Neoadjuvant trial of pre-operative exemestane or letrozole +/-celecoxib in the treatment of ER positive postmenopausal early breast cancer

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CHIEF INVESTIGATOR SIGNATURE PAGE

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Date: _____

PROTOCOL AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version

Amendment number	Date of amendment	Protocol Version number	Type of amendment (e.g. (non)substantial/administrative change)
01	19-Jan-2007	2.0	Substantial Amendment
02	21-Jun-2007	N/A-Changes to Clinical Trial Agreement	Substantial Amendment
03	25-Sep-2009	3.0	Substantial Amendment
04	01-Feb-2010	4.0	Substantial Amendment
05	10-Sep-2010	5.0	Substantial Amendment
06	24-Feb-2012	6.0	Substantial Amendment
07	10-Apr-2013	7.0	Substantial Amendment
08	05-May-2014	N/A-Change in Principal Investigator	Substantial Amendment
09	30-Nov-2017	8.0 Changes to End of Trial Definition, Chief Investigator and Trial Management Group	Substantial Amendment

GENERAL INFORMATION

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8. 1. KEY CONTACT DETAILS

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Clinical Trial Supplies

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Refer to NEO-EXCEL Pharmacy File/Investigator Site File for full pharmacy instructions and codebreak procedure.

2. TRIAL SUMMARY

ACRONYM: NEO-EXCEL
TITLE: A neoadjuvant trial of pre-operative exemestane or letrozole +/-celecoxib in the treatment of oestrogen receptor (ER) positive postmenopausal early breast cancer.

TRIAL OBJECTIVES
To determine whether the activity of aromatase inhibitors as primary neo-adjuvant endocrine therapy for early stage ER positive breast cancer in postmenopausal women may be enhanced by the addition of the selective COX 2 inhibitor celecoxib.

TRIAL DESIGN
Prospective phase III, multicentre, randomised clinical trial, with placebo-controlled comparisons.

OUTCOME MEASURES

Primary
- Objective clinical response (Complete Response (CR), Partial Response(PR)) to neoadjuvant treatment

Secondary
- Objective ultrasound-determined response (CR, PR) to neoadjuvant treatment
- Type of surgery (mastectomy, breast conserving surgery)
- Axillary lymph node involvement at surgery
- Complete pathological response
- Local recurrence-free survival
- Progression-free survival
- Overall survival

Translational sub-study (Optional)
- Biological profiling for prognostic and predictive indicators

SAMPLE SIZE
256 subjects

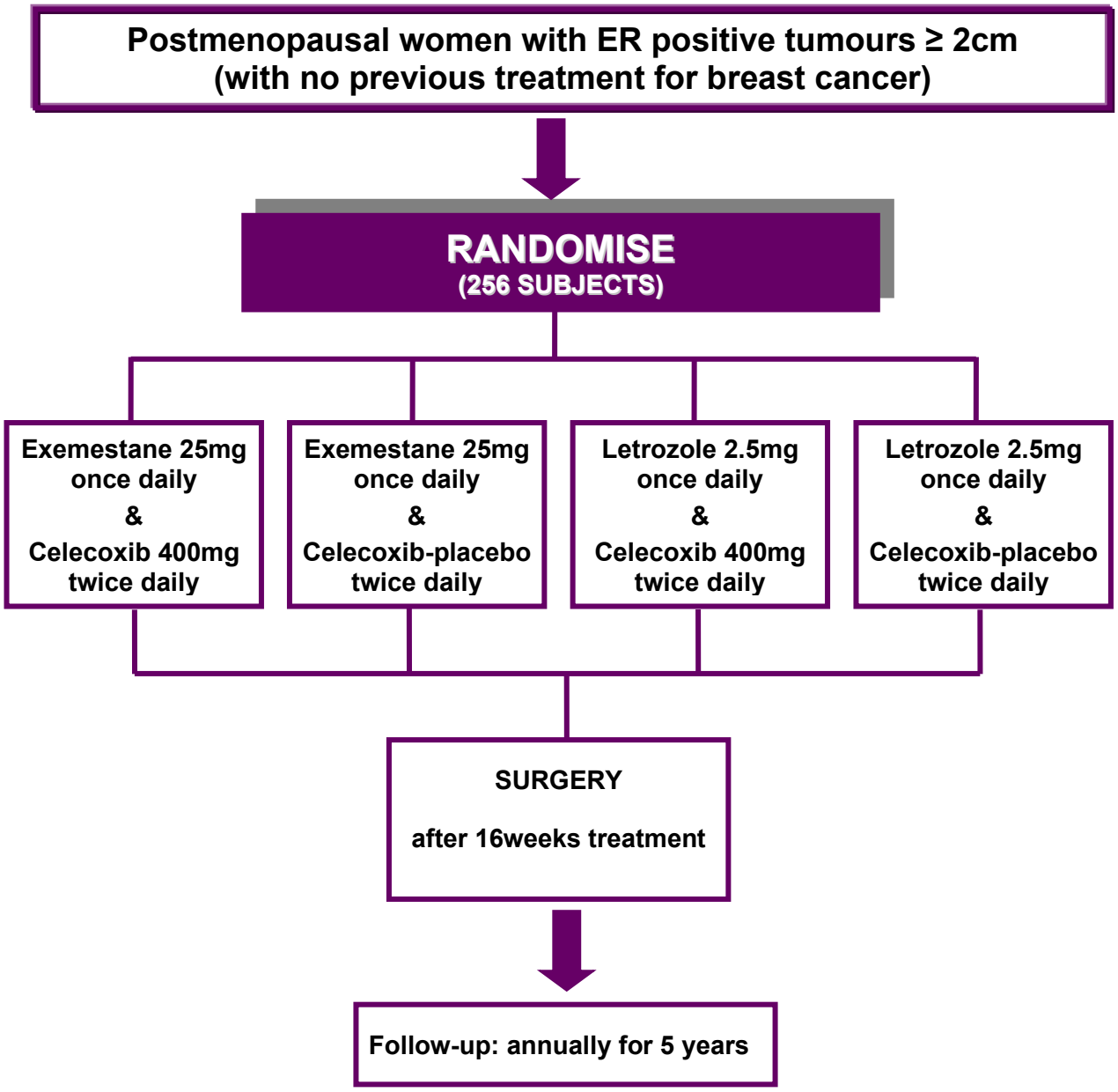
MAIN SELECTION CRITERIA
Women with a histological diagnosis of early invasive breast carcinoma, meeting the following criteria:

- Postmenopausal
- ER positive
- Breast tumour ≥ 2cm by clinical evaluation
- No previous treatment for breast cancer
- Adequate haematological, renal and hepatic function
- ECOG performance status 0, 1 or 2

RANDOMISATION
(9:00am till 5:00pm, Monday to Friday)

☎ 0800 371 969
🖷 0121 414 7989

Trial Schema



3. BACKGROUND AND INTRODUCTION

3.1 Current surgical treatment for postmenopausal early breast cancer

The management of early stage breast cancer remains sub-optimal. Standard treatment is primary surgery and subsequent selective adjuvant radiotherapy, hormone therapy and chemotherapy. Larger tumours and those close to the nipple are usually treated by mastectomy whilst smaller more peripheral tumours are often suitable for breast conserving surgery. There is good evidence that body image is just as important to postmenopausal women as it is to younger patients[1]. Neoadjuvant chemotherapy, usually offered to younger patients, can achieve overall survival identical to post-operative chemotherapy but with the additional benefit of less radical surgery[2-4]. Postmenopausal patients are not routinely given the opportunity of neoadjuvant chemotherapy to downstage their tumours and allow less disfiguring surgery because of concerns about the associated toxicity. Furthermore the large majority of these patients have oestrogen receptor-positive tumours and may thus be less likely to respond well to chemotherapy. However, the proven ability of aromatase inhibitors to downstage these tumours[5] pre-operatively, without the toxicity of chemotherapy, is currently infrequently utilised and is the subject of this study.

