of Cancer Research

Approval: Clinical Trials Advisory and Awards Committee (CTAAC)

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Coordinating Clinical Trials Unit: The Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU)

Endorsements: Association of Breast Surgery

PROTOCOL

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This trial is part of the National Institute for Health Research (NIHR) portfolio as a high priority trial.

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2. Biological Coordination

The future use of biological samples will be coordinated by the EPHOS-B Translational Research Sub-committee (part of the Trial Management group)

3. Clinical Trials Unit

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The Trial Management Group (TMG) will comprise members of the Protocol Development Group and Principal Investigators from a subset of participating centres. A copy of the current membership of the TMG can be obtained from the EPHOS-B Trial Manager within ICR-CTSU.

Protocol Authorised by:

Name & Role	Signature	Date
Professor Nigel Bundred (Chief Investigator)		4th September 2019

This protocol describes the EPHOS-B trial and provides information about procedures for entering patients. The protocol should not be used as a guide for the treatment of other patients; every care was taken in its preparation, but corrections or amendments may be necessary. These will be circulated to investigators in the trial, but centres entering patients for the first time are advised to contact ICR-CTSU to confirm they have the most recent version. Protocol amendments will be circulated to participating centres as they occur.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031). It will be conducted in compliance with the protocol, the Data Protection Act (Z6364106) and other regulatory requirements as appropriate.

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1. Trial Summary

Title:	**Effect of Perioperative AntiHER-2 Therapy on Early Breast Cancer Study – Biological Phase (EPHOS-B).**
Objectives	• Determine whether pre-operative treatment of HER-2 positive breast cancer patients with anti-HER2 therapy inhibits proliferation or increases apoptosis. • To compare the effects of trastuzumab, lapatinib and the combination of lapatinib and trastuzumab on the inhibition of proliferation or increase of apoptosis. • To determine whether perioperative anti-HER2 treatment reduces serum angiogenic factors.
Trial Design	The EPHOS-B trial is a multicentre, three group, randomised controlled trial conducted in two parts. Part 1 (protocol versions 1 to 4): HER2+ patients were allocated in a 1:2:2 ratio to control, perioperative trastuzumab only or perioperative lapatinib only. This part of the trial will be superseded by Part 2 following the approval of amendment 5 by West Midlands - Edgbaston NRES Committee on 15/05/2013 and subsequently in each centre. Part 2 (Implemented from protocol version 5): HER2+ patients are allocated in a 1:1:2 ratio to control, perioperative trastuzumab only or the combination of lapatinib and trastuzumab. This part is now active. Perioperative treatment consists of 11 days (up to +2 days or -1 day) pre-operative treatment and further post-operative treatment as specified below.
Treatment	**Group 1**: Control (i.e. no perioperative treatment) The control group will receive standard care. All local and systemic therapy for early breast cancer should be according to standard care protocols. **Group 2**: Trastuzumab 6mg/kg iv given on days 1 & 8 pre-surgery & one dose of 2mg/kg iv between days 15-19 post surgery. **Group 3**: Lapatinib 1000mg/day p.o. continuously for 28 days, in combination with trastuzumab 6mg/kg iv given on days 1 & 8 pre-surgery & one dose of 2mg/kg iv between days 15-19 post surgery. Both drugs should start 11 days (+2 or -1 day) before the scheduled surgery. <u>Standard care</u> All other local and systemic therapy for early breast cancer should be according to standard care protocols.

Target Patient Population	Newly diagnosed HER2 positive primary breast cancer patients suitable for surgery.
Number of Patients	A total of 250 patients will be randomised in EPHOS-B.
Main Eligibility Criteria	Women with operable breast cancer; with planned surgery, diagnosis by core biopsy, HER2 (3+); no prior systemic therapy; no evidence of metastatic disease; normal cardiac function (as assessed by ECG, and ECHO or MUGA); willing to undergo perioperative therapy with trastuzumab or combination of trastuzumab and lapatinib in addition to planned adjuvant therapy (including chemotherapy and further trastuzumab therapy); negative pregnancy test; written informed consent to participate in the trial and to the donation of tissue samples from the diagnostic biopsy and surgery and to the donation of blood samples for research.
Research Tissue Samples	Research tissue samples will be collected at the time of diagnostic core biopsy and at surgery. Samples should be embedded in paraffin blocks and separate samples in RNA-later® if sufficient material is available.
Research Blood Samples	Research blood samples will be taken at baseline, prior to surgery and at 28-30 days post surgery.
Biological Endpoints	Primary Change in apoptosis (activated caspase 3) and/or change in proliferation (Ki67). Secondary: • Relapse free survival • Time to local recurrence • Time to distant recurrence • Overall survival • Changes in the angiogenic serum markers VEGF-A, VEGF R1 and CD105, measured at baseline, surgery (plus also tumour CD31) and 28-30 days post surgery. • Changes in expression of molecular markers (EGFR, HER-3, IGF1R, c-myc, AKT, p-ERK, pS6 Kinase, Activated src, or truncated p95HER-2 expression) and whether pre-treatment values predict increases in apoptosis or decreases in proliferation in response to therapy.

2. Introduction

Use of anti-HER2 therapy perioperatively may improve the outcome of women with HER2 positive early breast cancer, more than a third of whom will be expected to have occult micrometastatic disease at the time of surgery. There is evidence that removal or irradiation of primary tumours, leads to an increase in the labelling index and size of concomitant metastases[1, 2], and anti-HER2 therapy may counter such effects. Patients often have a 6 week interval between diagnosis and starting chemotherapy; these patients may benefit from active anti-HER2 therapy during this time by helping to prevent eventual relapse of their tumour. Preoperative trials with SERMs have demonstrated that in oestrogen receptor (ER) positive cancers proliferation can be inhibited and apoptosis increased on therapy[3]. Suppression of proliferation in response to anti-HER2 therapy with lapatinib has also been observed in HER2 positive early breast cancers. However, no randomised controlled large comparative study has been performed with biological endpoints. Biological data are required to validate the underlying hypotheses before undertaking the clinical phase of a perioperative trial examining the effect on the prevention or delay of relapse.

The aim is to compare both agents and the combination of lapatinib and trastuzumab (with untreated controls) to confirm a short term increase in apoptosis or fall in proliferation on treatment and to use these changes to establish grounds for biological efficacy.

In the United Kingdom there are 40,000 breast cancers diagnosed each year, of which 20,000 are symptomatic cancers and around 15% of patients have HER2 positive tumours. Potentially therefore, there are around 6,000 cases (20,000 * 15% = 6,000) per year who might benefit from perioperative anti-HER2 treatment. Core biopsy can identify most of the HER2 positive primary breast cancers as HER-2 3+ by immunohistochemistry who would be eligible for such treatment In centres using FISH as an initial screen, FISH positive cancers would also be suitable for such treatment.

3. Background

HER2 (C-ErbB2) is a member of the Tyrosine Kinase Growth Factor Receptor family which includes HER-1 (Epidermal Growth Factor Receptor (EGFR)), HER-3 and HER-4[4, 5]. The four members of this family share a similar conserved structure - an extracellular ligand-binding domain, a single transmembrane-spanning domain, and an intracellular tyrosine kinase domain.

Upon binding of ligands, HER receptors become activated and undergo hetero- or homodimerization. No ligand has been identified for HER2 but it participates in dimerisation. Ligand-mediated dimerization of the HER receptors and subsequent autophosphorylation or transphosphorylation leads to their association with a variety of cytoplasmic phosphotyrosine binding proteins. This results in the initiation of a phosphorylation cascade and activation of several downstream pathways involved in cell growth [mitogen-activated protein kinase (MAPK); also known as Erk1/2] and survival [phosphatidylinositol-3-kinase (PI3K/Akt)][4, 5]. Stimulation of these pathways transmits a signal to the nucleus resulting in modification of gene transcription patterns that ultimately affects cellular processes, such as proliferation, apoptosis, adhesion, migration, and/or differentiation. No ligand has yet been identified for HER2 suggesting its primary role is to modulate signals after ligand binding to other HER-family receptors[4, 5].

Breast cancers which over express HER2 by gene amplification have a higher incidence of node positivity at diagnosis and a higher risk of recurrence and metastasis within the first five

years after diagnosis leading to poorer overall survival[6-10]. Menard *et al* have described a peak of early local and distant recurrences in the first four years after breast cancer surgery[9]. Data from node negative patients indicates that even for those patients who are T1 and below there is an increased risk of relapse in the first five years if a tumour is HER2 positive (by either IHC or FISH)[9, 11]. In Finnish women with T1 N0 moderately or poorly differentiated breast cancers less than 10mm, 9 year distant disease free survival dropped from 92% for those who were HER2 negative to 67% in those who were HER2 amplified or expressed[12]. This suggests that 33% of these good prognosis patients had occult metastatic disease at diagnosis compared with 8% of HER2 negative patients, a fourfold increase.

Rationale for Perioperative Approach

Removal or irradiation of primary tumours has been shown to lead to the labelling index and size of concomitant metastases increasing[1, 13]. Access of tumour cells to the circulation is a requirement for metastasis to occur, circulating tumour cells were detected in 1 of 18 patients preoperatively and 6 patients during surgery, but none after surgery[14]. Induction of tumour cell shedding into effluent venous blood vessels may occur during breast cancer surgery[14]. Preoperative surgical trials of short term treatment with SERMs have demonstrated that in oestrogen receptor (ER) positive cancers proliferation can be inhibited and apoptosis changed on therapy[3]. Perioperative chemotherapy significantly reduced relapse rate in breast cancer patients in the absence of other adjuvant therapy[15], indicating that treatment during the perioperative period can be beneficial.

HER2 positive tumours have an increased local and distant relapse rate relative to HER2 negative tumours which may be associated with surgery[8]. Incompletely excised breast cancers that required re-excision within 48 days of surgery showed a significant increase in proliferation if they were HER2 positive, but not if they were HER2 negative[11]. The increased growth could be blocked by trastuzumab in vitro and was related to increased EGF like and VEGF like growth factors present in post surgical serum samples[11]. Perioperative treatment of patients shown to be HER2 positive on core biopsy for 4-6 weeks (i.e. the two weeks prior to surgery and the post operative period) may prevent tumour cell implantation at both local and distant sites as well as the growth of distant metastases, improving relapse free survival and long term prognosis[16]. There is evidence that durations of therapy with trastuzumab of much less than one year can still deliver significant improvements in disease recurrence. The FinHer trial[17] included 232 HER2 positive early breast cancer patients randomly assigned to trastuzumab or not, trastuzumab being given during the first three cycles of chemotherapy. A hazard ratio favouring trastuzumab of 0.42 (95%CI: 0.21 to 0.83; P = 0.01) was observed for recurrence or death in this study. More recently, data provided from the PHARE trial comparing 6 months with 12 months' trastuzumab has not concluded that the 6 months is clearly less effective[18].

Thus there is a biological rationale for the hypothesis that perioperative anti-HER-2 monotherapy could prevent local and distant recurrence but randomised level 1 evidence that anti-HER2 therapy either increases apoptosis or decreases proliferation is currently unavailable. Furthermore given that there is evidence that significant improvements in recurrence rates can be achieved with 3-6 months of post-operative trastuzumab, we believe that it is a reasonable hypothesis to suggest that a short exposure to anti-HER2 therapy in the critical peri-operative period could improve long-term disease outcomes. This study is also designed to produce the necessary corroborative evidence of biological changes in

tumours when treated with anti-HER2 therapy in the peri-operative period, to support the design of a phase III trial to test that hypothesis.

Trastuzumab

The monoclonal antibody trastuzumab has been shown to prolong survival in HER-2 positive breast cancer which has metastasised and data from four major trials demonstrates a significant survival advantage for the use of trastuzumab as adjuvant therapy after breast cancer treatment[6, 8]. In a meta-analysis of the NSABP B31 and NCCT9831 trials, the four year DFS rate increased from 73%, for HER-2 positive patients given AC followed by taxane chemotherapy alone, to 86% for those given chemotherapy and trastuzumab[8, 10]. The global (non-US) HERA trial compared any chemotherapy, with or without radiotherapy, followed by a three weekly regime of trastuzumab for 12 or 24 months against an observation arm. A significant 50% reduction in distant disease free relapse was seen with trastuzumab which was highly significant and appeared to be consistent in all subgroups analysed[6]. The FinHER trial gave 9 weeks of trastuzumab immediately after primary surgery (concurrently with docetaxel or vinorelbine) achieving a reduction in relapse free survival of an equivalent amount to 52 weeks of trastuzumab therapy after chemotherapy[17]. Around 30-40% of HER2 positive tumours are ER negative and Colleoni et al[19] have shown delays in initiating chemotherapy of greater than three weeks in ER negative cancers can lead to a poorer survival. Primary chemotherapy combined with Herceptin has shown up to 50% complete pathological response rates, compared to the usual 20% complete response rates when trastuzumab is not combined with chemotherapy, indicating earlier treatment with trastuzumab may be more effective[20]. Using anti-HER2 therapy perioperatively may further improve the outcome for women with HER2 positive early breast cancer.

In HER2 positive metastatic breast cancers, trastuzumab given as monotherapy had a 48% clinical benefit rate[12] and on its own may prove to be a viable strategy, although further study is required before advocating this approach. In 35 patients with large, primary, HER-2 positive breast cancer, neoadjuvant trastuzumab monotherapy was associated with a 35% increase in apoptotic index within one week and a 23% partial response rate was seen by three weeks[21]. Small molecule HER2 inhibitors (ie lapatinib) induce increased tumour apoptosis in metastatic disease, but no data currently exists from randomised controlled trials confirming this effect objectively. Increases in apoptosis on lapatinib were associated with subsequent tumour responses and lapatinib was shown to induce tumour responses in both trastuzumab treated and naïve patients in the absence of chemotherapy[22].

Most HER2 positive breast cancer patients with metastatic disease undergoing trastuzumab therapy experience progression, the median time to progression is 12 months, hence there is a need for more effective anti-HER2 agents [12, 23] .

Safety of Trastuzumab

The incidence of grade 3 or 4 adverse events was greater in the trastuzumab arm of the HERA trial[6]. However, infection (1.3% in the trastuzumab group vs. 0.4% in the observation group) and vascular disorder (1.2% vs. 0.5% respectively) were the only grade 3 or 4 adverse events with an incidence greater than 1% in either group. One cardiac death occurred in the observation group and nine patients (0.54%) in the trastuzumab group had severe congestive heart failure. Symptomatic congestive heart failure, including these cases, was observed in 1.7% of patients in the trastuzumab group and 0.06% of patients in the

observation group. The rates of a decrease in LVEF, noted on at least one assessment, were 7.1% in the trastuzumab group and 2.2% of those in the observation group.