3.2 Aromatase inhibitors and breast cancer

Aromatase inhibitors are administered systemically to inhibit oestrogen synthesis in tissues by the aromatase enzyme which catalyses the conversion of androgens to oestrogens. Aminoglutethimide is a first-generation aromatase inhibitor and although effective as treatment for advanced disease and active as an adjuvant therapy in breast cancer,[6] it was poorly tolerated and was partially replaced by the better tolerated second-generation aromatase inhibitor formestane. However the drawbacks of this compound were that not only did it require parenteral administration, but it only suppressed oestradiol to 1/3 of baseline levels[7,8]. The current third-generation aromatase inhibitors fall into two categories, irreversible steroidal type I inhibitors such as exemestane and non-steroidal type II inhibitors such as letrozole and anastrozole. These drugs are far more potent than their predecessors in terms of oestradiol reduction. They are orally administered, and are in many respects better tolerated than tamoxifen[9,10].

There is increasing and compelling evidence to indicate that third-generation aromatase inhibitors are more effective than tamoxifen in the advanced and adjuvant setting. There is also accumulating evidence that aromatase inhibitors are superior to tamoxifen as pre-operative therapy.

Data from the Anastrozole, Tamoxifen Alone or in Combination (ATAC) trial demonstrates that anastrozole has a relapse-free survival benefit over tamoxifen in the adjuvant setting, with generally improved tolerability[11-13]. Exemestane has demonstrated superior efficacy compared to tamoxifen when introduced half way through a 5-year program of adjuvant hormone therapy[14]. Toxicity differences are similar but not identical to those seen in the ATAC study, with fewer gynaecological symptoms and a reduced incidence of endometrial cancer. However aromatase inhibitors are associated with a higher incidence of arthralgia and bone mineral density loss when used over long periods. Preliminary reports now indicate that letrozole is also more active than tamoxifen in the adjuvant setting with a similar hazard ratio. (B. J. Thurlimann, A. Keshaviah, H. Mouridsen, L. Mauriac, J. F. Forbes, R.

3.2.1 Neoadjuvant aromatase inhibitor use in breast cancer

Two randomised Phase III trials have compared pre-operative tamoxifen with an aromatase inhibitor. The P024 trial randomised 337 postmenopausal women with ER positive tumours to 16 weeks neoadjuvant letrozole (2.5mg/day) versus tamoxifen (20mg/day)[15]. The primary endpoint was to compare overall objective response (CR+PR). Secondary endpoints included rates of breast conserving surgery. Results clearly showed letrozole to be significantly more effective than tamoxifen in respect of objective response (55% versus 36%, $p<0.001$). Clinically determined complete response rates were 10% for letrozole and 4% for tamoxifen. Letrozole was at least as well tolerated (Table I). Letrozole permitted significantly more breast conserving surgery (45% versus 35%, $p=0.022$). Clinical response rate differences were confirmed by ultrasound and mammographic response assessments (35% versus 25% and 34% versus 16% respectively). In this study, pre-treatment biopsies were analysed and two potentially important correlations of response and molecular features have been reported[16]. Tumours expressing either HER-1 or HER-2 (ErbB-1/Erb-B2) or both markers were associated with poor response to tamoxifen, 4/19 (21%).This difference was statistically significant $p=0.004$ compared to 15/17 (88%). Response rates in tumours negative for both markers were 42/100 (42%) for tamoxifen and 55/101 (54%) for letrozole, which were not significantly different. The other observation was a higher response rate in tumours with higher levels of ER scored by Allred score, irrespective of treatment allocation. This appeared most striking for tamoxifen, with no responses seen with scores below 6, but responses were observed with letrozole in tumours with Allred scores as low as 3. This relationship was however not statistically different with small numbers of tumours in the low Allred score groups. In case series where only strongly positive tumours have been treated, no clear patterns of differential sensitivity between the aromatase inhibitors have emerged[17]. In any new large study of neoadjuvant therapy, central review of the quantitative steroid hormone receptor expression is therefore critical.

The IMPACT trial randomised 330 women and compared 12 weeks of anastrozole with 12 weeks of tamoxifen and the combination of both agents as neoadjuvant therapy[18]. The overall response rate was 37.2 % for anastrozole, 36.1% for the combination and 39.4% for tamoxifen. In larger tumours where pre-treatment mastectomy was thought to be required anastrozole was associated with a higher probability of downstaging to permit breast conservation. HER-2 expression was observed to predict low response to tamoxifen, with maintained response to anastrozole. However the sample size was small and statistically unreliable. As the P024 and IMPACT studies involved different treatment durations and different inclusion criteria it is not possible to make any firm conclusions from cross comparisons, but the clear difference in response rates between tamoxifen and anastrozole is encouraging.

Another study, the PROACT trial, randomised 451 women to receive 12 weeks of anastrozole or tamoxifen treatment before surgery in a double-blind trial in which some patients also received chemotherapy. While the presence of chemotherapy has somewhat complicated the interpretation of this study, anastrozole use was associated with a higher rate of breast conserving surgery over tamoxifen.

Exemestane has been less extensively evaluated as a neoadjuvant therapy. Recently reported studies include a multicentre French trial in which 38 ER positive postmenopausal women requiring mastectomy pre-treatment were treated with exemestane 25 mg/day for 16 weeks. The overall response rate was reported as 70.6%, with 45.2% of women able to undergo breast conserving surgery[19].

The Edinburgh group have reported a single centre randomised Phase II trial comparing tamoxifen with exemestane[20]. They report an 83% response rate (10/12 patients). More extensive experience is reported by the Russian group who have conducted a randomised Phase II trial. Here 73 postmenopausal women with ER positive tumours were randomised to tamoxifen or exemestane for 12 weeks. Exemestane was reported to have an overall response rate of 69.4% and tamoxifen 40.5%. The rate of breast conservation was 38.7 % for exemestane and 10.8 % for tamoxifen[21]. This group has also reported a further randomised Phase II trial comparing neoadjuvant chemotherapy, using doxorubicin and paclitaxel, against neoadjuvant endocrine therapy using anastrozole or exemestane. One hundred and forty six postmenopausal women with ER or PgR positive large primary breast tumours were randomised. Overall response was similar in all three groups; chemotherapy 76%, anastrozole 75.6% and exemestane 81.5%. Breast conserving surgery was more common in the endocrine treated group, 34% versus 24%, p=0.058. Endocrine therapy was clearly better tolerated in this study, and low toxicity has been observed in all studies of neoadjuvant exemestane[22].

3.3 COX 2 inhibitors and breast cancer

The potential for cyclo-oxygenase (COX) inhibition in cancer prevention and treatment is founded on epidemiology (reduction of colorectal cancers in aspirin users), animal experiments and molecular genetics. Non-steroidal anti-inflammatory drugs (NSAIDS) block endogenous prostaglandin synthesis from arachidonic acid through inhibition of cyclo-oxygenase enzyme activity, primarily that of COX 2. COX 2 is frequently overexpressed in tumours and is inducible by various agents such as growth factors and tumour promoters.

The role of COX 2 in carcinogenesis is thought to be related to its abilities to increase production of prostaglandins, convert pro-carcinogens to carcinogens, inhibit apoptosis, promote angiogenesis, modulate inflammation and immune function and increase tumour cell invasiveness[23]. The advent of specific COX 2 inhibitors which do not interfere with the cytoprotective constitutive COX I enzyme has opened up new therapeutic possibilities. A review of the role of COX 2 inhibitors in breast cancer[24] provides overwhelming evidence from molecular, animal and cell line studies supporting the ability of COX 2 inhibitors to prevent the development of breast tumours. Administration of increasing doses of the COX 2 inhibitor celecoxib inhibited mammary tumour incidence and multiplicity as well as tumour volume in a dose dependant manner in female Sprague-Dawley rats. The control rats had a higher incidence of tumours ($p<0.001$), higher tumour volume ($p<0.001$) and more tumours ($p<0.001$) than animals receiving celecoxib[25]. The effect of cyclo-oxygenase inhibition on tumour growth has also been studied in breast cancer cells in BALB/c mice. Microvessel density was reduced and tumour cell apoptosis was increased in primary tumours of mice treated with cyclo-oxygenase inhibitors. In vitro, cyclo-oxygenase inhibition decreased vascular endothelial growth factor production and increased apoptosis of cells[26].

Epidemiological evidence shows that long-term use of NSAIDS appears to reduce the risk of developing breast cancer, with a risk reduction of nearly 25% in the most recent studies[27-29]. Celecoxib has been licensed by the FDA for the prevention of colorectal carcinoma in the USA.

COX 2 inhibition enhances the apoptotic effect of chemotherapy[30] and the combination of the two treatments is currently under investigation in Phase III trials in several tumour types. Linkage between the COX 2 and aromatase enzyme systems in malignancy suggest that COX 2 inhibition with aromatase inhibition may also be more effective than either therapy alone.

In a previously reported case controlled study (BMC Cancer 2006) a significant reduction in the risk of human breast cancer due to intake of selective COX 2 inhibitors has been observed. Chemopreventive effects against breast cancer were associated with recommended daily doses of celecoxib (median dose=200 mg) or rofecoxib (median dose=25 mg) for an average duration of 3.6 years. Nevertheless, even in the short window of exposure to these compounds, the selective COX 2 inhibitors produced a significant (71%) reduction in the risk of breast cancer, underscoring their strong potential for breast cancer chemoprevention.

3.4 Aromatase inhibitors with COX 2 inhibitors in breast cancer

COX 2 expression and aromatase expression have been found to correlate in breast cancer tissue[31]. The COX 2 product prostaglandin E2 (PGE2) and cytokines such as interleukin-6 (IL6) can up regulate aromatase expression through interaction with the 1.3 promoter of the aromatase gene[32], with the resulting possibility that aromatase inhibition and COX 2 inhibition treatments may be more effective when prescribed together [33].

The Glasgow group has reported good tolerability and encouraging activity in a Phase II feasibility study of the combination of exemestane and celecoxib in postmenopausal women with ER positive advanced breast cancer[34]. The CAAN trial reported at San Antonio 2003 that exemestane plus celecoxib is well tolerated in patients with locally advanced breast cancer. However, evidence of the therapeutic superiority of this combination compared to exemestane alone as anticancer treatment is currently lacking.

3.5 Celecoxib: clinical pharmacology

Celecoxib is a nonsteroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. The mechanism of action of celecoxib is believed to be due to inhibition of prostaglandin synthesis, primarily via inhibition of COX 2 and at therapeutic concentrations in humans, celecoxib does not inhibit the COX 1 isoenzyme. In animal colon tumour models, celecoxib reduced the incidence and multiplicity of tumours (Pfizer data on file).

3.5.1 Pharmocokinetics

Absorption: Peak plasma levels of celecoxib occur approximately 3hrs after an oral dose. Under fasting conditions, both peak plasma levels (Cmax) and area under the curve (AUC) are roughly dose proportional up to 200 mg twice a day (BID); at higher doses there are

less than proportional increases in Cmax and AUC. Absolute bioavailability studies have not been conducted. With multiple dosing, steady state conditions are reached on or before day 5.

Distribution: In healthy subjects, celecoxib is highly protein bound (~97%) within the clinical dose range. In vitro studies indicate that celecoxib binds primarily to albumin and, to a lesser extent, α1-acid glycoprotein. The apparent volume of distribution at steady state (Vss/F) is approximately 400L, suggesting extensive distribution into the tissues. Celecoxib is not preferentially bound to red blood cells.

Metabolism: Celecoxib metabolism is primarily mediated via cytochrome P450 2C9. Three metabolites, a primary alcohol, the corresponding carboxylic acid and its glucuronide conjugate, have been identified in human plasma. These metabolites are inactive as COX 1 or COX 2 inhibitors. Patients who are known or suspected to be P450 2C9 poor metabolisers based on a previous history should be administered celecoxib with caution as they may have abnormally high plasma levels due to reduced metabolic clearance.

Excretion: Celecoxib is eliminated predominantly by hepatic metabolism with little (< 3%) unchanged drug recovered in the urine and faeces. Following a single oral dose of radiolabeled drug, approximately 57% of the dose was excreted in the faeces and 27% was excreted into the urine. The primary metabolite in both urine and faeces was the carboxylic acid metabolite (73% of dose) with low amounts of the glucuronide also appearing in the urine. It appears that the low solubility of the drug prolongs the absorption process making terminal half-life (t1/2) determinations more variable. The effective half-life is approximately 11 hours under fasted conditions. The apparent plasma clearance (CL/F) is about 500mL/min (Pfizer data on file).

3.5.2 Cardiovascular safety of celecoxib

At the end of 2004, as a result of a small but statistically significant excess of myocardial and cerebrovascular events, in a trial using the COX 2 inhibitor rofecoxib versus placebo as secondary prophylaxis in patients with colorectal polyps, rofecoxib was voluntarily withdrawn from sale. The increased risk did not emerge until patients had been exposed to the drug for more than 18 months and the risk appears to be a chronic effect. The aetiology of the increased cardiovascular risk is as yet unclear and the magnitude of the increased risk is small. The clinical data which prompted the withdrawal of rofecoxib relates specifically to rofecoxib.

There is however a substantial body of evidence supporting the safety of celecoxib:
A pooled analysis of 30,000 patients who completed arthritis trials (including the CLASS and SUCCESS trials) indicates that celecoxib did not increase the incidence of thromboembolic events versus placebo, or in comparison with traditional NSAIDS[35]. In the CLASS trial, a long-term (12 month) prospective study, celecoxib, even at 2-4 times the approved dose for arthritis and pain, was not associated with an increased risk for serious cardiovascular events such as heart attack, stroke or unstable angina compared to non-specific NSAIDS[36].

A recent FDA funded retrospective analysis of 1.4 million patients who were treated with COX 2 inhibitors or traditional NSAIDS showed that celecoxib demonstrated no increase in the relative risk of acute cardiac events when compared with those who had not taken any NSAID for at least 60 days[37].

In a cohort analysis of over 138,000 people aged 65 or over, patients on celecoxib had a significantly less chance of being hospitalised for congestive heart failure than users of traditional NSAIDS and rofecoxib[38].