Lapatinib

Lapatinib is an orally active, reversible small-molecule dual kinase inhibitor targeting both HER1 and HER2 receptors. It works inside the cell and directly targets the tyrosine kinase (TK) domain[22]. Lapatinib reversibly binds to the cytoplasmic adenosine triphosphate- (ATP) binding site of the kinase and blocks receptor phosphorylation and activation, thereby preventing subsequent downstream signalling events (simultaneous activation of Erk1/2 and P13K/Akt)[22]. There are several theoretical advantages of dual HER2 inhibition, i.e. an inhibitor of both HER1 and HER2 compared with inhibitors of either one of these TKs alone. While small molecule inhibitors of HER1 can block signalling through HER1 homodimers, these agents may not be as effective at inhibiting heterodimers containing both HER1 and HER2. In addition to HER:HER heterodimers, other growth factor receptors have been shown to form heterodimers with HER receptors, notably, insulin-like growth factor receptor 1 (IGFR-1)[19]), which forms heterodimers with both HER1 and HER2. In this regard, co-expression of IGFR-1 with HER2 overexpression has been shown to mediate resistance to trastuzumab in preclinical models and in patients[19, 24]. However, the presence of IGFR-1 and HER2 overexpression appears to predict for sensitivity of breast cancer cells to the anti-tumour effects of lapatinib[13, 16]. This suggests that in tumour cells expressing multiple HER family members as well as other growth factor receptors (e.g., IGFR-1), incomplete blockade of HER1 and HER2 receptor signalling may still enable growth and/or survival signals to be transmitted.

Efficacy and Safety of Lapatinib

Lapatinib in combination with capecitabine is licensed for the treatment of HER positive advanced or metastatic breast cancer previously treated with an anthracycline, taxane and trastuzumab in the metastatic setting[25]. The efficacy and safety of lapatinib both as a single agent and in combination with other anti-cancer treatments continues to be assessed in several clinical phase I-III studies of metastatic and early breast cancer. In an open-label, randomized, two-arm study (EGF20009) of lapatinib 1500mg once daily (OD) or 500mg twice daily (BD) as first-line treatment, an interim analysis (including independent radiology review) was performed in 40 patients followed for a minimum of 12 weeks. Results included a partial response (PR) in 14 (35%) patients, an unconfirmed PR in 2 (5%) patients, stable disease in 14 (35%) patients, and progressive disease or unknown efficacy in 5 (12.5%) patients each. These data are similar to those of trastuzumab monotherapy reported in a similar patient population (34% response rate in a FISH-positive subgroup)[21]. Gomez et al have shown response rates of 24% and 31% of patients derived clinical benefit (CR, PR, or stable disease for ≥24 weeks) in HER-2 positive breast cancers treated first line with lapatinib[16]. However two recent reports of studies in metastatic breast cancer (Canadian MA31 and CEREBEL)[26, 27] suggest that monotherapy lapatinib in combination with chemotherapy may be inferior to monotherapy trastuzumab (given with the same chemotherapy), whilst neo-adjuvant studies (NEO-ALTTO and CHER-LOB)[28, 29] have reported higher pathological Complete Response (pCR) rates when these two agents were combined as compared to given as monotherapy in combination with chemotherapy.

A phase I study of lapatinib in patients with trastuzumab refractory breast cancer has shown clinical activity. Several potential explanations for this exist and include the presence of a

truncated variant of the receptor p95 HER2 which lacks the extra cellular receptor domain (and thus cannot be blocked by trastuzumab) but maintains a functionally active intracellular active tyrosine kinase domain. In preclinical models, cells expressing p95 HER2 are trastuzumab resistant and lapatinib sensitive[22, 30]. A study of the cardiac safety of lapatinib was undertaken in 3689 patients entered into 44 phase I trials, in which lapatinib was administered as monotherapy or in combination, the median duration of therapy being less than 3 months. A small number of these patients (1.5%) experienced clinical signs of some heart damage but many of these patients had previously had other treatments for their cancers. This suggests the incidence of serious cardiac events is low[31].

The pivotal phase III trial that established the role of lapatinib in the treatment of trastuzumab resistant breast cancer was first reported in 2006. In this trial, lapatinib plus capecitabine was compared with capecitabine alone in women with HER2-positive locally advanced or metastatic breast cancer (MBC) that had progressed after treatment with anthracycline-taxane- and trastuzumab-containing regimens[23, 32]. Following publication of this study, approval has now been obtained for use of lapatinib in combination with capecitabine for the treatment of patients with advanced or MBC whose tumours overexpress HER2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab in the metastatic setting. No data existed on the relative efficacy of peri-surgical/neoadjuvant lapatinib in vivo at the time of initiating EPHOS-B. Recent evidence (DeCensi, MAPLE, LPS study) however, has shown a biological effect of lapatinib if given 2-3 weeks pre-surgery[33-35]. Patients in the Neo-ALTTO trial showed evidence of tumour shrinkage with 6 weeks anti-HER2 therapy which would support a worthwhile biological effect being detectable in EPHOS-B [28]. Specifically, these trials have demonstrated a reduction in proliferation and an increase in apoptosis. The data from the small number of patients in each of these trials will be confirmed by the EPHOS-B trial from the lapatinib-only arm ('EPHOS-B Part 1'), which will close to recruitment following the implementation of amendment 5. The introduction of a combination arm (lapatinib and trastuzumab) post amendment 5 ('EPHOS-B Part 2'), is likely to have a superior effect on proliferation and be more therapeutically-active.

Efficacy and Safety of Lapatinib-Trastuzumab Combination

Since the initial development of EPHOS-B in 2007 more evidence in relation to safety and efficacy of anti-HER2 therapies are now available, and in particular, a growing body of evidence that combinations of two anti-HER2 therapies are more effective than monotherapies. Therefore this study has been amended to include a regimen most relevant therapeutically. Specifically:

i) Four major trials support the therapeutic superiority of combinations of anti-HER2 therapy (NeoALTTO, NeoSphere, CLEOPATRA and EGF104900[28, 36-38]) in the metastatic and neoadjuvant setting and the safety profile for patients receiving combination therapy seems acceptable [38].

ii) Evidence supports ability of short term lapatinib to induce relevant and consistent biomarker changes[33, 34, 39].

iii) Evidence supports the cardiac safety of short term use of combination trastuzumab-lapatinib [28, 38].

Amendment 5 thus proposes adding trastuzumab to lapatinib thus replacing the lapatinib monotherapy group with a combination lapatinib-trastuzumab treatment group. This will constitute part 2 of the EPHOS-B trial.

EPHOS-B will remain a 3-group trial and the total sample size will remain the same (250 patients). However the allocation ratio will be altered for patients entered after the implementation of this amendment. The new allocation ratio will allow more patients to be recruited in the combination arm in order to obtain more precise estimates of the size of the effect.

EGF104900 (metastatic breast cancer trial) [36] was used as a comparator to the EPHOS-B proposal, as patients received prolonged combination anti-HER2 therapy in the absence of chemotherapy. Adverse event (AE) rates were similar in both the lapatinib-trastuzumab and monotherapy lapatinib groups, and SAEs reported were slightly elevated at 26% (lapatinib-trastuzumab) vs. 16% (monotherapy).
Grade 3 diarrhoea was reported similarly in both groups, although incidence of grade 1 / 2 diarrhoea was increased with lapatinib-trastuzumab (62%) vs. monotherapy (48%). It is proposed that supportive medication guidelines for diarrhoea are included in the protocol. Rash was reported less in the lapatinib-trastuzumab group - potentially as a result of the reduced lapatinib dose.

NeoALTTO (early breast cancer neoadjuvant trial) [28] was used as comparator to EPHOS-B - as patients had no prior exposure to chemotherapy enabling review of cardiac toxicities. No major cardiac dysfunctions were reported and there was no difference in AEs between lapatinib-trastuzumab and lapatinib monotherapy groups. It was noted that there were more hepatic enzyme changes in the lapatinib alone group (compared with lapatinib-trastuzumab) – potentially as a result of the higher lapatinib dose. The NeoALTTO data also supported that from EGF104900 in which diarrhoea grade 3 was similar across the groups - 23% lapatinib-trastuzumab vs. 21% lapatinib monotherapy [36]. Overall toxicity was not increased in the lapatinib-trastuzumab group compared to the lapatinib monotherapy group, because of the reduction of the lapatinib dose used. There were no cardiac events in NeoALTTO and the most common side effects found were of grades 1 / 2 diarrhoea, which are routinely corrected with supportive medications [28].

Molecular Markers of Biological Response to Anti-HER2 Therapy

Several predictors of response to anti-HER2 therapy have been proposed[5, 37].

Patients with metastatic breast cancer whose tumours over expressed epidermal growth factor receptor and phospho Erk were significantly more likely to respond to trastuzumab whilst those who expressed IGF-1 receptor and PS6 kinase were less likely to respond to trastuzumab[38]. Others have reported HER3 (cerbB3)/cerbB2 co-expression alters downstream signalling by increasing pAKT and prevents response to trastuzumab[39]. Scaltriti M et al[30] have reported that tumours that express a truncated form of the HER2 receptor (P95 HER2) do not bind trastuzumab but retain tyrosine kinase activity.

It could be hypothesised that tumours expressing EGFR and which are IGF1R and PS6 kinase negative will be more likely to respond to trastuzumab anti-HER2 therapy and that those tumours that express the P95 HER2 truncated form of the receptor will be more likely

to respond to lapatinib rather than trastuzumab. Trans-EPHOS will evaluate the frequency of truncated P95 HER2 receptor expression in invasive HER2 positive cancers.

In addition, this study will also permit analyses of the possible roles of other dimerisation partners of HER2, such as HER3, in the response to either lapatinib, trastuzumab or their combination. RNA samples will allow us to use microarray to identify new markers of response by identifying genes showing marked change and subsequently determining if they predict survival in the subsequent clinical phase of a perioperative trial. If markers to identify preferential response to either lapatinib, trastuzumab or the combination of lapatinib and trastuzumab could be identified they would have clinical utility in both adjuvant and metastatic settings, detection of breast cancers unlikely to respond to therapy is important in view of the cost of therapy. Pre-surgical biomarker changes may enable identification of HER2 positive breast cancers unlikely to respond to therapy, this is important in view of the cost of therapy. Indeed, if markers to identify preferential response to either lapatinib or trastuzumab could be identified they would have clinical utility in both adjuvant and metastatic settings. Collection of RNA samples will allow us to use microarrays to identify new markers of response by identifying genes showing marked change and subsequently determining if they predict survival in any future clinical study which follows on from the EPHOS-B study.

EPHOS-B

EPHOS-B aims to test the hypothesis that inhibiting HER2 increases apoptosis or lowers proliferation and to determine which anti-HER2 agent to use in a subsequent clinical study to determine whether early treatment with anti-HER blockade prevents local and distant metastasis at 5 years from breast cancer. Assuming changes in either Ki67 or apoptosis are found, we intend to determine whether they relate to clinical benefit (clinical study) and whether we can identify predictors of pre-surgical biological response to anti-HER2 therapy.

4. Trial Rationale

The two part EPHOS-B study will determine whether significant biological changes can be achieved with short (11 day) peri-operative treatment. Originally (Part 1) the perioperative treatment was either lapatinib or trastuzumab, however with the implementation of Protocol version 5 (Part 2), this was amended to be either trastuzumab, or a combination of lapatinib and trastuzumab. Part I of the trial was important to establish the feasibility of the trial based on the rapid availability of HER2 testing on core biopsies and the ability of centres to deliver trastuzumab treatment in the timescales required. It also provided evidence of the safety (including cardiac safety) of using anti-HER2 therapy prior to chemotherapy. As Part 1 has now demonstrated feasibility an application was submitted to CRUK (CTAAC) in July 2012 to request support for an amendment to the study design (Part 2).

If the results from the analyses of EPHOS-B demonstrate statistically significant changes in tumour apoptosis and/or proliferation following 11(-1 or +2) days' anti-HER2 therapy perioperatively without clinically significant increases in cardiac toxicity during subsequent chemotherapy, then approval and funding will be sought to conduct a phase III trial to test the potential improvement in relapse-free survival or improvement in the prediction of treatment benefit that could occur with this perioperative anti-HER2 intervention. EPHOS-B will thus inform the design of any subsequent clinical study and allow the optimum treatments to be selected for the main clinical study which will assess the impact of anti-HER2 therapy on disease outcome. EPHOS-B will be followed for disease-related endpoints such as relapse-free survival and overall survival. If the overall trial is positive for the early introduction

of anti-HER2 therapy, this could be easily implemented in routine practice. Evidence which is obtained from EPHOS-B will also be valuable in defining subgroups which demonstrate a differential effect of anti-HER2 therapy; these subgroups can then be tested prospectively both within the current trial and in other anti-HER2 trials such as ALTTO. Finally, the majority of metastatic breast cancer patients treated with trastuzumab develop resistance within 1 year[12], so there is a need to identify the molecular mechanisms underlying primary (and secondary) resistance, and the translational aspects of this study will increase our understanding of resistance to anti-HER2 therapies. This trial therefore has an important role to play within the international endeavour to treat HER2+ breast tumours effectively.

5. Trial Objectives

- To determine whether pre-operative treatment of HER-2 positive breast cancer patients with anti HER2 therapy inhibits proliferation or increases apoptosis
- To compare the effects of trastuzumab, lapatinib and the combination of lapatinib and trastuzumab on the inhibition of proliferation or increase of apoptosis
- To determine whether perioperative anti-HER2 treatment reduces serum angiogenic factors.
- To identify molecular predictors of biological response to anti-HER2 therapy

6. Trial Hypotheses

The principal hypotheses to be tested in EPHOS–B are as follows:

- perioperative anti-HER2 therapy (trastuzumab, lapatinib or the combination of lapatinib and trastuzumab) causes a significant increase in tumour apoptosis;
- there is a statistically significant difference between 1) lapatinib alone, 2) trastuzumab alone and 3) and the combination of lapatinib and trastuzumab as indicated by the changes they induce in primary tumour apoptosis;
- perioperative anti-HER2 therapy (trastuzumab, lapatinib or the combination of lapatinib and trastuzumab) causes a significant decrease in tumour cell proliferation;
- there is a significant difference in inhibition of proliferation between 1) lapatinib alone, 2) trastuzumab alone and 3) the combination of lapatinib and trastuzumab.;
- circulating biomarkers of cell death and/or angiogenesis correlate with changes in tumour Ki67, apoptosis or angiogenesis;
- it will be possible to determine in tumour and/or blood (if changes in tumour proliferation or apoptosis are seen with either therapy) markers predictive for this response/resistance.

7. Trial Design:

Part 1

Part 1 (Protocol versions 1 to 4): This part of the trial will be superseded by Part 2 following regulatory approval of amendment 5 , and approval by the West Midlands – Edgbaston NRES Committee on 15/05/2013 and subsequently in each centre.

See Protocol Version 4: 1 October 2012 for details of Part 1 of the EPHOS-B trial.

Part 2 (post-amendment 5)

A randomised multi-centre trial in women diagnosed with HER2 positive breast cancer judged to be 3+ by immunohistochemistry or FISH positive on core cut biopsy, due to undergo surgery*. HER2+ patients will be allocated in a 1:1:2 ratio to control, perioperative trastuzumab or the combination of lapatinib and trastuzumab.

Randomisation should be completed within one month of the decision to treat the patient with surgery*.