A retrospective study of more than 54,000 elderly patients published in the journal, Circulation, showed that celecoxib was not associated with an increased risk for acute myocardial infarct compared with rofecoxib, traditional NSAIDS and no NSAID therapy[39].

In December 2004 important safety information was reported from the Independent Data Safety Monitoring Boards (IDSMB) of the US National Cancer Institute monitoring the Adenoma Prevention with Celebrex (APC) and the Prevention of Spontaneous Adenomatous Polyps (PreSAP) trials. Results from a third long-term celecoxib study, a US National Institutes Aging of Alzheimer's Prevention trial (ADAPT), were also recently reported. The results of these 3 studies are summarized below.
The cancer prevention studies used the same cardiovascular review board (commissioned by the Data Safety Monitoring Boards of the two respective trials) to adjudicate the results and used the same analysis methods. Patients in the studies were treated for up to 4 years.

APC
In the Adenoma Prevention with Celecoxib (APC) Trial, celecoxib demonstrated a statistically significant increased cardiovascular risk over placebo. The doses of celecoxib in this trial were 400-800mg per day. These findings were unexpected and not consistent with other reported findings from the PreSAP trial. Patients taking 400 and 800mg of celecoxib daily had an approximately 2.5-fold increase in their risk of experiencing a major fatal or non-fatal cardiovascular event compared to those taking placebo.

PreSAP
In the Prevention of Spontaneous Adenomatous Polyps (PreSAP) trial there has been no increased risk for celecoxib patients taking 400mg daily compared with placebo. The two studies, which are following patients for 5 years, have enrolled a total of about 3,600 patients.

ADAPT
The third trial (ADAPT) compared celecoxib to either naproxen sodium or placebo in a group of patients at risk for Alzheimer's disease treated for up to 3 years. Preliminary safety results (not yet adjudicated) from that study indicate an increased cardiovascular risk with naproxen sodium but not celecoxib relative to placebo.

As a result of this new data, in February 2005 the Committee on Safety of Medicines issued the following advice for all selective COX 2 inhibitors (celecoxib, etoricoxib, valdecoxib and parecoxib) which has been entirely incorporated into the NEO-EXCEL protocol:

'Patients with established ischaemic heart disease or cerebrovascular disease should be switched to alternative treatment: in addition the existing contraindication for severe heart failure is now extended to include moderate heart failure (NYHA class II-IV). For all patients the balance of gastrointestinal and cardiovascular risk should be considered before prescribing a COX 2 inhibitor, particularly in those with risk factors for heart disease and those taking low dose aspirin, for whom gastrointestinal benefit has not been clearly demonstrated. The lowest effective dose of COX 2 inhibitor should be used for the shortest

necessary period. Periodic re-evaluation is recommended, especially for osteoarthritis patients who may only require intermittent treatment.'

There is a wealth of data supporting gastrointestinal safety and the tolerability profile of celecoxib. In a retrospective observational study of 144,000 elderly patients, celecoxib demonstrated a reduced risk of hospitalisation due to upper GI haemorrhage versus traditional NSAIDS, misoprostol plus diclofenac and rofecoxib[40].

3.6 Letrozole / Exemestane: clinical pharmacology

3.6.1 Letrozole

Letrozole is a highly potent, orally active non-steroidal competitive inhibitor of the aromatase enzyme system. It effectively inhibits the conversion of androgens to oestrogens in both in vitro and in vivo. It is currently approved for use in women with locally advanced and metastatic breast cancer as first-line use and after antioestrogen failure. It is also approved for treatment of ER positive operable primary breast cancer to downstage the disease to facilitate less extensive surgery. In addition letrozole is also licensed for use after adjuvant tamoxifen as extended adjuvant therapy.

Preclinical pharmacology
Letrozole competitively inhibits the human placental aromatase enzyme in vitro (IC_{50}11.5nM, Ki 2.1nm), is 170 times more potent than aminoglutethimide[41] and does not significantly affect adrenal steroidogenesis. In vivo studies have shown letrozole to be over 10,000 times as potent as aminoglutethimide[42].

Clinical pharmacology
In postmenopausal women with advanced breast cancer letrozole suppressed plasma levels of oestradiol oestrone and oestrone sulphate by 75-95% of baseline in a dose dependent manner with 0.5mg and higher suppressing oestrogen oestrone and oestrone sulphate below the limits of detection. There is no detectable effect of letrozole on adrenal corticosteroid synthesis, aldosterone synthesis or androgen levels in healthy postmenopausal volunteers[43].

Letrozole is rapidly and completely absorbed from the gastrointestinal tract (mean absolute bioavailability of 99.9%). Food slightly decreases the rate of absorption (median tmax:1 hour fasted versus 2 hours fed; and mean Cmax: 129+/- 20.3nmol/l fasted versus 98.7+/- 18.6nmol/l fed) but the extent of absorption (AUC) is not changed. The minor effect on the absorption rate is not considered to be of clinical relevance and therefore letrozole may be taken without regard to meal times. Clearance is via conversion to an inactive hydroxymetabolite. Elimination is almost exclusively via the kidneys as the inactive metabolite, clearance is however not dependent on renal function.

Letrozole in advanced breast cancer treatment
Early phase experience with letrozole has been reproduced in large Phase III trials where the drug was generally well-tolerated, with the most frequently reported adverse events being bone pain, hot flushes, back pain, nausea and arthralgia, These were similar in both first-line and second-line settings[9,44] In the neoadjuvant setting letrozole is well-tolerated and safe, with hot flushes, arthralgia and nausea the most frequently reported adverse events. In the adjuvant context, letrozole has been compared to placebo after prior tamoxifen exposure. In this case hot flushes and musculoskeletal complaints were more common with letrozole. Vaginal bleeding was less common. In addition osteoporosis was more common with letrozole and fractures were numerically, but non-significantly, more common[45].

Letrozole (2.5mg/day) was compared to tamoxifen (20mg/day) in a large Phase III trial in women with locally advanced or metastatic breast cancer[44]. In this study of 907 patients, letrozole was associated with a superior median time to progression of 9.4 months versus 6.0 months, p <0.0001. Overall response was 32% versus 21%, p = 0.0002. Letrozole (2.5 mg/day) has also been compared with megestrol acetate in women with advanced breast cancer failing prior tamoxifen. In this trial letrozole was the more active agent, with a response rate of 23.6% versus 16.4%, p=0.04. Here letrozole, at the lower dose of 0.5mg/day, was also less active than the 2.5mg cohort.

Letrozole as adjuvant therapy

Letrozole has also been studied in the adjuvant context. A large trial comparing tamoxifen for 5 years with letrozole for 5 years and two sequential arms in which the two drugs are administered in sequence, switching after 2.5 years to the alternative agent, has been conducted and preliminary data are expected shortly. Letrozole has also been compared with placebo after the completion of 5 years adjuvant therapy. In this study of 5187 women an improvement in disease-free survival emerged after a median follow-up of 2.4 years when the study was unblinded. The Hazard Ratio (HR) was 0.58, with an estimated 4 year disease free survival of 93% versus 87%. Preliminary data from a complex adjuvant letrozole trial, BIG 1-98, has also been released. This large randomised study compares tamoxifen for 5 years with letrozole for 5 years and sequential tamoxifen/letrozole and letrozole/tamoxifen. Data from all four arms has been analysed to provide a comparison of tamoxifen versus letrozole, with patient data censored at the crossover point within the switching arms. This shows superiority for letrozole versus tamoxifen, HR 0.81 (95% CI 0.7-0-0.93). While generally well-tolerated this trial has reported a non-significant excess of fatal myocardial infarctions in the letrozole treated group (13 versus 6 in over 8000 patients). As it is non-significant and was not seen in the MA-17 trial the importance and potential cause is speculative and unlikely to be relevant to a short-term 16 week exposure to letrozole[46]

Neoadjuvant tolerability

The clinical efficacy of neoadjuvant letrozole was discussed earlier; letrozole is well-tolerated with serious adverse events only rarely encountered (one pulmonary embolus reported in the P024 study). Adverse events occurring in >2% of patients are reproduced from this study in Table 1.