Perioperative treatment commences 11 days (+2 or -1day) prior to surgery and consists of:

- trastuzumab given on Day 1 and 8 prior to surgery and between Day 15 and Day 19 i.e. after surgery
- combination of lapatinib and trastuzumab: lapatinib will be administered continuously for 28 days (perioperatively), and trastuzumab will be given on Day 1 and 8 prior to surgery and between Day 15 and Day 19

For the two treatment arms, day 1 is the first day of trastuzumab treatment. For control arm patients, day 1 is counted as either 10, 11, 12 or 13 days prior to surgery.

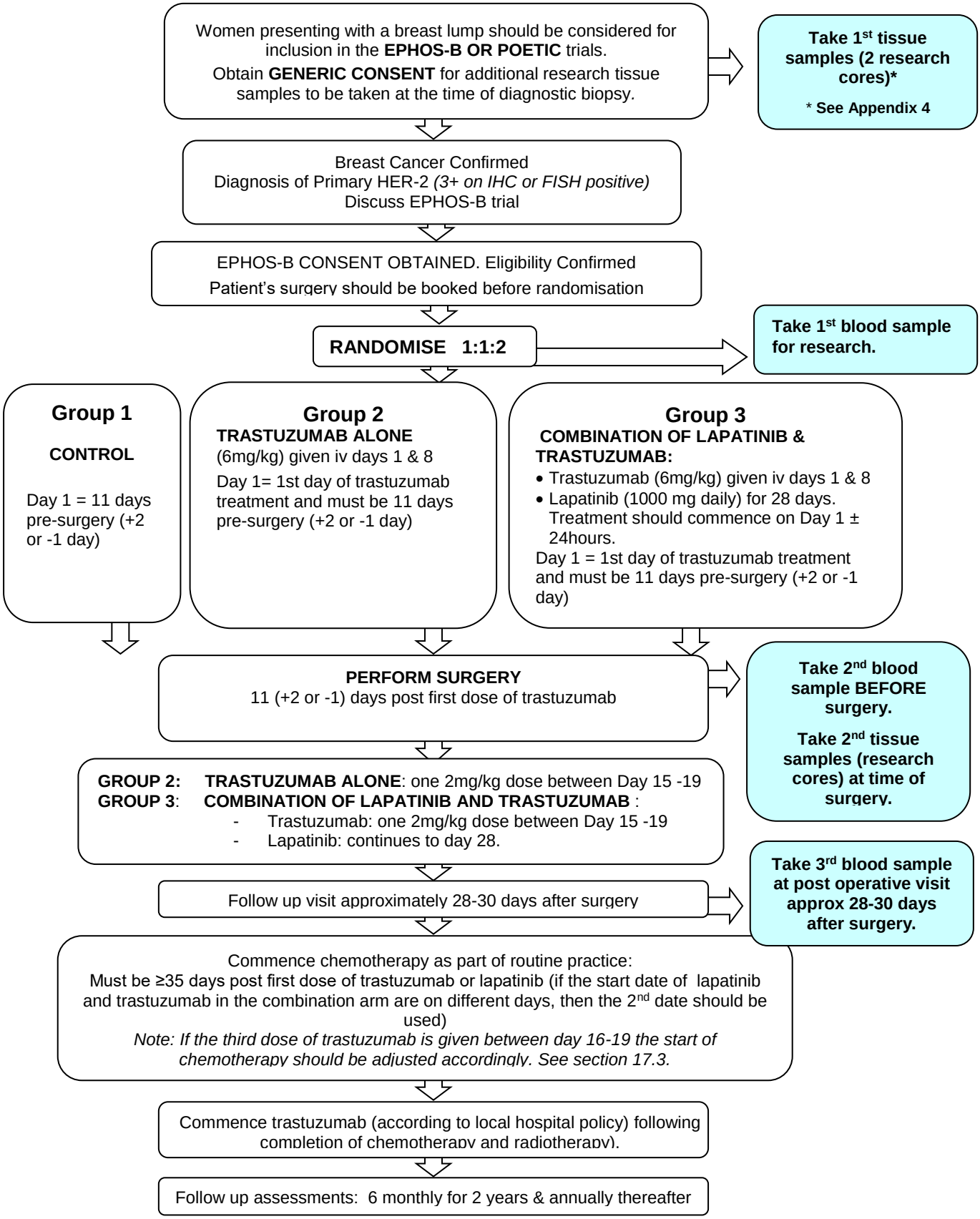
* Cancer waiting time standards mandate that there should be a maximum of one month wait from the date of decision to treat to first treatment for breast cancer. If a patient has agreed to enter a National Institute for Health Research trial such as EPHOS-B then the trial protocol will determine which treatments are classed as first treatments and they will be assigned as such under cancer wait standards.

For the purposes of national cancer waiting times, allocation to anti HER2 therapy or no anti HER2 therapy (following randomisation into EPHOS-B) is classed as first treatment.

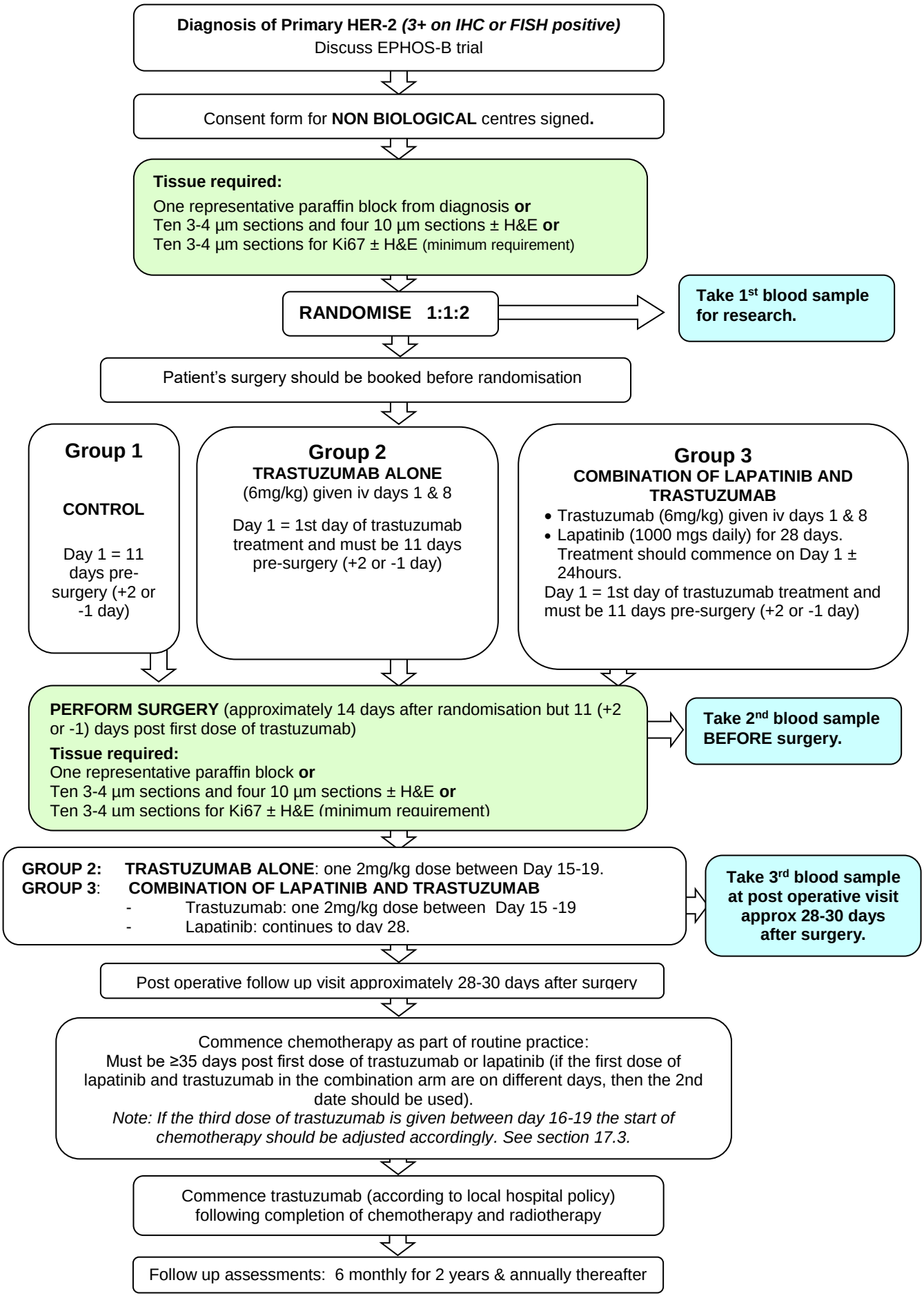
8. Study Schema

For clarification regarding the difference between Biological Pathway A and B, please refer to Appendix 4 – page 56.

8.1 Biological Centres



8.2 Non Biological Centres



9. Endpoints

Primary Endpoints:

1. Increase in apoptosis: Change in the tumour (morphological apoptosis and activated caspase 3) measured at diagnosis and at surgery.
2. Fall in proliferation between diagnosis and surgery: Change in proliferation measured by Ki67 immunohistochemical assessment (%) at diagnosis and at surgery

Secondary Endpoints

1. Relapse free survival
2. Time to local recurrence
3. Time to distant recurrence
4. Overall survival
5. Changes in the angiogenic serum markers VEGF-A, VEGF R1 and CD105, measured at diagnosis, surgery (plus also tumour CD31) and 28-30 days post surgery.
6. Pre-treatment and/or surgical expression of Molecular Markers (EGFR, Her-3, IGF1R, c-myc, AKT, p-ERK, pS6 inase, activated src, or truncated p95HER-2 expression)

10. Patient Selection and Eligibility

10.1 Source of Patients

Women with HER2 positive primary breast cancer will be recruited from breast cancer clinics within participating UK centres.

10.2 Number of Patients

Approximately 250 patients will be required.

10.3 Inclusion Criteria

- Women aged ≥18 years.
- HER2 positive (3+ on IHC or amplification proven by FISH*) operable invasive breast cancer diagnosed by core biopsy.
- Planned surgery.
- Liver function tests (LFTs) should be normal for the institution (gamma GT, ALT and alkaline phosphatase). Serum creatinine and bilirubin <2 times the upper limits of normal for the institution, or creatinine clearance >60mL/min. (Marginally abnormal test results should be repeated).
- ECOG performance status 0, 1, or 2 (Karnofsky ≥ 60%).
- Non pregnant and non-lactating with no intention of pregnancy during study treatment. Women of childbearing potential must agree to use adequate non-hormonal contraception for the duration of the treatment phase of the study (adequate contraceptive measures include intra-uterine device, barrier method e.g. diaphragm and condoms used in conjunction with spermicidal jelly). Women of childbearing potential must have a negative blood serum pregnancy test within 28 days prior to randomisation.
- Patients must be candidates for and willing to undergo adjuvant chemotherapy and trastuzumab post surgery.
- Written informed consent obtained for trial and to donation of tissue and blood samples.

*** FISH cases should be categorised as follows:**

Using a two probe system in which at least 100 cells are counted the ratio of the HER2 signal to the control centromeric probe on chromosome 17 is derived.

- Negative: a HER2/centromeric control probe ratio of less than 1.8
- Borderline negative: between 1.8 and 2
- Borderline positive: between 2 and 2.2
- Positive: 2.2 or greater and grossly amplified >5 where the HER2 signal is clumped and cannot be properly counted but is clearly present in large amounts.

For borderline counts further counting should be performed on more fields but if the ratio remains the same all counts of 2 and above are regarded for treatment purposes as positive and those below as negative.

10.4 Exclusion Criteria

- HER2 negative cancers and those with unknown HER2 status.
- Inoperable breast cancer (T4 category) or suspicion of distant metastases.
- Diagnosis of inflammatory breast cancer.
- Clinical evidence of metastatic disease.
- Prior trastuzumab therapy within the last 12 months or local (radiotherapy) cancer treatments.
- Previous cancer at any other site that has been treated within the last 6 months (except previous basal cell carcinoma and cervical carcinoma *in situ)*
- Have current active hepatic or biliary disease (with exception of patients with Gilbert's syndrome, asymptomatic gallstones or stable chronic liver disease per investigator assessment).
- Impaired gastro-intestinal function thought sufficient to reduce lapatinib absorption.
- Contra-indicated to receive adjuvant chemotherapy and /or trastuzumab (ECOG performance status >2).
- Known immediate or delayed hypersensitivity, reaction to drugs chemically related to trastuzumab or lapatinib.
- Other concomitant investigational agents or concurrent anti-cancer therapy.
- Use of herbal (alternative) therapies within 2* weeks of study entry (see Appendix 1). NB: vitamin and / or mineral supplements are allowed.
- If patients are taking any of the prohibited medication as listed in Appendix 1.
- Regular use of systemic steroids or other agents that could influence study endpoints (inhaled steroids are allowed).
- Any altered mental state that would preclude obtaining written informed consent.
- Clinically significant cardiac abnormalities or uncontrolled hypertension.
- Previous myocardial infarction, heart failure, or significant angina. Cardiac function should be assessed by physical examination, ECG, and baseline LVEF should be ≥55% as measured by echocardiography or MUGA.

***Note:** Herbal or dietary supplements should ideally be stopped from the start of screening but may be discontinued up to the day before randomisation.

Cessation of HRT

- Oestrogen receptor positive patients on HRT must either continue HRT or must not have taken HRT within the last three weeks before the baseline research biopsy is taken. The possible benefits and risks of HRT must be discussed with the patient.
- Oestrogen receptor negative breast cancer patients may enter the trial whether or not they have taken HRT within the last four weeks.

11. Biological Specimen Collection and Storage

11.1 Tissue Collection

Centres may participate in EPHOS-B as either biological or non biological centres.

Biological Centres

Biological centres will meet any regulatory requirements for the storage of tissue for unspecified future research, and will provide samples in RNA-*later*® and one paraffin block for all consenting patients, taken prior to study entry and at surgery. Biological centres may enter patients into the study via Pathway A or Pathway B (See Appendix 4).

Pathway A: requires tissue for future research to be taken from patients at the same time as the diagnostic core biopsy, and stored in accordance with any regulatory requirements. Generic consent for research core biopsy samples should be gained prior to diagnostic core biopsy. The patient should be offered the trial as soon as HER2 positive breast cancer and eligibility are confirmed.

In addition to the core-cut biopsies taken for the purposes of diagnosis	2 core-cut biopsies should be taken for research. One biopsy should be placed in normal buffered formalin, labelled as "research core" and sent for embedding in paraffin to the local Histopathology Department The second research core should be placed in a tube containing "RNA-*later*®" (will be provided).
At the time of surgery	2 further research core-cut biopsies should be taken and treated in the same manner as the biopsies taken at diagnosis.

Pathway B: should be considered for patients whose diagnostic core biopsy was performed before the opportunity to consider possible entry to EPHOS-B e.g. at another hospital. Patients consenting to the trial should have their research biopsy core taken after written informed consent to the EPHOS-B study, and before randomisation. All patients must be asked to consent to this research biopsy core, however patients who decline consent to a further biopsy core but wish to enter the trial may do so, providing sufficient tissue is remaining in the diagnostic core for research.

As the primary end-point of EPHOS-B is the measurement of increased apoptosis or a fall in proliferation, the repeat research biopsy or access to primary core material is essential.

At the time of surgery patients will provide 2 research core-cut biopsies. One biopsy should be placed in normal buffered formalin, labelled as "research core" and sent for embedding in paraffin to the local Histopathology Department. The second research core should be placed in a tube containing "RNA-*later*®" (will be provided).

Laboratories that hold an HTA research licence and have their own established tissue bank with Ethics Committee approved consent procedures can store samples indefinitely until the patient enters EPHOS-B. Centres without an HTA licence may store the research tissue samples until a diagnosis has been made, as it may be necessary to use research tissue for diagnostic purposes in cases where the diagnostic tissue was insufficient. If the patient is

eligible for EPHOS-B the samples should be sent to the Paterson Institute for Cancer Research.

If the patient is not eligible for EPHOS-B or other perioperative studies i.e. POETIC, the samples should be sent to the Paterson Institute for Cancer Research, or if this is not possible, the samples should be destroyed.

Non Biological Centres (Pathway C)

Non Biological centres are not required to provide samples in RNA-l*ater*®. Paraffin embedded tissue must be available from diagnostic tissue already taken at diagnosis and during surgery.

Centres who wish to participate in EPHOS-B as a non biological centre should routinely take ≥ 3 cores of tissue at diagnosis.

11.2 Use of Tissue Sample Collection beyond EPHOS-B

Samples from patients who consent to tissue donation but do not ultimately enter EPHOS-B will be transferred to the Manchester Cancer Research Biobank for storage. The Biobank holds generic ethics approval to allow research using Biobank samples without the need for further ethical review. To ensure the scientific quality of applications requesting to use Biobank samples, there is a Biobank Access Policy, where projects are reviewed and scored by internal and external reviewers. Only projects meeting a threshold in quality will be approved. All samples will be used in accordance with the consent under which they were obtained for dedicated cancer research projects. The information sheet offered to patients at the time of donating this tissue makes clear that donated tissue together with essential data may be made available to other researchers.

11.3 Blood Sample Collection

As part of the study blood samples will be collected

At randomisation	3 x blood samples are required 1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum
On the day of (& prior to) surgery	3 x blood samples 1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum
Approximately 28-30 days after surgery	3 x blood samples 1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum

Blood kits required for the study will be provided.

Further details on storage and transfer of blood and tissue samples are given in Appendix 5 and full details of blood and tissue sampling collection and storage are given in trial specific Trial Guidance Notes, which should be followed for all samples taken for the EPHOS-B trial.

12. Randomisation Procedure

All patients must be randomised **before** perioperative therapy begins. Sufficient time (≥ 1 day) should be allowed for the patient to decide on trial entry.

An eligibility checklist must be completed by the Principal Investigator/Research Nurse and consent obtained prior to randomisation.

The following information will be required at randomisation:

- name of centre, consultant and person randomising the patient into the trial;
- patient's full name, hospital number, postcode, date of birth and NHS number;
- confirmation that an eligibility checklist has been completed including confirmation that written informed consent has been obtained;
- date of planned surgery.

The caller (PI or research nurse) will be given the patient's unique randomisation number (Trial ID). The Trial ID together with the patient's initials, date of birth and hospital number should be used on all Case Report Forms (CRFs) and correspondence relating to the patient.

To randomise a patient telephone:

ICR Clinical Trials and Statistics Unit (ICR-CTSU), The Institute of Cancer Research

On: 020 8643 7150

09.00-17.00 Monday to Friday

Allocation of Treatment

Treatment allocation will be 1:1:2 (control / trastuzumab alone / combination of lapatinib and trastuzumab), using a minimisation algorithm incorporating a random element. Immediately following randomisation a fax will be sent to the research nurse and relevant pharmacy department identifying the patient by initials and date of birth and confirming the Trial ID together with the allocated treatment.

13. Treatment Plan

Patients agreeing to enter the study will have surgery booked prior to randomisation for around two weeks ahead.

Patients will be randomised to one of three groups of patients in a ratio of 1:1:2

Group 1: control (i.e. no pre-surgical treatment),

Group 2: trastuzumab alone 6mg/kg iv dose given on days 1^1 & 8^2 pre-operatively and **2mg/kg** iv dose on day 15^3 (or up to day 19) post surgery.

Group 3: Combination of lapatinib and trastuzumab

- Lapatinib: **1000mg/day** continuously for 28 days[4].
- Trastuzumab: **6mg/kg** iv dose given on **days 1[4] & 8[2]** pre-operatively and **2mg/kg** iv dose on **day 15[3]** (or up to day 19) post surgery.

Note:

[1] Day 1 of treatment in both treatment arms is the day of first dose of trastuzumab and should be 11 (+2 or -1 day) prior to surgery.

[2] The 2nd trastuzumab dose may also be given on day 7 or day 9 in exceptional circumstances. Please contact the ICR-CTSU Trials Office to confirm any change to the timing of the Day 8 treatment. If the 2nd dose is given on day 9, then surgery must be no sooner than 11 days post Day 1 of treatment.

[3] The 3rd trastuzumab iv dose may be given between day 15 and day 19. This is to allow some flexibility with post operative recovery and scheduling.

[4] Lapatinib treatment is expected to start on the same day as the first dose of trastuzumab (Day 1) but may be given on Day 1 ± 24 hours as long as the start of lapatinib is 11 (+2 or -1 day) prior to surgery.

Therapeutic surgery should be carried out approximately 14 days after randomisation, or as near to this time as possible.

13.1 Perioperative Trial Treatment: Route and Dose Schedule

13.1.1 Trastuzumab

Day 1 is defined as the first date of trastuzumab treatment and the first dose of trastuzumab should be administered 11 (+2 or -1) days before the day of surgery.

Patients should be administered trastuzumab in an outpatient setting and given at a dose of **6mg/kg** intravenously over 90 minutes **on days 1 and 8**, and **2mg/kg** on **day 15**. If the initial loading dose was well tolerated, the subsequent doses can be administered as a 30-minute infusion.

Patients should be observed for at least 4-6 hours after the start of the first dose of trastuzumab. If no adverse events occur with the first infusion the observation period for the second infusion may be shortened to 2 hours after the start of the infusion.

13.1.2 Lapatinib (Combination arm only)

Lapatinib treatment is expected to start on the same day as the first dose of trastuzumab (Day 1) but may be given on Day 1 ± 24 hours as long as the start of lapatinib is 11 (+2 or -1 day) prior to surgery.

It is strongly recommended that the Research Staff discuss the planned start and stop dates of the medication with the patient to ensure the patient has understood the treatment plan.

Treatment start and stop dates should be recorded on the Patient Card as a reminder to patients when they should start and stop taking their tablets.

Lapatinib tablets are dispensed as 250mg tablets.

At the time of randomisation each patient will be given TWO bottles of lapatinib containing 90 tablets sufficient for a 28 day supply and some overage.

13.1.3 Pre-operative Treatment with Lapatinib

Patients should be advised to take all four tablets at once as one dose (i.e. total daily dose 1000mg). Tablets should be taken early in the evening at the same time each day and on an empty stomach either one hour (or more) before or one hour (or more) after a meal and according to the instructions on the bottle.

If vomiting occurs shortly after the lapatinib tablets are swallowed the dose should be replaced only if all the intact tablets can actually be seen and counted. Otherwise the next dose should be taken the following day, as per schedule. Missed doses should not be replaced and the dosing should resume with the next scheduled daily dose.

NOTE: Lapatinib should NOT be taken with grapefruit or grapefruit juice. Grapefruit and grapefruit juice is not permitted for the duration of the study.

(See Appendix 1 for pharmaceutical information).

13.1.4 Post-operative Treatment with Lapatinib

Patients should be instructed to continue taking lapatinib, (including the evening of surgery) so that each patient takes lapatinib for a total of 28 days. Patients should be asked to return both bottles of lapatinib to the research team at the 28-30 day post surgery follow up visit for assessment of compliance. The research team should then return both bottles to pharmacy for accountability purposes.

14. Drug Supplies and Labelling

14.1 Trastuzumab

Trastuzumab is manufactured by ROCHE and should be taken from hospital pharmacy stock (please refer to Section 13 of the protocol for dosing of trastuzumab).

As this study is using commercially available supplies, the pharmacist should follow local procedures for reconstitution and administration. In addition to the local pharmacy label, the trastuzumab infusion bag should be labelled as a minimum with the trial identifier and statement 'for clinical trial use only'*. Records of trastuzumab administration to each patient should be according to the local practice of the centre.

The pharmacy should follow the Summary of Product Characteristics (SPC) documentation from ROCHE for trastuzumab. An appropriate dose modification in the study of 6mg/kg loading dose on Day 1 and a further 6mg/kg at Day 8 should be followed by a further maintenance dose of 2mg/kg at Day 15(or up to day 19)[38] for patients in both treatment arms of the study.

NOTE: Trial treatment with trastuzumab ends on Day 15 (or up to day 19). Trastuzumab given to patients after chemotherapy is not a part of the EPHOS-B study.

* As per exemption in article 46 of The Medicines for Human Use (Clinical Trials) Regulations SI1031

14.2 Lapatinib

14.2.1 Drug Manufacture/Distributor

Lapatinib is manufactured by GlaxoSmithKline* and will be provided free of charge to participating centres. Supplies of lapatinib will be distributed from GSK* to Catalent, their

distribution company. Catalent is responsible for supplying lapatinib to participating centres free of charge.

As soon as each participating centre has site specific approval (SSA), R&D approval and a centre agreement is in place, ICR-CTSU will contact Catalent to arrange for the study supplies to be sent to the relevant pharmacy.

14.2.2 Packaging and Labelling

The Co-Sponsors of EPHOS-B have delegated the responsibility of packaging and ensuring compliance with GMP to GSK*.

Lapatinib will be provided by GSK* in high-density polyethylene (HDPE) bottles with a child resistant closure, each containing 90 of 250mgs tablets of lapatinib. Labelling is in accordance with UK regulations and certified by the Qualified Person (QP) within Catalent.

***Note: In March 2015 the GSK oncology portfolio was acquired by Novartis.**

14.2.3 Storage

The bottles should be stored at controlled room temperature (15°C-30°C).

The nominated pharmacist is responsible for ensuring that the study medication is stored in a secured area. Lapatinib supplied for EPHOS-B must not be used outside the context of the protocol.

14.2.4 Pharmacy Responsibilities

At the time of the dispensing, the pharmacist should complete the required details on each IMP label. **This will include the number of tablets to be taken.**

Lapatinib will normally be dispensed immediately following randomisation, however patients will not start treatment until 11 (+2 or -1) day prior to surgery. The start and stop dates of treatment will be recorded on a patient's card provided by the Research Team following randomisation. (Patients allocated to the combination of lapatinib and trastuzumab treatment group should have both lapatinib and trastuzumab dates recorded.) At the time of randomisation each subject will be given two bottles of lapatinib, each bottle will contain 90 tablets, allowing for a 28 day supply and some overage.

Each pharmacy department must designate a person responsible for ensuring that:

- investigational products are handled and stored safely and properly
- investigational products are dispensed only to trial patients and in accordance with the protocol
- any unused products are destroyed according to local practice

It is the responsibility of the pharmacist to contact ICR-CTSU when further stock of lapatinib is required. Lapatinib order forms will be provided by ICR-CTSU for use in this trial.

14.2.5 Drug Accountability and Destruction

Patients in the lapatinib arm of the study should be asked to bring with them all their trial medication at the post surgical visit (at 28-30 days after surgery) for the purposes of drug

accountability. The unused medication should be returned to pharmacy by the Research Nurse for destruction according to local practice.

The Sponsors' responsibility for drug accountability is delegated to the local pharmacies. Local pharmacies should maintain accurate drug accountability and destruction records for lapatinib and accountability records for trastuzumab within the EPHOS-B trial. Drug accountability and destruction forms will be provided by the ICR-CTSU for local use, however centres may use their own accountability forms, as long as the information recorded is consistent with that required on the forms provided.

Whichever local method of drug accountability is used, pharmacies are responsible for ensuring that it satisfies the requirements of any MHRA audit or inspection.

15. Safety Considerations

15.1 Supportive Care Guidelines

Dermatological Events: In a recent analysis of 2093 patients participating in 9 completed metastatic breast and non breast cancer clinical trials, 928 patients received lapatinib as monotherapy and 491 patients received lapatinib in combination with either capecitabine or paclitaxel. In patients exposed to lapatinib (n-1419) 52% of patients (n=928) reported skin events (defined as skin, hair and nail toxicities). Most skin events developed early between days 1 to 14 of treatment, and (87%) did not require a lapatinib dose reduction or treatment interruption. Only 1% of skin events resulted in discontinuation of therapy[39].

Rash: Skin rash (usually grade 1-2) has been observed during the first several days of treatment with EGFR inhibitors. Anecdotal reports of improvement have occurred with several agents. In patients with severe rash, treatment may need to be discontinued. However the rash resolves when the treatment is discontinued and in most cases the patient should be able to continue treatment for 11+1 days. Anecdotal reports of improvement have occurred with any of the following: minocycline, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone (short course).

Diarrhoea: Diarrhoea-related events in connection with lapatinib therapy have been studied by Crown et al in a pooled analysis of 9 completed phase II and III studies including 2,093 patients with locally advanced or metastatic breast and non breast cancer[31]. Lapatinib-induced diarrhoea was usually low-grade, self-limiting, and manageable. Severe (grade 3) diarrhoea occurred in <10% of patients and grade 4 diarrhoea rarely occured. Diarrhoea generally occurred early in the course of treatment (<1 week) and was usually of limited duration (median of 4–5 days). Most diarrhoea events resolved and did not require lapatinib dose reduction, interruption, or discontinuation[40] .

Diarrhoea is also seen with other EGFR inhibitors. In general EGFR inhibitor-induced diarrhoea has been transient, usually not of sufficient severity to hinder administration of the agents, and responsive to loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg q 2–4 hr until diarrhoea free for 12 hr.

Nausea: Routine pre-medication for nausea is not necessary, but symptomatic patients should be treated with standard anti-nausea/antiemetic therapy as necessary. If the patient vomits after taking the tablets, the dose is replaced only if the tablets can actually be seen and counted.

Cardiovascular events: Congestive heart failure has been reported in the clinic with trastuzumab, a monoclonal antibody directed against the HER2 receptor. This cardiomyopathy has been seen at a higher incidence in patients that previously received anthracyclines and is characterised by dilatation and thinning of the ventricular walls with decreased left ventricular ejection fraction.

Left ventricular ejection fraction (LVEF) decreases have been reported in approximately 1% of patients receiving lapatinib and were asymptomatic in more than 90% of cases. LVEF decreases resolved or improved in more than 60% of cases on discontinuation of treatment with lapatinib. Symptomatic LVEF decreases were observed in approximately 0.1% of patients who received lapatinib monotherapy. Observed symptoms included dyspnoea, cardiac failure and palpitations. All events resolved promptly on discontinuation of lapatinib.

In a pooled analysis of 44 phase I-III studies conducted between 2001 and 2006 lapatinib was administered to 3689, patients either as monotherapy or in combination. Asymptomatic cardiac events were reported in 53 patients (1%) and symptomatic cardiac events in 7 (0.2%). Cardiac events were largely reversible decreases in LVEF and occurred at similar rates in patients who were not pre-treated with anthracyclines or trastuzumab. No cardiac deaths occurred among patients treated with lapatinib[31].