TABLE 1: Adverse Events with neoadjuvant letrozole and tamoxifen

ADVERSE EVENTS	Letrozole, n= 157	Tamoxifen, n=170
Hot Flushes	20%	24%
Nausea	5%	5%
Asthenia	2%	3%
Fatigue	3%	2%
Increased sweating	2%	3%
Weight gain	2%	2%
Leukorrhea	0%	4%
Pruritis	1%	2%
Headache	3%	1%
Dyspepsia	1%	2%
Hair Thinning	2%	1%

3.6.2 Exemestane

Exemestane is a very potent, orally active, selective and long lasting steroidal, irreversible inactivator of aromatase. In in vitro studies exemestane appeared to be 2.8 and 156 times more potent than the steroidal formestane and the non-steroidal aminoglutethimide (AG), respectively, in inhibiting human placental aromatase[47].

In vivo studies of aromatase inactivation indicate that exemestane, by the oral route, is several times more potent than formestane and suppresses plasma oestrogen by approximately 98% compared with baseline[48]. Exemestane has no noteworthy binding to oestrogen, progesterone, glucocorticoid or mineralocorticoid receptors and only a very low binding to the androgen receptor (Relative Binding Affinity, RBA, 0.2% from that of dihydrotestosterone, DHT)[49]. However, its metabolite FCE 25071 (17-hydro-exemestane) was found to have a binding affinity to the androgen receptor (100-fold higher than that of exemestane (RBA 27% from and 0.28% that of DHT, respectively) (Pfizer Inc, data on file).

Exemestane in the treatment of advanced breast cancer
Exemestane (25mg/day) has been compared to tamoxifen (20mg/day) in first-line therapy for advanced breast cancer in the EORTC 10951 randomised Phase III study. Preliminary data shows a significant response rate advantage to exemestane (46% versus 31%, p=0.005). Time to progression was also longer, 10 months versus 6 months. This was not significant in the primary analysis using a log rank test, p=0.121, but was significant with a secondary Wilcoxon sensitivity analysis, p=0.028. Exemestane was well-tolerated with hot flushes, bone pain and gastrointestinal adverse events the most commonly reported events.

Exemestane (25mg daily) was evaluated in a Phase III, randomised double-blind, comparative study of postmenopausal women with advanced breast cancer who had disease progression after hormonal treatment with antioestrogens (primarily tamoxifen) for metastatic disease or as adjuvant therapy. In this study, 769 patients were randomised to receive exemestane 25mg once daily (n=366) or megestrol acetate 40mg four times daily (n=403). Response rate for exemestane was 15.0% versus 12.4% for megestrol acetate. Time to progression was longer with exemestane (20.3 weeks versus 16.6 weeks, p=0.037) and survival was superior, median survival for exemestane was not reached versus 123 weeks for megestrol acetate, p=0.039[50].

Exemestane as adjuvant therapy
The Intergroup Exemestane Study (IES) randomised women with early breast cancer who were disease free after 2-3 years of prior tamoxifen therapy to either continue to complete 5 years of adjuvant tamoxifen or to commence exemestane (25mg) once daily. This double-blind study of over 4,700 patients has recently published interim findings after a median follow-up of 30 months. This study, in a different population, has shown a somewhat larger difference than the ATAC study with a HR of 0.68. This translates to an absolute advantage of 4.77% at 36 months[14]. Within the receptor positive subgroup the hazard ration rises to 0.64 (95% CI 0.52-0.79).

In this study exemestane was well-tolerated, with arthralgia and hot flushes again the commonest reported adverse events, but diarrhoea was also seen here. Osteoporosis was

more commonly reported with letrozole and fractures were numerically but not significantly more common (Table 2).

The TEAM trial is a large multinational study initially designed to compare 5 years of exemestane with 5 years of tamoxifen. This trial has been revised in the light of the IES data to compare exemestane as initial therapy versus a switching policy in which women initiated on tamoxifen cross over to exemestane after 2-3 years.

Exemestane tolerability

Exemestane has been reported as well-tolerated in the small neoadjuvant studies described but a detailed breakdown of the adverse events has not been reported. Table 2 summarises data from the IES trial, which has provided the most detailed comparative information on the tolerability of exemestane and tamoxifen. The most significant toxicities that are worse with exemestane are joint pains and diarrhoea. The TEAM study has also reported short-term tolerability data in the adjuvant setting which demonstrates predominantly better tolerability with exemestane[51].

TABLE 2: Adverse events in the IES trial significantly different, or different by >1% or common (>5%)

ADVERSE EVENTS (ANY GRADE)	Exemestane % (n = 2309)	Tamoxifen % (n = 2332)	P value
Arthralgia	5.4	3.6	**0.01**
Diarrhoea	4.3	2.3	**0.001**
Throboembolic AEs	1.0	2.0	0.01
Thromboembolic SAEs	1.3	2.4	0.005
Vaginal bleeding	4.0	5.6	**0.01**
Other gynaecological symptoms	5.8	9.0	**<0.001**
Endometrial cancer	0.21 (5 events)	0.46 (11 events)	Not stated
Other malignancy	22	42	Not stated
Cramp	2.8	4.4	**0.0007**
Sweating	18.6	18.1	
Headache	18.6	16.4	0.09
Dizziness	12.5	12.1	0.93
Visual disturbance	7.4	5.8	**0.04**
Depression	5.2	4.0	
Osteoporosis	7.4	5.7	**0.05**
Clinical fracture	3.1	2.3	0.08

4. STUDY RATIONALE / TREATMENT SELECTION

The hypotheses to be addressed in this Phase III trial is that the activity of aromatase inhibitors as primary neoadjuvant endocrine therapy for early stage ER positive breast cancer in postmenopausal women may significantly be enhanced by the addition of the selective COX 2 inhibitor, celecoxib.

The Investigators feel that further exploration of their clinical activity of neoadjuvant endocrine therapy in this population is long overdue and has the potential to impact on clinical practice, not withstanding the benefits of breast cancer screening.

The conventional tool for the development of new systemic therapies is presently the randomised Phase III clinical trial. Conventionally, in the adjuvant setting this endeavour

requires 2-3000 patients and may take 10 years from inception, funding, launch, execution, follow-up and analysis. By contrast, randomised trials of systemic therapy in the primary or neoadjuvant setting offer economies of scale and the opportunity for time-scale compression. Over recent years compelling evidence has accumulated to link various measures of primary tumour response to eventual disease-free survival, thus validating their candidacy as surrogate endpoints. Two recent trials demonstrated that primary hypotheses tested in the neoadjuvant setting have the power to answer therapeutic questions with a relatively small number of patients because the biological predictors of response are likely to be strong.