Because of the known effects of trastuzumab on cardiac function and the observed rare transient reductions of LVEF seen among patients receiving lapatinib, only patients with normal LVEF will be eligible for study. All patients should therefore be screened by echocardiogram or MUGA prior to randomisation. Only patients with an ejection fraction ≥55% will receive anti-HER2 therapy.

Hepatic Events: In a review of the GlaxoSmithKline (GSK) safety database, as of 31 December 2007, 39 out of 8702 patients developed hepatic events (predominantly transaminase elevations) where causal associations to lapatinib could not be ruled out, giving a crude incidence of 0.4%. Rarely hepatoxicity has been severe, with only a small number of liver related deaths. In the majority of cases, patients have recovered when lapatinib use was discontinued.

Based on the possible causal relationship between hepatobiliary disorders (specifically) transaminase elevations) and lapatinib, liver function tests (LFTs) will be done prior to randomisation. Only patients with normal LFTs as defined in this Protocol's inclusion/exclusion criteria at baseline will be eligible for study entry. LFTs should be repeated prior to surgery and 28-30 days post surgery. Any patients found to have evidence of hepatobiliary toxicity at the time of surgery or at any other time whilst taking lapatinib, should have their treatment immediately discontinued. The criteria for stopping therapy are as for other lapatinib trials, namely ALT >3 × ULN and total bilirubin >2.0 × ULN.

16. Study Evaluations

First Surgical Appointment Visit* (Pathway A patients only)

- Patients should be considered for entry into EPHOS-B at the time of the first outpatient visit, once a diagnosis of primary breast cancer is suspected. Generic consent should be obtained for research core biopsy/ies prior to confirmation of trial eligibility.

Follow up Visit for Results of Biopsy*

- Patients with a confirmed diagnosis of HER-2 (3+) primary breast cancer should have their surgery planned (approximately 2 weeks after randomisation).
- Patients should be offered entry into EPHOS-B with appropriate information and discussion.

* Some patients will have had their diagnostic core biopsy performed before the opportunity to consider possible entry to EPHOS-B e.g. at another hospital. These patients will enter EPHOS-B via Pathway B and should have their research biopsy core taken after written informed consent, and before randomisation. All patients must be asked to consent to this research biopsy core, however patients who decline consent to a further biopsy core but wish to enter the trial may do so, providing a sufficient tissue is remaining in the diagnostic core for research.

Pre-Treatment Assessments/Evaluations (baseline)

- Written informed consent for EPHOS-B should be obtained
- Relevant past and current medical history
- ECOG performance status
- Required staging investigations will be minimal in keeping with standard UK practice in breast cancer management. All patients should have a full blood count (FBC) and biochemical screen to include liver function tests (obtained within 28 days prior to randomisation). Chest X-ray or a chest CT scan should be performed if standard practice. Further staging investigations will be performed as clinically indicated.
- Blood serum pregnancy test within 28 days prior to randomisation (if appropriate)
- All patients should have an ECG and ECHO or MUGA to confirm eligibility (LVEF ≥ 55%) prior to randomisation.

Procedures/Assessments on Day of Surgery

- Document compliance with lapatinib medication (if applicable)
- Record new concomitant medication(s) (if applicable)
- LFTs*

* If a subject experiences ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required), then the following actions must be taken:

1) Immediately and permanently discontinue lapatinib. For patients in the combination lapatinib and trastuzumab treatment group, trastuzumab should be continued and ICR-CTSU should be informed.

2) Complete the EPHOS-B SAE form and send to ICR-CTSU Trials Office within 24 hours of knowledge of the event.

Post Operative Follow-Up Assessment (approximately 28-30 days after surgery) *

- Wound assessment
- LFTs
- Compliance with allocated lapatinib treatment
- Record use of any new concomitant medication(s)

Pre-Adjuvant Chemotherapy

- All patients should have an ECG and ECHO or MUGA before adjuvant chemotherapy is started. Patients receiving perioperative treatment should have finished their treatment. Control arm patients (or those that discontinue all perioperative treatment), should not have the cardiac screening conducted until the 2nd day after surgery.

Assessments at Month 6, 12, 18 & 24 Following Randomisation *

- Cardiac toxicity assessment
- Clinical examination
- Evidence of local & or metastatic relapse

* NB please refer to section 19 for further details for reporting study treatment toxicities

16.1 Follow-Up Investigations

Patients should be followed-up as per local practice, but the minimum must include, for patients in all groups of the study, assessment of cardiac toxicity at 6-monthly clinic visits until the end of year 2 .Thereafter, clinical cardiology review will be conducted during routine follow up appointments.

16.2 Annual Follow-Up

Annual follow-up data will be collected for at least 10 years after completion of recruitment and will include as a minimum sites of recurrence, time of recurrence, mortality, and date and cause of death. Information on second primary breast cancers and other second primary tumours will also be recorded.

If necessary, annual follow-up post 5 years may be performed as a telephone consultation in the first instance, with patients attending hospital for imaging and biochemical investigations where recurrence or other significant clinical problems are suspected.

Should a patient be discharged to GP care or another hospital, all reasonable attempts should be made by the randomising hospital to collect follow up information from these sources and sent to the ICR-CTSU trials office at the time points stipulated in the protocol. If the GP or other hospital are unable to provide follow up information the ICR-CTSU trials office should be informed. The trials office will then apply to a national records office to either trace the patients' new GP *or* give notification in the event of their death.

When collection of routine data from registries via electronic methods is considered reliable and complete, follow up data may be collected via these methods, subject to the necessary approvals being in place.

16.3 Relapse

The date of relapse is taken as the date of first confirmed recurrence by an appropriate investigation such as cytology, histology, or imaging wherever possible. In the absence of such confirmation, the date of first clinical suspicion will be taken provided that suspicion leads to a change or re-introduction of anti-cancer therapy. The management of recurrence will be at the discretion of the clinician. Follow-up information should continue to be provided until the patient dies.

17. Non Investigational Therapy

All patients will also receive non HER2 targeted standard adjuvant systemic therapy. Standard adjuvant therapy (including the potential for entry into further trials of adjuvant systemic therapy) will include endocrine therapy for hormone sensitive cancers and an expectation to receive chemotherapy, with each patient's treatment dictated by local guidelines. Choice of therapy will be independent of whether the patient received perioperative antiHER2 treatment (NB guidelines defining both criteria for treatment and choice of specific agents will be declared to the ICR-CTSU at the outset of trial, with changes to such guidelines also notified). Since HER2 status, grade and ER status will have been measured on the core biopsy, it is very unlikely that there will be confounding of patients between the trial arms in terms of choice of standard adjuvant therapy.

17.1 Surgical Treatment

Patients may undergo either breast conservation surgery or mastectomy in accordance with local protocols and patient choice. Patients entered into this study must have confirmation of axillary node status at the time of surgery. The axilla should be staged by axillary sampling, sentinel node biopsy (SNB) or axillary clearance. If sampling or SNB identifies axillary node involvement, the axilla should be cleared or axillary radiotherapy undertaken in accordance with local protocols. In patients treated by breast conservation, clear margins should be achieved with a minimum of 1mm clearance. Further re-excision to extend the margin of clearance should be in accordance with local protocols.

Primary breast reconstruction and other oncoplastic procedures to improve cosmetic outcome is acceptable in the EPHOS trial.

17.2 Adjuvant Endocrine Therapy

All ER positive patients will be treated in accordance to local policy at the time, with either tamoxifen or an aromatase inhibitor or each sequentially for a combined minimum of 5 years after chemotherapy is completed. It is recognised that this is a changing field and policies may change during the course of the trial.

17.3 Adjuvant Chemotherapy

Patients in the treatment groups should commence chemotherapy ≥ 35 days after the first pre-operative dose of trastuzumab, lapatinib or the combination of lapatinib and trastuzumab, as part of routine practice. This assumes at least 7 days lapatinib washout. Although only expected in exceptional circumstances, patients receiving combination therapy may have 2 different treatment start dates, please use the later of the two dates when calculating ≥ 35 days. If the third dose of trastuzumab is given between day 16-19 the start of chemotherapy should be adjusted accordingly i.e. patients receiving trastuzumab on day 19 should start chemotherapy ≥39 days after the first preoperative dose of trastuzumab. If an anthracycline containing regime will be used cardiac monitoring should be performed as per local protocol.

17.4 Radiotherapy

Radiotherapy should be given to all patients following breast conservation, and if required as per local agreed protocols post mastectomy, after chemotherapy or surgery in keeping with local practice.

17.5 Adjuvant Trastuzumab

Within EPHOS-B it is anticipated that patients will receive further trastuzumab treatment after adjuvant chemotherapy. Centres may choose at their discretion whether to take into account that some patients have already received up to 4 weeks of perioperative anti-HER2 therapy when prescribing their standard adjuvant trastuzumab.

18. Trial Schedule of Events

	First Surgical Visit	Prior to Randomisation	Perioperative Treatment period 11 (+2 or -1) day before surgery	Surgery Approx 14 days after Randomisation	Postoperative Treatment period (prior to chemotherapy)	Non Investigative Therapy	Post Chemotherapy Trastuzumab Treatment	Follow–up at month 6,12,18 & 24
Medical History	**X**							
Generic consent (Pathway A patients only)	**X**							
Research biopsies	**X**			**X**				
HER2+ breast cancer confirmed discuss EPHOS-B trial		**X**						
Consent for EPHOS-B, baseline screening		**X**						
Clinical cardiovascular evaluation		≤ 28 days prior to randomisation			After treatment has stopped, but prior to chemotherapy			
Pregnancy test (If applicable)		**X**						
Research blood samples		**X** (after consent)		Before surgery	At 28-30 day post op visit			
FBC, Biochemistry		**X**						
LFTs		**X**		**X**	At 28-30 day post op visit			

	First Surgical Visit	Prior to Randomisation	Perioperative Treatment period 11 (+2 or -1) day before surgery	Surgery Approximately 14 days after Randomisation	Postoperative Treatment period (prior to chemotherapy)	Non Investigative Therapy	Post Chemotherapy Trastuzumab Treatment	Follow–up at month 6, 12, 18 & 24
Clinical examination		**X**						**X**
Toxicity assessment								**X**
Perform ECG and ECHO or MUGA		**X**					Performed before start of trastuzumab treatment according to local practice	
Record of new concomitant medication				**X**	At 28-30 days post op visit			
Wound assessment					To be performed 28-30 days after surgery			
Commence chemotherapy as part of routine practice						Should commence on or after day 35 (Day 1 is the 1st day of perioperative treatment*		
SAE reporting		**X**	**X**	**X**	To be reported as described in 19.1.2			

* If the third dose of trastuzumab is given between day 16-19 the start date of chemotherapy should be adjusted accordingly i.e. patients receiving trastuzumab on day 19 should start chemotherapy ≥39 days after the first preoperative dose of trastuzumab

19. Pharmacovigilance

19.1 Definitions

19.1.1 Definition of an Adverse Event (AE)

For the purpose of this trial any untoward medical occurrence or effect that occurs after randomisation and within 30 days of the last administration of perioperative trastuzumab or lapatinib, which is not unequivocally attributable to breast cancer or primary surgery, should be considered an Adverse Event (AE). The event does not necessarily have a causal relationship with the treatment.

19.1.2 Definition of a Serious Adverse Event (SAE)

An SAE in the EPHOS B trial is defined as any untoward medical occurrence that occurs after the commencement of randomised treatment and within 30 days of the last administration of the last dose of perioperative trastuzumab or lapatinib and results in:

- death;
- is a life-threatening condition (i.e. immediate risk of death);
- requires inpatient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity: results in a significant or persistent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life;
- any untoward medical occurrence requiring medical intervention to prevent permanent impairment or damage;
- is a congenital anomaly/birth defect.

As cardiac dysfunction, pneumonitis and hepato-biliary events have been seen in subjects taking lapatinib and other tyrosine kinase inhibitors, as a precaution the following will be reported as an SAE in the EPHOS-B study **if they occur after the commencement of randomised treatment and before day one of cycle two of adjuvant chemotherapy treatment or after the commencement of lapatinib and within 30 days of completing treatment, whichever is the later:**

- any signs and symptoms of pneumonitis that are ≥ grade 3 (CTCAE v4.0). (Patients who experience ≥ grade 3 pneumonitis must be withdrawn from trial medication).
- any signs and symptoms of deterioration in left ventricular cardiac function that are ≥ grade 3 (CTCAE v4.0) or a 20% decrease in left ventricular ejection fraction relative to baseline. These patients must be withdrawn from trial medication.
- ALT >3 × ULN and total bilirubin >2.0 × ULN (>35% direct; bilirubin fractionation required).

NOTE:

- bilirubin fractionation should be performed if testing is available. If testing is unavailable and a patient meets the criterion of total bilirubin >2.0 × ULN, then the event should still be reported as an SAE. Other hepatic events should be documented as an AE or an SAE as appropriate.
- SAEs only apply to patients who receive perioperative treatment, (i.e. not control patients)

19.1.3 Definition of a Serious Adverse Reaction (SAR)

A SAR is defined as a SAE that has a definite, probable or possible causal relationship to the study drugs (trastuzumab and lapatinib).

Many adverse events that occur in this study, whether they are serious or not, will be known treatment related toxicities due to lapatinib or trastuzumab. For this trial known treatment related toxicities shown in Tables 5 & 6 which meet the definition of serious should be reported on the EPHOS-B SAR form.

Table 5: A summary of 'known' adverse reactions as stated in the lapatinib Investigator's Brochure are listed below:

Common
Diarrhoea which may lead to dehydration Nausea Fatigue Rash (including dermatitis acneiform) Vomiting Anorexia

Table 6: A summary of 'known' adverse reactions as stated in the trastuzumab SmPC are listed below:

Common	**Uncommon**
Nausea Vomiting Fatigue Rigors Headache Dizziness Rash	Dyspnoea Hypotension Tachycardia Bronchospasm Wheezing Reduced oxygen saturation

In summary, the SAEs described below should be recorded on the EPHOS-B SAR form and forwarded to ICR-CTSU in the usual timeframes for SAE reporting.

1. Known adverse reactions to lapatinib as stated in table 5 which meet the definition of serious*.
2. Known adverse reactions to trastuzumab as stated in table 6 which meet the definition of serious*.

* SARs listed in table 5 and 6 that are life-threatening or result in death should be reported on the EPHOS-B SAE form.

All other SAEs, whether related to the study drugs or not, should be recorded on the EPHOS-B SAE form and forwarded to ICR-CTSU in the usual timeframes SAE reporting.

19.1.4 Definition of Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any adverse reactions that have a suspected relationship to the study drugs that are both serious and unexpected, as judged by the Chief Investigator.

19.1.5 Definition of Causality (relatedness)

The assignment of causality for serious adverse events should be made by the investigator responsible for the care of the patient. If there is any doubt about the causality the investigator should inform ICR-CTSU who will notify the Chief Investigator.

Table 4: Definitions for Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial drug
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment)
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments)
Probable	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

19.2 Reporting Procedures

19.2.1 Procedure for reporting adverse events (AEs) to ICR-CTSU

Wherever one or more signs/symptoms correspond to a disease or well defined syndrome only the main disease/syndrome should be reported. The severity of adverse events should be graded according to the NCIC-CTCAE version 4. For each sign/syndrome the highest grade observed should be reported.

All adverse events (AEs) must be reported on the Case Report Forms (CRFs) and sent to the ICR-CTSU as soon as they have been completed.

19.2.2 Reporting of Serious Adverse Events/Reactions (SAEs/SARs) to ICR-CTSU

SAEs and SARs should be reported from the time of randomisation and up to 30 days following the last dose of perioperative trastuzumab or lapatinib.

All SAEs and SARs should be reported within 24 hours of the investigator becoming aware of the event, by completing the EPHOS-B SAE or SAR form and faxing it to:

Please fax SAE and SAR forms for the attention of the EPHOS-B Trial Manager to the ICR-CTSU Safety Desk

Fax: +44 (0)20 8722 4368 (Monday – Friday 09.00-17.00)

All SAE and SAR forms must be completed, signed and dated by the Principal Investigator or designated representative.

19.3 Review of Serious Adverse Events

Events reported using an SAE form will be reviewed by the Chief Investigator (or designated representative) for causality and expectedness.

Centres should respond as soon as possible to requests from the CI or designated representative (via ICR-CTSU) for further information that may be required for final assessment.

19.4 Expedited Reporting of SUSARs

If an SAE is defined as a SUSAR by the Chief Investigator and is fatal or life threatening, ICR-CTSU will report this to the MHRA, the Main REC, and to the Co-Sponsors within 7 days of being notified of the event.

If an SAE is defined as a SUSAR by the Chief Investigator and is not fatal or life threatening, ICR-CTSU will report this to the MHRA, the Main REC and to the Co-Sponsors within 15 days of being notified of the event.

The Principal Investigator at all actively recruiting centres will be informed of any SUSARs occurring within the trial.

ICR- CTSU will report the following events to Novartis (formerly reported to GSK within the timelines specified in Table 8.

Table 8: ICR-CTSU Reporting Timelines to Novartis (formerly reported to **GSK.**

Type of Event	**Timeframe of Reporting**
SAEs** that are fatal or life threatening or result in persistent or significant disability/incapacity	Within 24 hours*
Liver function abnormalities**	Within 24 hours*
Pregnancy**	Within 2 weeks*

* of ICR-CTSU becoming aware of the event

** from patients in the combination of lapatinib and trastuzumab arm of the trial only

19.5 Follow up of Serious Adverse Events

Information on outcome of the SAE which may not be available at the time the SAE was initially reported should be completed on the relevant part of the original SAE form within 15 days of the initial report of the event and faxed to ICR-CTSU. However Centres should continue to send follow up of SAEs until clinical recovery is complete and laboratory results have returned to normal, or until disease has stabilised.

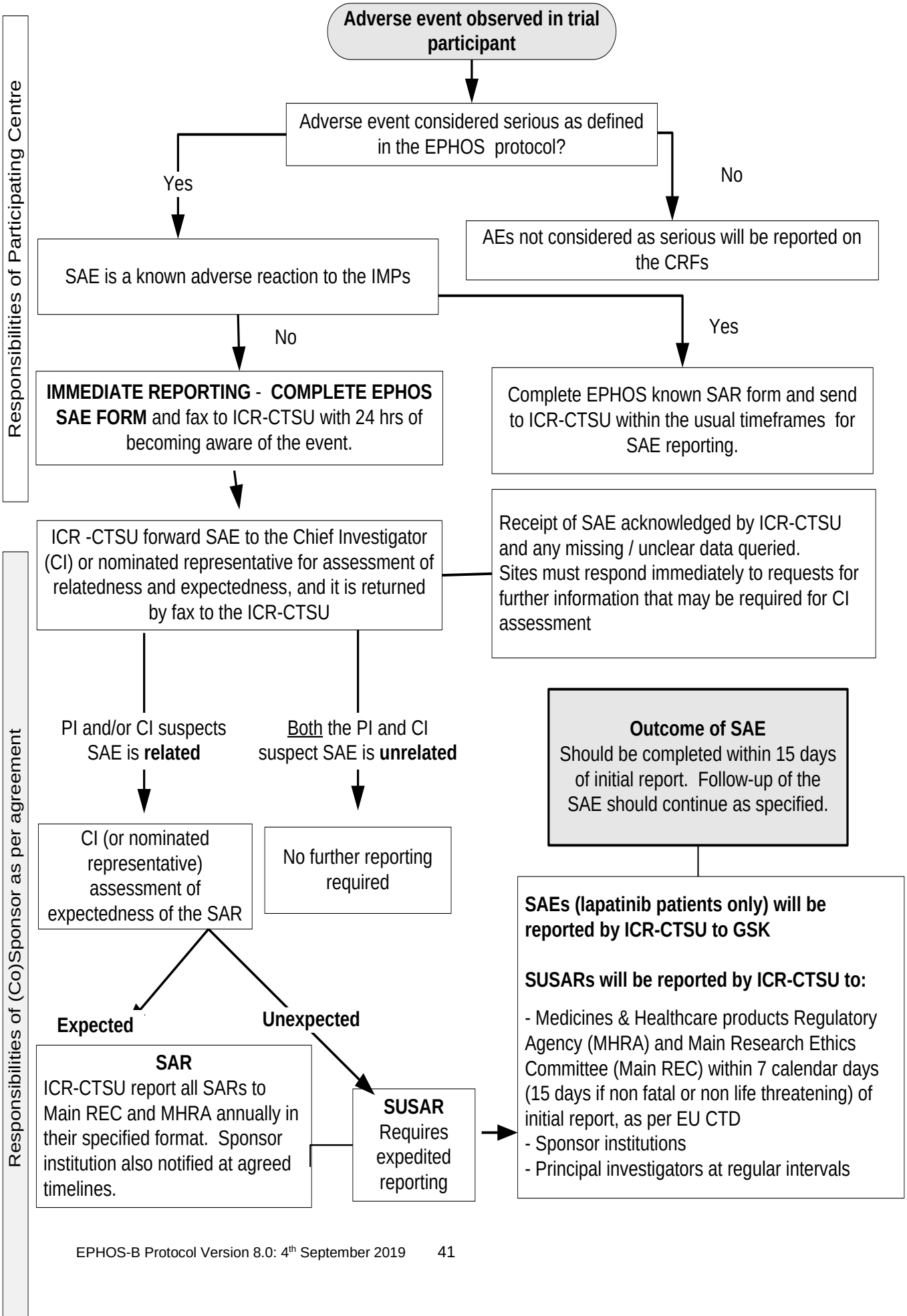
19.6 Annual Reporting of Serious Adverse Reactions

An annual report will be provided to the MHRA and the Main REC at the end of the reporting year. This will be defined as the anniversary of the date when the Clinical Trials Authorisation (CTA) was obtained. This will include all related events reported on SAE and SAR forms, and a report from the Independent Data Monitoring Committee (IDMC).

19.7 Reporting Pregnancies

Any pregnancy that occurs up to 30 days after the completion of perioperative treatment should be reported to ICR-CTSU on the EPHOS-B SAE form within 2 weeks of the Investigator becoming aware of the event. The pregnancy should be followed up to determine outcome, including any spontaneous or voluntary termination, details of the birth and the presence of birth defects or congenital abnormalities.

Flow diagram for SAE reporting, and action following report



20. Statistical Considerations

20.1 Treatment Allocation

Participants will be randomised via minimisation in a 1:1:2 ratio to control or trastuzumab alone or combination of lapatinib and trastuzumab. Stratification will be by centre.

20.2 Sample Size

EPHOS-B Part 1 consisted of:

- a three group randomised trial comparing trastuzumab alone vs. lapatinib alone vs. control

As external evidence now supports an effect of lapatinib on proliferation and a general biological effect of both trastuzumab alone and the combination, EPHOS-B Part 2 will consist of:

- a three group randomised trial comparing trastuzumab alone vs. the combination of lapatinib+trastuzumab vs. control.

Results from the lapatinib versus control comparison in HER2+ patients from MAPLE will also be incorporated in the lapatinib versus control comparison within EPHOS-B.

Hypothetical adjusted powering considerations are given below. The exact numbers achievable in each group will be influenced by the time at which the trial is amended. Treatment allocation will be adjusted via minimisation at that point so that a higher proportion of patients are treated with the combination group (trastuzumab and lapatinib) to gain greater information on the efficacy of dual therapy. This will ensure that across parts 1 and 2 of the trial a similar number of patients have received combination therapy or trastuzumab alone.

Number of patients	**Estimated number of patients randomised until March 2013**	**Number of patients needed from April 2013 forwards**	**Total**
Groups			
Control	20	35	55
Lapatinib	40	Not recruiting	40
Trastuzumab	40	35	75
Trastuzumab + Lapatinib	-	80	80
Total	100	150	250

The new combination group (trastuzumab + lapatinib) will be compared only with newly randomised patients of the control or trastuzumab groups to respect the randomisation.

Comparisons for primary outcomes of proliferation & apoptosis	No. patients eligible
Trastuzumab vs. Control	75 vs. 55
Trastuzumab vs. *Trastuzumab + Lapatinib* (new randomisation)	35 vs. 80
Trastuzumab + Lapatinib vs. *Control* (new randomisation)	80 vs. 35
Lapatinib vs. Control	40 vs 20

External evidence allows us to increase the false positive rates for each of the above comparisons from 0.85% to 2.5% on a heuristic basis. Each comparison would have a power of 85% or more with a one-sided p-value of 0.025, to allow for multiple comparisons, given the following assumptions:

It is expected that only 5% or less of control patients will show a 30% rise in apoptosis (or 30% fall in proliferation) but we anticipate 30% or more of trastuzumab or trastuzumab+ lapatinib patients will show such a rise. It would be possible to detect differences of 30%, e.g.

60% vs. 30% with more than 80% power when comparing the trastuzumab and trastuzumab-lapatinib arms.

Illustrative Amended Powering Considerations:

Comparisons:	Trastuzumab vs. Control Trastuzumab + Lapatinib vs. Control	Trastuzumab + Lapatinib vs. Trastuzumab
Alpha (one-sided)	0.025	0.025
Power	85%	80%
1st proportion	0.05	0.30
2nd proportion	0.30	0.60
Allocation ratio	2	2
Numbers needed per group	Control 35 Trastuzumab 35 Trastuzumab + Lapatinib 80	
Total needed	150	

20.3 Analysis Plan

A statistical analysis plan will be finalised prior to the main analysis taking into account the current opinion at the time of analysis. The endpoints will be interpreted in the light of all existing evidence from similar studies. The analysis of apoptosis and proliferation will be from the date of randomisation to the date of surgery using non-parametric statistics to compare the log (surgical/pre- treatment) scores. The combination group of lapatinib and trastuzumab will be compared only with concomitantly randomised patients of the control or trastuzumab groups to respect the randomisation. Treatment comparisons will be tested with and without adjustment for baseline prognostic factors. In the absence of major confounding factors the latter will be considered secondary endpoints. Analyses will be based on the intention to treat principle, patients excluded from analysis because of missing data will be assumed to have shown no change.

Baseline characteristics will be described by randomised treatment group and correlations between baseline characteristics and biological markers and marker changes will be investigated using Spearman Rank correlation. Comparisons will be performed using simple parametric, exact, non-parametric or chi-squared tests as appropriate. Tests will be two-sided and 95% confidence intervals will be used, adjusted confidence intervals (97.5%) will also be presented for the primary endpoints. An exploratory investigation of treatment compliance with randomised treatment will be undertaken. Relapse Free Survival (RFS) is defined as time from randomisation to local, regional, or distant tumour recurrence or death from any cause. Second primary cancers will be treated as censoring events. Patients who are alive and disease free will be censored at the date last seen alive. Overall survival will be measured from date of randomisation to date of death from any cause. All survival endpoints will be analysed using the logrank test and Cox regression methods. The proportional hazards assumption will be checked to validate the use of these methods. Probabilities of relapse free and overall survival will be presented as Kaplan-Meier survival curves with fixed term survival estimates.

Serum angiogenic markers will be tested at the more stringent p-value of 0.001. This would, for example, give 80% power in the comparisons with control if 5% of control patients showed a fall compared with 41% of combination treated patients. Descriptive estimates of the

cardiac event rates will be provided for each of the treatment groups, typical estimates would be 2/100 = 2%(95%CI:0.2 to 7.0); 5/100 = 5%(95%CI:1.6 to 11) and 10/100 = 10%(95%CI:4.9 to 18).

The link between the translational data and the patient data will be maintained by requesting that the sample acquisition and transfer process is fully audited. When samples are sent from the participating hospital to the testing laboratory the hospital will simultaneously dispatch a letter to ICR-CTSU detailing that the sample has been sent. When the laboratory receives the sample it will also notify ICR-CTSU, verifying the sample ID and their own internal laboratory identifier for the sample. This linkage will ensure the trial complies with the Human Tissue Act and will also enable the sample to be destroyed at the end of the study or if consent is withdrawn. The translational data will be linked with the patient data by using the trial number as the key identifier, in addition the laboratory number/trial identifier correspondence will also act as a back up linkage.

20.4 Interim Analysis and Role of Independent Data Monitoring Committee (IDMC)

An IDMC will be instigated to monitor the progress of the trial, it will meet in confidence at regular intervals (but at least six monthly) and will be consulted at the time of each interim analysis. An interim analysis will coincide with the analysis of part 1 and will be performed once all part 1 patients have been evaluated for the primary endpoint. . Biological changes in apoptosis or proliferation between groups will first be analysed using a highly conservative p-value for significance of 0.0002. This analysis will provide an opportunity to validate the methods of analysis and refine them if necessary. It will also will provide support (or otherwise) for the extension of the trial beyond 250 patients up to the beginning of the clinical phase.

Collaboration will be sought with MAPLE trialists so that lapatinib versus nil pre surgical data in HER2+ patients from the two trials can be combined. Interim analysis of compliance and toxicity will be conducted on a regular basis and presented in confidence to the IDMC. A report of the findings and recommendations will be produced following each meeting. This report will be submitted to the Trial Management Group and Trial Steering Committee, the main REC and the MHRA, as required.

Interim analyses will be supplied in strict confidence by the trial statistician to the IDMC together with any other analyses that the IDMC may request. No results will be made available to investigators or any other party until at least six weeks after the last patient is entered unless the IDMC determines that it would be unethical to withhold the interim results.

The main criterion for early stopping of the trial by the Trial Steering Committee upon suggestion from the IDMC and request from the Trial Management Group will be that evidence from the trial and from other sources suggests a) proof beyond reasonable doubt that for all, or for some types of patient, the trial question has been answered. Criteria for the above, where this trial alone is concerned, will usually be a difference at any stage significant at $p < 0.0002$ (Haybittle-Peto interim stopping rule adjusted for 6 endpoints). Use of these criteria will not materially affect the overall alpha in the final analysis.

The IDMC will however reserve the right to release any data on outcome or side-effects through the Trial Steering Committee to the Trial Management Group (and if appropriate to participants) if it determines at any stage that the combined evidence from this and other studies justifies it.