5. TRIAL DESIGN / OBJECTIVES AND OUTCOME MEASURES

TRIAL OBJECTIVES

To determine whether the activity of aromatase inhibitors as primary neo-adjuvant endocrine therapy for early stage ER positive breast cancer in postmenopausal women may be enhanced by the addition of the selective COX 2 inhibitor celecoxib.

TRIAL DESIGN

Prospective phase III, multicentre, randomised clinical trial, with placebo-controlled comparisons.

OUTCOME MEASURES

Primary

- Objective clinical response (Complete Response (CR), Partial Response(PR)) to neoadjuvant treatment

Secondary

- Objective ultrasound-determined response (CR, PR) to neoadjuvant treatment
- Type of surgery
- Axillary lymph node involvement at surgery
- Complete pathological response
- Local recurrence-free survival
- Progression-free survival
- Overall survival

Translational sub-study (Optional)

- Biological profiling for prognostic and predictive indicators

6. PATIENT SELECTION

6.1 Inclusion criteria

The study population consists of postmenopausal women diagnosed with resectable breast cancer, who meet the following eligibility criteria:

- Biopsy proven, ER positive invasive breast cancer (where ER positive is defined as equivalent to an ER "Quick or Allred score" of 3 or greater)
- Tumour, measured clinically, as ≥ 2 cm in diameter
- Postmenopausal, defined as:

Any Age:-	bilateral surgical oophorectomy amenorrhea ≥ 5 years (any cause)
Age ≥ 50 yrs:-	natural amenorrhea for ≥ 1 year
Age <50 yrs: -	if amenorrhea < 5 years or hysterectomy without bilateral surgical oophorectomy, then FSH, and/or LH and/or oestradiol must be assayed to confirm postmenospausal status

- Adequate haematological, renal and liver function, defined as a platelets of >100 x 10^9/l, white blood cell count of >3 x 10^9/l, creatinine <110 µmol/l, AST and/or ALT < 1.25 x upper limit of normal
- Patients must be fit to complete surgery for their breast cancer
- Written informed consent
- ECOG performance status 0,1 or 2

6.2 Exclusion criteria

- Bilateral breast cancer
- Evidence of distant metastases (M1)
- Patients who have received previous treatment for invasive breast cancer
- Concomitant active malignancy except for adequately treated carcinoma in situ of the uterine cervix or basal cell carcinoma of the skin
- Co-morbid disease which would preclude safe surgical treatment of the primary cancer
- Other physical or psychiatric disorder that may interfere with subject compliance, adequate informed consent or determine the causality of adverse events
- Contraindications to celecoxib: active peptic ulcer disease, renal impairment, asthma exacerbated by NSAIDs, congestive cardiac failure (NYHA II-IV*), ischaemic heart disease, cerebrovascular disease, uncontrolled hypertension

**NYHA classification*

Class I: Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea or anginal pain.

Class II: Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnoea or anginal pain.

Class III: Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnoea or anginal pain.

Class IV: Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

- Patients with an ongoing requirement for regular NSAID or COX 2 inhibitor therapy (Asprin 75mg daily is permitted)
- Regular selective COX 2 inhibitor use in the 2 years prior to randomisation
- History of hypersensitivity to celecoxib, exemestane or letrozole or to any of the excipients
- Known hypersensitivity to sulphonamides
- Patients who have experienced asthma, acute rhinitis, nasal polyps, angioneurotic oedema, urticaria or other allergic-type reactions after taking acetylsalicylic acid or NSAIDs including COX-2 inhibitors
- Inflammatory bowel disease
- Patients with ongoing requirements for fluconazole or ketoconazole therapy
- Patients with ongoing requirement for lithium therapy
- Patients with ongoing requirement for ACE inhibitor therapy
- Patients who are on warfarin or heparin

6.3 Informed Consent

It is the responsibility of the Investigator to obtain written informed consent for each patient prior to performing any trial related procedure. It is anticipated that patients will be approached regarding participation in NEO-EXCEL following their diagnostic biopsy. Patient Information Sheets are provided to facilitate the informed consent process:

- Patient Information Sheet and Informed Consent Form version A should be utilised by sites who are not participating in Trans NEO-EXCEL.
- Patient Information Sheet and Informed Consent Form version A and B should be utilized by sites participating in Trans NEO-EXCEL. Version A being used for patients who are deemed to be unsuitable for entry into the Trans NEO-EXCEL sub-study by their Investigator while version B should be used for all other patients

Patients participating in TRANS NEO-EXCEL are asked to donate tissue from their baseline core biopsy. There are two ways in which consent for this can be obtained:

1. Prior to diagnostic biopsy patients may give consent for the collection of additional tissue for research purposes (unrelated to NEO-EXCEL). Sites that routinely collect research tissue should use their own patient information sheets and consent forms for this purpose in line with standard practice. Sites that do not have standard patient information sheets and consent forms for the collection of research tissue can utilise the Patient Information Sheet - Donating Tissue For Research and Research Tissue Consent Form provided by the NEO-EXCEL Study Office. The research tissue collected can be donated to the TRANS NEO-EXCEL sub-study once the patient has given written informed consent for participation in NEO-EXCEL.
2. Sites that do not collect additional research tissue at diagnostic biopsy should be prepared to donate any excess diagnostic tissue to the TRANS NEO-EXCEL sub-study. If there is insufficient tissue or the responsible pathologist is unwilling to relinquish the tissue patients may need to have an additional biopsy to collect research material specifically for the trial. This possibility should be made clear to the patient during the informed consent process.

When obtaining Informed Consent Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time. The patient should be given ample time (e.g. 24 hours) to read the appropriate Patient Information Sheet and to discuss their participation with others outside of the site research team. The patient must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the trial without giving a reason must be respected.

If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the appropriate Informed Consent Form. The Investigator must then sign and date the form. A copy of the Informed Consent Form should be given to the patient, a copy should be filed in the hospital notes, and the original placed in the

Investigator Site File (ISF). Once the patient is entered into the trial the patient's Trial Number (TNO) should be entered on the Informed Consent Form maintained in the ISF. In addition, if the patient has given explicit consent a copy of the signed Informed Consent Form should be sent in the post to the Neo-Excel Study Office for review.

Details of the informed consent discussions should be recorded in the patient's medical notes, this should include date of, and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Electronic copies of the Patient Information Sheet - Donating Tissue For Research and Research Tissue Consent Form, Patient Information Sheets and Informed Consent Forms are available from the Neo-Excel Study Office and should be printed or photocopied onto the headed paper of the local institution.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent their General Practitioner (GP) should also be informed that they are taking part in the trial. A GP Letter is provided electronically for this purpose. All patients randomised into the trial must to given a patient contact card on which the patient trial number, allocated treatment and contact details for emergency unblinding must be recorded. Patients must be encouraged to keep the contact card with them at all times.

7. RANDOMISATION

An Eligibility Form and a Randomisation Form must be completed prior to randomisation. These details should be telephoned or faxed through to the Randomisation Office at the Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham.

RANDOMISATION
(9:00am till 5:00pm, Monday to Friday)

☎ 0800 371 969
🖷 0121 414 7989

During the randomisation procedure eligibility criteria and randomisation details will be confirmed and verification that the patient has signed the NEO-EXCEL Informed Consent Form will be requested. The patient's full name will be collected over the phone with their prior consent. A unique sequential TNO will be allocated. This number should be used on all Case Report Forms (CRF) and all subsequent correspondence relating to that patient. The TNO and allocated treatment must also be recorded on the Randomisation Form, and the Form then signed and dated. The completed original Forms should be sent to the NEO-EXCEL Study Office, with copies retained at site. Confirmation of the randomised treatment allocation will be sent to the Investigator and appointed Pharmacist.