There are no planned subgroup analyses, but hypotheses may be generated by other studies such as MAPLE. In the absence of prospective hypotheses p-values for interaction will be required to satisfy $p<0.01$ before subgroup results are considered to override overall trial results. This rule will be employed when testing biological/translational hypotheses. Although

the within patient changes (Log (Surgical score/pre-treatment score)) will be compared using the Mann-Whitney test, tests of normality will also be performed.

20.5 Milestones

The EPHOS-B trial is a three group, randomised controlled trial conducted in two parts.

In Part 1 of the trial HER2+ patients were allocated in a 1:2:2 ratio to control, perioperative trastuzumab only or perioperative lapatinib only (EPHOS-B protocol Version 1-4). In December 2012 Cancer Research UK, Clinical Trial Awards and Advisory Committee confirmed support of an amendment to replace the lapatinib monotherapy group of the trial with a lapatinib combination group on the basis of the evidence that has emerged since the original application was submitted in 2007.

Part 1 of the trial will therefore be superseded by Part 2 following the approval of amendment 5 by West Midlands - Edgbaston NRES Committee on 15/05/2013 and subsequently in each centre. In Part 2 of the trial HER2+ patients are allocated in a 1:1:2 ratio to control, perioperative trastuzumab only or and the combination of lapatinib and trastuzumab

It is anticipated that approximately 125 patients will be randomised into Part 1 and Part 2 of the trials respectively and that 250 patients will be recruited into this trial by the end of 2014.

20.6 Data Sharing

Collaboration will be sought with other trialists i.e. The Breast Cancer International Research Group BCIRG. We will also approach MAPLE trialists so that the lapatinib versus nil pre surgical data in HER2+ patients from the two trials can be combined (possibly stratified by trial). It is recognised that we share a common interest with these groups and that biological changes seen in these trials may support subgroup analyses in adjuvant trials such as ALLTO.

The Trial Management Group will aim to adhere to the principles set out in the NCRI data sharing policy. We aim to report the results according to the recently agreed REMARK guidelines [41](38) and the NCRI Bioinformatics guidelines (www.cancerinformatics.org.uk) in order to facilitate data sharing and validation.

21. Study Organisation

21.1 Trial Management Group

A Trial Management Group (TMG) will be set up and will include the Chief Investigator, co-investigators and identified collaborators, the trial statistician and the trial co-ordinator(s). Principal Investigators and key study personnel will be invited to join the TMG as appropriate to ensure representation from a range of centres and professional groups. Notwithstanding the legal obligations of the Co-Sponsors and Chief Investigator, the TMG have operational responsibility for the conduct of the trial. Where possible membership will include a lay/consumer representative. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU and based on MRC Good Clinical Practice (MRC GCP).

21.2 Trial Steering Committee (TSC)

The trial will be monitored by the generic ICR-CTSU Breast Systemic Therapy Trials Steering Committee (TSC) The TSC will meet at least annually although there may be periods when more frequent meetings are necessary. It is the role of the TSC to monitor progress of the

trial and to ensure there is adherence to the protocol and the principles of Good Clinical Practice. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU and based on MRC Good Clinical Practice (MRC GCP).

22. Data and Sample Acquisition

CRF completion guidelines are contained in the Trial Guidance Notes. The Trial Management Group reserves the right to amend or add to the CRFs as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by centres in accordance with the guidelines provided by ICR-CTSU.

Should a patient be discharged to GP care or have their care transferred to another hospital, all reasonable attempts should be made by the randomising hospital to collect follow up information from these sources and send it to the trials office at the prescribed time points laid down in the Trial Guidance Notes. Should the GP or other hospital be unable to provide this information, the Trials Office should be informed.

On receipt at ICR-CTSU, completed CRFs will be reviewed for data anomalies and their receipt recorded. Any data queries arising from initial review will be sent to the relevant centre for resolution. Following initial review, data items from the CRFs will be entered centrally into the clinical study database at ICR-CTSU.

ICR-CTSU will be responsible for monitoring transfer and receipt of biological specimens. Tracking forms will be sent by centres to ICR-CTSU to monitor the transfer of all biological samples. The Paterson Institute for Cancer Research will notify ICR-CTSU of all samples received from centres. All data will be handled, computerised and stored in accordance with the Data Protection Act 1998.

23. Patient Protection and Ethical Considerations

The study was approved by West Midlands Research Ethics Committee. Before entering patients, the Principal Investigator at each site is responsible for gaining Site Specific Assessment and Research and Development approval of this protocol.

Patients should be asked to sign the trial consent form after receiving both verbal and written information about the trial. All consent forms must be countersigned by the Principal Investigator or a designated individual. A record listing the designated individuals and the circumstances under which they may countersign consent forms must be clearly documented at the research site as part of the Delegation of Responsibilities Log. This log, together with original copies of all signed patient consent forms, should be filed in the EPHOS-B site file and must be available for inspection.

The EPHOS-B patient information sheet should be provided in addition to any standard patient information sheets that are provided by the centre and used in routine practice.

23.1 Liability

Indemnity for participating hospitals is provided by the usual NHS indemnity arrangements.

23.2 Patient Confidentiality

Patients will be asked to consent to their full name being collected at randomisation in addition to their date of birth, hospital number and NHS number (CHI in Scotland) (to allow tracing through the GP and national records to assist with long term follow up). The personal

data recorded on all documents will be regarded as confidential, and any information which would allow individual patients to be identified will not be released into the public domain.

Each investigator should keep a separate log of all patients' Trial IDs, names, addresses and hospital numbers. The investigator must maintain trial documents, which are to be held at the participating centres (e.g. patients' written consent forms), in strict confidence. The investigator must ensure the patients' confidentiality is maintained.

ICR-CTSU will maintain the confidentiality of all patients and will not reproduce or disclose any information by which patients could be identified. Representatives of the ICR-CTSU and the regulatory authorities will be required to have access to patients notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times. In the case of special problems and/or competent authority queries it is also necessary to have access to the complete study records provided that patient confidentiality is protected.

23.3 Data Protection Act (DPA)

ICR-CTSU will comply with all aspects of the DPA 1998. Any requests from patients for access to data about them held at ICR-CTSU should be directed to the Trial Coordinator in the first instance, who will refer the request to the Data Protection Officer at The Institute of Cancer Research.

24. Withdrawal of Patients from Trial Treatment

Patients who do not receive their allocated treatment for any reason should be treated at the discretion of their clinician. Unless the patient requests otherwise, all CRFs, including long term follow-up, should be completed, regardless of treatment actually received, as analyses of all outcome efficacy data will be on the basis of intention to treat (i.e. all randomised patients). A trial deviation form should be completed to record details of deviation from treatment allocation. Patients are asked prior to randomisation to consent to follow-up should they withdraw from their allocated treatment (see patient information sheet and consent form), and any patient unwilling to give that assurance prior to trial entry should not be randomised. Patients are however free to reverse that decision at any time without giving a reason (see below).

24.1 Withdrawal of Patients from Trial Follow-Up

A trial deviation form should be completed in the unlikely event that the patient withdraws consent for further follow-up data to be collected. If this situation is suspected, clarification should be sought to ensure that the patient is not simply withdrawing from allocated treatment (as above). In the extremely unlikely event that the patient wishes to have their data removed from the trial completely (the implications of this should be discussed with the patient to ensure that this is their intent) this should be indicated as such on the trial deviation form.

25. Research Governance

25.1 Trial Administration & Logistics

The co-sponsors are The Institute of Cancer Research (ICR) and University of Manchester/ Manchester University NHS Foundation Trust, the Chief Investigator's host institution. Sponsorship activities and delegated responsibilities are shared between ICR and University of Manchester/ Manchester University NHS Foundation Trust, in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended and in line with the

UK Policy Framework for Health and Social Care and the principles of GCP. Both parties agree to allow inspection of their premises by the competent authorities. The responsibilities of the co-sponsors are set out in an agreement letter between ICR and University of Manchester/ Manchester University NHS Foundation Trust**.** . The responsibilities of the participating site are defined in an agreement between the individual participating centre and the co-sponsors.
ICR is responsible for administering funding and co-ordinating any required legal agreements and investigator statements.

The delegation of sponsorship responsibilities does not impact on or alter standard NHS indemnity cover. The agreement of delegated responsibilities is viewed as a partnership and as such it is necessary to share pertinent information between ICR and the Chief Investigator, including proposed inspections by the MHRA and/or other regulatory bodies.

25.2 Protocol Compliance

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031). It will be conducted in compliance with the protocol, the Data Protection Act (Z6364106) and other regulatory requirements as appropriate.

Before activating the trial, participating centres are required to sign an agreement accepting responsibility for all trial activity which takes place within their centre.

Sites may commence recruitment once centre agreements have been signed by both parties, trial documentation is in place and a site initiation (visit or teleconference) has taken place. Site initiation visits will be conducted at sites where the Principal Investigator has requested one or where ICR-CTSU deems it is appropriate. Site initiation visits are not considered necessary for sites where staff have attended the Investigator Launch Meeting.

25.3 Local Data Quality Assurance and On-Site Monitoring

On-site monitoring, or auditing, will be based on a risk-based strategy. ICR-CTSU staff may visit centres to confirm that agreements are being adhered to, specifically to carry out source data verification and confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki 1964 as amended October 1996. By participating in the EPHOS-B trial, the Principal Investigators at each centre are confirming agreement with his/her local NHS Trust to ensure that:

- sufficient data is recorded for all participating patients to enable accurate linkage between hospital records and CRFs;
- source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit visits;
- all staff at their centre who are involved with the trial will meet the requirements of the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031). original consent forms are dated and signed by both patient and investigator and are kept together in a central log together with a copy of the specific patient information sheet(s) given at the time of consent;
- copies of CRFs are retained for 15 years to comply with international regulations; and
- staff will comply with the protocol and Trial Guidance Notes for the EPHOS-B trial.

25.3.1 Central Data Monitoring

ICR-CTSU will monitor receipt of CRFs and evaluate incoming CRFs for compliance with the protocol, inconsistent or missing data.

Data monitoring will primarily be conducted using central statistical monitoring, and any systematic inconsistencies identified may trigger monitoring visits to centres. Further monitoring visits may be conducted at the request of participating centres, or by random selection. Monitoring visits that include source data verification will be conducted at a random sub-set of participating centres during the follow-up phase of the trial, and the extent and timing of this exercise will be influenced by central statistical monitoring.

Site monitoring will be conducted at a proportion of participating centres at least once during the course of the trial. If a monitoring visit is required ICR-CTSU will contact the centre to discuss dates of proposed visit. Once a date has been confirmed a list of patients whose notes will be monitored during the visit will be sent to the centre. This list will be sent out in advance to give sufficient time for the notes to be made available. The Trial Statistician will advise what percentage of patients is to be monitored.

If any problems are detected in the course of the monitoring visits then the Principal Investigator ICR-CTSU and the Chief Investigator (where required) will work together to resolve issues and, if necessary, to determine the centre's future participation in the study.

25.4 Completion of the Study and Definition of Study End Date

The study end date is deemed to be the date of last data capture.

25.5 Archiving

Essential documents are documents that individually and collectively permit evaluation of the conduct of the trial and substantiate the quality of the data collected. Essential documents will be maintained at ICR-CTSU and at the Investigator Sites in a way that will facilitate the management of the trial, audit and inspection. They should be retained for a sufficient period (at least 15 years) for possible audit. Documents should be securely stored and access restricted to authorised personnel.

25.6 Financial Matters

The trial is investigator designed and led and has been approved by Clinical Trials Advisory & Awards Committee (CTAAC) of Cancer Research UK, and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

The trial has received funding from Cancer Research UK. Additional financial support for one ECHO / MUGA per patient has been received from Novartis (formerly from GSK). If further funding is received from any other source this will be made apparent in the patient information sheet and to the approving Main REC and CTAAC, but will not require a protocol amendment.

NCRN (or regional equivalent) network resources should be made available for EPHOS-B, as the trial is part of the NCRI portfolio by virtue of its approval by CTAAC.

26. 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 Trial Management Group, and high accruing clinicians. All participating centres and clinicians will be acknowledged in this publication together with staff from the ICR-CTSU. All presentations and publications relating to the trial must be authorised by the

Trial Management Group, on whose behalf publications should usually be made. Authorship of any secondary publications e.g. relating to the various biological studies will reflect the intellectual and time input into these studies, and will not be the same as on the primary publication. No investigator may present or attempt to publish data relating to the EPHOS trial without prior permission from the Trial Management Group.

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Appendix 1: Potential Drug Interactions & Prohibited Medication list

Potential Drug Interactions:

Lapatinib is a substrate for CYP3A4. Inducers and inhibitors of CYP3A4 may alter the metabolism of lapatinib. The list of CYP3A4 inducers and inhibitors below are prohibited from screening through discontinuation from study. Additionally, there may be potential interaction between GW572016 and warfarin. Patients have experienced elevated INRs and bleeding with warfarin and quinazolines. Patients on warfarin and GW572016 should have more frequent INR/PT determinations after starting GW572016 (e.g. weekly for the first month and weekly for a minimum of 2 weeks following discontinuation of GW572016).

GW572016 Prohibited Medication List

Gastric pH Modifiers. GSK is no longer prohibiting gastric pH modifiers (i.e. H2 blockers and PPIs). This is based on collection of concurrent medication use and the observation that PK was no different between patients on gastric pH modifiers and those not taking gastric pH modifiers.

Lapatinib is a substrate for CYP3A4. Inducers and inhibitors of CYP3A4 may alter the metabolism of lapatinib. The following list of CYP3A4 inducers and inhibitors are prohibited from screening through discontinuation from study. Additionally, medications that modify gastric pH are included in the table below:

Drug Class	**Agent**	**Wash-out[1]**
CYP3A4 Inducers		
Antibiotics	all rifamycin class agents (e.g., rifampicin, rifabutin, rifapentine)	14 days
Anticonvulsants	phenytoin, carbamezepine, barbiturates (e.g., phenobarbital)	
Antiretrovirals	efavirenz, nevirapine	
Glucocorticoids[2] (oral)	cortisone (>50 mg), hydrocortisone (>40 mg), prednisone (>10 mg), methylprednisolone (>8 mg), dexamethasone (>1.5 mg)[2]	
Other	St. John's Wort, modafinil	
CYP3A4 Inhibitors		
Antibiotics	clarithromycin, erythromycin, troleandomycin	7 days
Antifungals	itraconazole, ketoconazole, fluconazole (>150 mg daily), voriconazole	
Antiretrovirals, Protease Inhibitors	delaviridine, nelfinavir, amprenavir, ritonavir, indinavir, saquinavir, lopinivir	
Calcium channel blockers	verapamil, diltiazem	
Antidepressants	nefazodone, fluvoxamine	
GI Agents	cimetidine, aprepitant	

Other	grapefruit, grapefruit juice	
	amiodarone	6 months
Miscellaneous		
Antacids	Mylanta, Maalox, Tums, Rennies	Excluded 1 hour before and after dosing
Herbal or dietary supplements	All	14 days[3]

1. At the time of screening, if a patient is receiving any of the above listed medications/substances, the medication or substance must be discontinued (if clinically appropriate) for the period of time specified prior to administration of the first dose of lapatinib and throughout the study period in order for the patient to be eligible to participate in the study.
2. Glucocorticoid daily doses (oral) $\leq$ 1.5 mg dexamethasone (or equivalent) are allowed. Glucocorticoid conversions are provided in parentheses.
3. Should ideally be stopped from the start of screening but may be discontinued up to the day before randomisation.

Appendix 2: List of Abbreviations

AC	doxorubicin (Adriamycin®) and cyclophosphamide
ATP	Adenosine Triphosphate
BASO	British Association of Surgical Oncology
CI	Chief investigator
CRF	Case Report Form
CTA	Clinical Trials Authorisation
CTAAC	Clinical Trials Advisory and Awards Committee
CXR	Chest X-Ray
DCIS	Ductal Carcinoma in Situ
DFS	Disease Free Survival
DNA	DeoxyriboNucleic Acid
DPA	Data Protection Act
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor
ER	Oestrogen receptor
ErbB	Epithelial Growth Factor Receptor
EU	European Union
FISH	Fluorescence In Situ Hybridisation
FBC	Full Blood Count
GCP	Good Clinical Practice
GP	General Practitioner
HER2	Human Epidermal Growth Factor Receptor 2
H&E	Haemotoxylin and Eosin
HRT	Hormone Replacement Therapy
HTA	Human Tissue Authority
ICR-CTSU	The Institute of Cancer Research Clinical Trials and Statistics Unit
IDMC	Independent Data Monitoring Committee
IGFR	Insulin like growth receptor
IHC	Immunohistochemistry
IMPs	Investigational Medicinal Products
ISRCTN	International Standard Randomised Controlled Trial Number
LFTs	Liver Function Tests
LVEF	Left Ventricular Ejection Fraction
MAPK	Mitogen-Activated Protein Kinase
Main REC	Main Research Ethics Committee
MHRA	Medicines & Healthcare products Regulatory Agency
MUGA	Multi-Gated Acquisition
NCRI	National Cancer Research Institute
NCRN	National Cancer Research Network
NIHR	National Institute for Health Research
OS	Overall Survival
p-Tyr	Phosphorylated tyrosine

p.o.	Route: Oral (by mouth)
RFS	Relapse Free Survival
RNA	RiboNucleic Acids
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SERMs	Selective Oestrogen Receptor Modulators
SUSAR	Suspected Unexpected Adverse Reaction
SDV	Source Data Verification
SNB	Sentinel Node Biopsy
SUSAR	Suspected Unexpected Serious Adverse Reaction

List of Abbreviations

TGF	Transforming Growth Factor
TMG	Trial Management Group
TSC	Trial Steering Committee
VEGF	Vascular Endothelial Growth Factor

Appendix 3: Biological Studies

Markers of Proliferation and Apoptosis

At diagnosis (pathway A) and at surgery: 2 core cuts (14-gauge) should be collected*. One should be placed into formalin and one should be placed in RNA-*later*® in the pots provided. All samples should be sent to the local histopathology department where all of the formalin fixed cores will be embedded in paraffin wax.

* or immediately before randomisation for pathway B patients.

IHC for the Ki67-proliferation associated antigen will be carried out on sections of the routinely processed material following heat mediated antigen retrieval and with the appropriate primary antibodies (MIB-1 anti-Ki67 antibody supplied by DAKO)[1, 3, 13]. Apoptotic cells will be detected also in sections of the routine processed material using the activated caspase 3 immnohistochemistry.

In both procedures, an insoluble brown precipitate is produced in cells expressing the antigen or which are undergoing apoptosis, which allows quantification of positive versus negative cells using light microscopy. Sections will be scored if the initial H & E stained section shows invasive cancer with clearly identifiable malignant epithelial cells and/or invasive tumour. Fields for scoring will be selected out of focus at low power and cells at x400 magnification with a graticule and cell counter. The presence of positive or negative staining will be noted and the number of stained cells counted per 1,000 cells. At least 1,000 invasive cancer cells will be counted. If fewer than 1,000 cells are present in a given section, additional serial sections will be cut from the same block until the desired number of cells can be counted. The percentages of positive cells will then be calculated for invasive components[3, 42, 43]. Activated Caspase 3 will be measured pre-treatment, before and after surgery, as previously described[44].

Expression of ERα, PR and -HER2, will be determined by IHC on sections of routinely processed pre- and post-treatment samples using similar methods to those described above. Invasive tumour ERα and PRα content will be expressed as a percentage of the total cells counted after scoring at least 1000 cells whereas a semiquantitative scale (0-3+) based on the proportion of positively stained cells and the intensity of staining will be used to define HER2 expression[31, 40, 43].

Measurement of Circulating Biomarkers of Apoptosis

M30 and M65 are ELISAs that detect different circulating forms of cytokeratin 18 (CK18). M30 detects a neoepitope mapped by two positions (387-396) of a 21kD fragment of CK18 that is only revealed after caspase cleavage of the protein and is postulated as a selective biomarker of apoptotic cell death[45]. M30 levels rise in plasma after drug treatment induced apoptosis, correlating with both the amplitude and timescale of the induction of apoptosis before falling again.

M65 detects a common epitope of CK18 as well as the 21KD caspase cleaved fragment and is thus believed to measure, in addition to apoptosis, intact CK18 that is being released as tumour cells undergo necrosis[45]. M65 levels are initially higher than M30 levels and correlate with tumour growth kinetics, falling in response to treatment and rising on progressive tumour growth. M65 is stable in plasma for 2 years whereas M30 levels increase in a proportion of patients after 6 months storage.

In parallel to measuring tumour Ki67 and CC3 changes in response to therapy, we intend to measure M30, M65 circulating apoptosis assays to assess whether these assays validated in CEP are circulating markers of apoptosis and necrosis, predict changes in the tumour and allow early identification of responding tumours to trastuzumab or lapatinib.

Angiogenic Markers

Microvessel counts in tumours from women with invasive cancer will be visualised by immunostaining tumour sections with antibodies against CD31. This technique has been well reported by us in reference[2]. Microvessel density will be quantified by light microscopy of labelled slides without knowledge of patient details. The most vascular areas in the tumour (i.e. the hot spots) will be located at low magnification and the vessels in these regions countered with the use of a Chalkley point eyepiece graticule at x400 magnification. Any brown staining endothelial cells or group of cells in contact with the spot in the graticule will be counted as an individual vessel. The mean of four Chalkley counts for each tumour will be calculated and used in statistical analysis. Levels of CD31 between tumours will be compared on the operative specimen and correlated with serum VEGF R1 and VEGF-A and CD105.

Measurement of Circulating Biomarkers of Angiogenesis

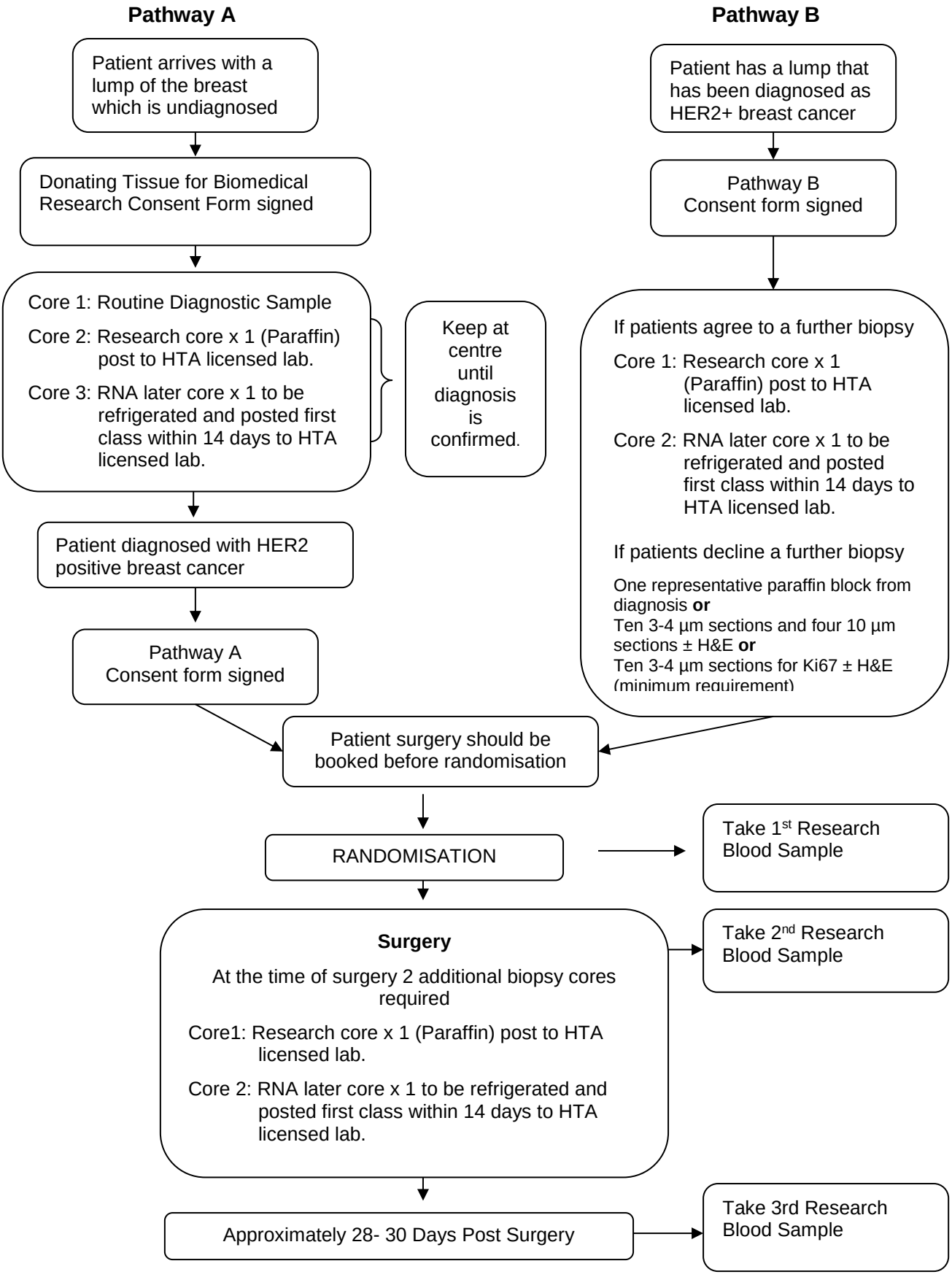
Several validated circulating biomarkers (in CEP) of angiogenesis (VEGF R1, VEGF R2, VEGF A, Ang1, Ang2, sTie2, PIGF, CD105) will be evaluated and compared to changes in tissue apoptosis and proliferation. Serum markers will be measured at baseline, at 14 days (surgery) and at 28-30 days after surgery. Samples would be stored at -20^{o}C and measured by ELISAs in a central laboratory.

3 blood samples will be collected by venepuncture at randomisation, within 24 hours prior to surgery and 28-30 days post surgery. The same 3 assays will be assayed, CD31 will be assayed using validated ELISAs in the clinical experimental pharmacological laboratory at the Paterson Institute for Cancer Research (as detailed in their SOPs). Serum VEGF-A will be measured on platelet depleted serum. The Trial Management Group reserves the right to amend or add to the CRFs as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by centres in accordance with the guidelines provided by ICR-CTSU.

Biological substudies

Additional funding will be sought to facilitate collection of material and preparation of tissue microarrays. Subsequent scientific studies will require additional research grant funding, and material from the EPHOS biobank will be made available to researchers both within the trial framework and outside. A pre-requisite for use of material will be supply of information back to the Trial Management Group on a case-by-case basis for inclusion in the trial database.

Appendix 4: Biological Centres - Patient Pathways "A" and "B"



Appendix 5: Tissue Sample & Blood Collection

A: The following samples will be collected:

1. At diagnosis (pathway A) or immediately after consent but before randomisation (pathway B): 2 core cuts (14-gauge) should be collected ***in addition*** to those taken for diagnostic purposes. One should be placed into formalin and one should be placed in RNA-*later*® in the pots provided. All samples should be sent to the local histopathology department where all of the formalin fixed cores will be embedded in paraffin wax:

a. ***For Pathway A*** – once it is confirmed that tissue taken for research will not be required to confirm diagnosis, the sample in RNA-*later*® and the paraffin block, without prior sectioning, should be sent to the Paterson Institute for Cancer Research. Any laboratories that hold an HTA licence and have their own established tissue bank with ethics committee approved consent procedures may, following prior agreement with the Chief Investigator and biological studies lead investigator, store samples locally until the patient enters EPHOS.

b. ***For Pathway B*** - the sample in RNA-*later*® and the paraffin block, without prior sectioning, should be sent to the Paterson Institute for Cancer Research. These samples may be sent together with samples taken at surgery (see below).

c) For Pathway C - **Non Biological Centres**

Non Biological centres are not required to provide samples in RNA-*later*®. Paraffin embedded tissue must be available from diagnostic tissue already taken at diagnosis and during surgery.

Centres who wish to participate in EPHOS-B as a non biological centre must routinely take ≥ 3 cores of tissue at diagnosis.

2. At randomisation: 3 blood samples should be taken; (1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum) .

3. Up to 24 hours before surgery: 3 blood samples - (1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum)

4. At surgery: 2 core-cuts (14-gauge) should be taken immediately the tumour has been excised. One core-cut should be placed into formalin and one should be placed in RNA-*later*® in the pots provided. The 2 samples should be sent to the local histopathology department where the formalin fixed core will be embedded in paraffin wax.

5. At first follow-up visit after surgery: 3 blood samples: (1x 6ml EDTA, 1x 8.5ml PAXgene tube, and 1x 5ml serum)

All tissue samples should be posted to the Paterson Institute for Cancer Research unless they are stored locally and alternative arrangement have been agreed with the Biological Studies Lead Investigator (Professor Andrew Hanby). Kits for the collection of samples in RNA-*later*® are provided, and instructions on packaging, labelling and tracking provided in the Trial Guidance Notes should be followed.

All blood samples should be posted to the Paterson Institute for Cancer Research on the same day the sample is taken. Blood kits are provided, and instructions on packaging, labelling and tracking provided in the Trial Guidance Notes should be followed.

Patient consent procedures provided in the Trial Guidance Notes should be followed.

B. Sample analysis:

The formalin fixed-cores will be sectioned at the central laboratory (for the analysis of the proliferation marker, Ki67, by immunohistochemistry. Other sections will be stored after coating with paraffin wax for assessment of additional biomarkers that are candidates as being involved in response or resistance to hormonal treatment or in other aspects of breast cancer biology.

The cores contained in RNA-*later*® will be analysed for RNA profiles and/or changes in the DNA of the tumour.

On receipt, EDTA-preserved blood samples will be centrifuged and the plasma stored frozen for the analysis of oestrogen levels. Residual plasma will be stored frozen for the analysis of biomarkers that may be related to the efficacy of treatment or the prognosis of the patient.

The PAXgene-preserved blood sample will be stored for future analysis of germ-line DNA in relation to disease outcome, biological response (e.g. change in Ki67) to the aromatase inhibitor or tolerability of treatment.