Randomisation will be stratified by tumour size (≥ 2-5cm, >5cm), grade (I, II, III), ER Q-score (3-4, 5-6, 7-8), Her2 (-ve, +ve, not determined), and low dose aspirin use (yes, no). This information therefore must be available at randomisation. A computerised minimisation algorithm, developed by the CRCTU, will be used to ensure that allocation of treatment to patients is balanced within these strata.

8. TREATMENT DETAILS

8.1 Trial plan

Treatment should begin within 5 working days of randomisation. The planned duration of treatment is 16 weeks.

Subjects will be randomised (1:1:1:1) to receive either:

Exemestane + Celecoxib
Patients will receive exemestane 25mg, one tablet daily and celecoxib 200mg, two capsules twice daily

Exemestane + Celecoxib-Placebo
Patients will receive exemestane 25mg, one tablet daily and celecoxib-placebo, two capsules twice daily

Letrozole + Celecoxib
Patients will receive letrozole 2.5mg, one tablet daily and celecoxib 200mg, two capsules twice daily

Letrozole + Celecoxib-Placebo
Patients will receive letrozole 2.5mg, one tablet daily and celecoxib-placebo, two capsules twice daily

Treatment in all arms will continue up to 16 weeks (patients should not take study medication after requirement of 'nil by mouth' for anaesthetic purposes). The surgical date should be planned in advance and will be at 16 weeks from the commencement of treatment.

Dose modifications of trial treatment are not permitted. Patients who are unable to tolerate the trial mediation should discontinue treatment.

Patients whose tumours progress during the neoadjuvant phase should discontinue trial treatment and be treated according with local practice.

8.2 Concomitant medication

Patients on oestrogen replacement therapy (HRT) at diagnosis should discontinue this at the time they start trial treatment. Treatment of menopausal symptoms may be initiated as per local protocol during the treatment phase. Systemic oestrogen therapy is prohibited and if required patients must discontinue trial treatment Aspirin.
NSAIDS and COX 2 inhibitors (except as study medication) are prohibited during the study, except for low dose aspirin. Celecoxib is not a substitute for acetylsalicylic acid for prophylaxis of ischaemic heart disease because of the lack of effect on platelet function. Because celecoxib does not inhibit platelet aggregation antiplatelet therapies should not be discontinued. Therefore continuation of low dose aspirin (75mg daily) is permitted.

Fluconazole & Ketoconazole
Clinical studies have identified potentially significant reactions of celecoxib with fluconazole and ketoconazole. Concomitant administration of fluconazole at 200mg once daily resulted in a 2-fold increase in celecoxib plasma concentration. This increase is due to the inhibition of celecoxib metabolism via CYPP450 2C9 by fluconazole. Patients on these medications are not eligible.

ACE inhibitors
There is a potential for interactions with ACE inhibitors as inhibition of prostaglandins may diminish their antihypertensive effect. Patients taking ACE inhibitors are excluded from this study.

Lithium
In healthy subjects, lithium concentrations are increased approximately 17% in subjects receiving lithium together with celecoxib. Patients on lithium are not eligible for this study.

Diuretics
Clinical studies have shown that NSAIDS, in some patients, can reduce the natriuretic effect of frusemide and thiazides by inhibition of renal prostaglandin synthesis.

Warfarin/Heparin
Because of possible interference with INR and also due to multiple biopsies being required, patients on warfarin/heparin are ineligible. Tumour measurements may also be affected by excessive bleeding.

Caution is advised when patients are receiving drugs with a narrow therapeutic index and which are mainly dependant on cytochrome CYP 450 enzymes 2A6 and 2C19.

8.3 Surgery

Surgery appropriate to the tumour size determined at the end of treatment will be performed according to national and local guidelines.

Complete clinical response
All patients require surgery. Even if no tumour is palpable or visible on ultrasound to allow an ultrasound marker then a wide local excision of the tumour site should be performed. Some centres with appropriate expertise may choose to insert clips at the same time as the day 14 biopsy in smaller tumours so that if complete clinical response occurs wide local excision can be centred on the clip.

8.4 Postoperative management

Postoperative management will depend on operative pathology and be determined by local protocol. It is expected that all patients will receive a total of 5 years endocrine therapy; continuation of aromatase inhibitor therapy is recommended.

9. SCHEDULE OF ASSESSMENTS

The schedule of assessments and investigations required is described below and summarised in Figure 1. This information should be recorded in the patient notes where not explicitly required in the CRF.

At Screening

- Relevant medical history including:
  - Cardiac history
  - Thromboembolic history
  - Rheumatological history
  - Other serious acute or chronic conditions
- Concomitant medication (description of other medication prescribed for more than 7 days and taken within one month of randomisation)
- ECOG performance status (Appendix 1)
- Clinical examination, including height and body weight
- Uni-dimensional tumour measurement by calipers of the longest dimension
- Tumour assessment by ultrasound
- Baseline blood tests: full blood count; urea + creatinine + electrolytes; liver function tests
- Blood pressure measurement
- NYHA classification (see section 6.2)

At Baseline

- Clinical examination
- Core biopsy*
- Sites participating in the optional TRANS NEO-EXCEL sub-study should also collect pre-treatment bloods:
- Sites participating in the optional Fresh Tissue TRANS NEO-EXCEL sub-study should also collect frozen core biopsy

* Baseline core biopsy material should be collected where possible for all patients. Patients recruited from sites who not taking part in the optional TRANS NEO-EXCEL sub-study will be asked to donate any excess diagnostic material (with the approval of the responsible pathologist). Patients recruited from sites taking part in TRANS NEO-EXCEL who have insufficient material left from the diagnostic core biopsy (and for whom separate consent for collection of research tissue has not been obtained) may need to have an additional biopsy prior to commencement of trial treatment.

At Day 14

- Treatment side-effects
- Sites participating in the optional TRANS NEO-EXCEL sub-study should also collect:
    - TRANS NEO-EXCEL bloods
    - Core biopsy
- In addition, sites participating in the optional Fresh Tissue TRANS NEO-EXCEL sub-study should also collect frozen core biopsy

Every 4 weeks, on treatment

- Clinical examination
- Treatment side-effects and events
- Uni-dimensional tumour measurement by calipers of the longest dimension

On completion of treatment (no more than 2 weeks prior to surgery)

- Clinical examination
- Treatment side-effects and events
- Uni-dimensional tumour measurement by calipers of the longest dimension
- Tumour assessment by ultrasound – response will be assessed by RECIST target lesion classification (Appendix 4)
- Routine blood tests including full blood count; urea + creatinine + electrolytes; liver function tests
- Formalin-fixed tumour tissue cut from surgical specimen by pathologist
- Sites participating in the optional TRANS NEO-EXCEL sub-study should also collect bloods
- In addition sites participating in the optional Fresh Tissue TRANS NEO-EXCEL sub-study should also collect additional tumour tissue that has been cut from surgical specimen by pathologist and frozen

Please refer to separate TRANS NEO-EXCEL laboratory manuals for full details of sample preparation and collection

Disease progression
Disease progression measured by ultrasound is as an increase in the largest uni-dimensional tumour measurement by > 20%. If disease progression does occur, taking into account accepted operator variability, patients will be withdrawn from trial medication and a biopsy taken as soon as possible and before commencement of alternative therapy. Full details of disease progression/death should be recorded on the CRF.

A summary of treatment offered at progression will also be requested. Sites are strongly urged to take serum/plasma samples, core biopsies (and for sites participating in the frozen tissue sub-protocol, an additional core biopsy to be frozen) as these may help provide invaluable information about the mechanisms of treatment failure. Although participation in the Translational (TRANS NEO-EXCEL) sub-study is strongly encouraged it is optional.

Follow-up after completion of treatment
All patients, provided that they have not withdrawn consent for follow up, should have long-term follow-up of at least 5 years, irrespective of whether they have discontinued trial treatment prematurely. The anticipated follow-up frequency is once every year, unless otherwise clinically indicated. Follow-up after relapse should be according to local policy.

The following information will be collected:

- Local/distant relapse
- New primary cancer
- Details of treatment of recurrent/progressive tumour
- Toxicity
- Survival
- Important information relevant for the trial (unforeseen circumstance that may have led to changes in interpretation of the results, for instance, cause of death)

FIGURE 1: FLOWCHART OF ASSESSMENTS

THIS TABLE PROVIDES A SUMMARY OF THE TRIAL-RELATED PROCEDURES	VISIT							
	S[1]	**B[2]**	**2**	**3**	**4**	**5**	**6**	**FOLLOW-UP**
Timeframe		**Day 1**	**Day 14**	**4 wks**	**8 wks**	**12 wks**	**16 wks**	Post surgery follow-up should be carried out annually for 5 years, and thereafter according to local policy
Medical history	**X**							
Clinical examination	**X**			**X**	**X**	**X**	**X**	**X**
Events[3]				**X**	**X**	**X**	**X**	**X**
Adverse Events/Serious Adverse Events[4]			**X**	**X**	**X**	**X**	**X**	
Tumour assessment by calipers	**X**			**X**	**X**	**X**	**X**	
Tumour assessment by ultrasound	**X**						**X**	
Routine bloods	**X**						**X**	
Core biopsy		**X**	**X†**					
Formalin fixed tumour tissue cut from surgical specimen							**X***	
TRANS NEO-EXCEL Bloods		**X†**	**X†**				**X†**	
TRANS NEO-EXCEL Frozen Tissue Sub-Protocol		**X****	**X****				**X***	

The TRANS NEO-EXCEL sub-protocol is optional

Notes:

1. Screening visit
2. Baseline visit (to take place *up to* 5 days before treatment start)
3. Breast cancer events and survival (disease progression or recurrence, death with cause)
4. As described in section 13
5. Tumour assessment by callipers and ultrasound must be performed on the same lesion

* From surgical specimen **Frozen core biopsies † TRANS NEO-EXCEL only

- Medical history to include clinical examination, height, weight, ECOG status, concomitant medication.
- Patients should continue to take their trial medication up to 16 weeks (patients should not take study medication after requirement of 'nil by mouth' for anaesthetic purposes)
- If patients are withdrawn due to disease progression a core biopsy should be performed prior to initiation of alternative treatment and TRANS NEO-EXCEL blood samples should also be taken
- If for any reason TRANS NEO-EXCEL blood samples cannot be taken always send all pathology samples

- The frozen tissue sub-protocol will only apply to sites that are participating in frozen tissue collection

10. TRIAL MANAGEMENT / DATA COLLECTION

NEO-EXCEL will be coordinated by the CRCTU at the University of Birmingham according to the current guidelines for Good Clinical Practice. Participating sites may be monitored by CRCTU staff to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki (Appendix 3).

10.1 Trial start-up and core documents

Interested sites should contact the NEO-EXCEL Study Office to obtain information on the core documentation required prior to trial participation. All Principal Investigators taking part in the trial will be asked to sign a Clinical Study Agreement (which will detail the responsibilities of the participating site) and should provide a current signed and dated *Curriculum Vitae* prior to trial activation.

It is recommended that all clinic and study-related personnel should attend a start-up meeting for training on trial procedures and data collection methods. Staff from sites that have attended the trial launch meeting will not require a start-up visit unless specifically requested by the local Investigator or NEO-EXCEL Study Office.

The Principal Investigator at each site must submit this protocol, any supporting documentation, and any subsequent amendments for Site-Specific Assessment from their Local Research Ethics Committee and, if locally required, Institutional Review Boards. Investigators must acquire ethical approval and Research and Development Trust approval before they commence recruitment.

10.2 Case Report Forms

Data collection will be kept to a minimum. The CRF must be completed and signed/dated by the Investigator or one of their authorised staff members as soon as the required information is available. The completed originals should be sent to the NEO-EXCEL Study Office, with a copy held by the Investigator at site. In all cases it remains the responsibility of the Investigator to ensure that they have been completed correctly and that the data are accurate. Entries should be made in ballpoint pen preferably in black ink and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not clear why a change has been made, an explanation should be written next to the change. Typing correction fluid should not be used. Data reported on each Form should be consistent with the source data or the discrepancies should be explained. All sections are to be completed before returning to the NEO-EXCEL Study Office. If information is not known, this must be clearly indicated by entering "NK" on the form. All missing and ambiguous data will be queried.

The CRF may be amended as appropriate; this will not constitute a protocol amendment. The revised CRF should be used by all participating sites with immediate effect.

Case Report Forms will include:

Form	Summary of Data Collected
Eligibility	Confirmation patient meets inclusion and exclusion criteria
Randomisation	Patient details (including date of birth, hospital number, and NHS/CHI number), Investigator, site, stratification variables, nodal involvement, hormonal status
On-Study	GP details, patient's medical history, tumour measurements, baseline investigations, concomitant medication
Two weeks - On Treatment	Details of trial medication and compliance
Four weeks - On Treatment	Details of trial medication and compliance, tumour measurements
Eight weeks - On Treatment	Details of trial medication and compliance, tumour measurements
Twelve weeks - On Treatment	Details of trial medication and compliance, tumour measurements
End of Treatment	Details of trial medication and compliance, tumour measurements
Post-Operative	Details of surgery, tumour pathology, receptor status
Year One Assessment	Details of adjuvant treatment, patient status
Annual Follow-Up (years 2+)	Patient status, hormonal therapy
Adverse Event (AE) Form	Details of event, causality, outcome, concomitant medication
Serious Adverse Event (SAE) Form	Patient details, details of event, causality, outcome, concomitant medication
Deviation	Date, type and reason for deviation
Withdrawal	Reason for treatment discontinuation, date of last dose
Relapse Form	Date, site, treatment for relapse
Death Form	Date, cause of death
Concomitant Medication	Dates, type, dose, frequency, indication

10.3 Early discontinuation of trial treatment and patient withdrawal

Patients should discontinue trial treatment if:

- There is evidence of disease progression
- They are unable to tolerate the trial mediation
- They are required to commence systemic oestrogen therapy

Patients can be withdrawn at the discretion of the Investigator or at the patient's own request. *Patients have the right to withdraw from the trial at any time for any reason*. Full details of the reason(s) for discontinuation of treatment/withdrawal should be recorded on the CRF if clinician-initiated, otherwise a simple statement reflecting patient preference will suffice.

Please note that patients who discontinue trial treatment will not be regarded as having withdrawn consent for ongoing follow-up and data collection unless clearly specified at time of discontinuation, and should therefore be followed-up in accordance with the protocol.

10.4 Data monitoring

The Trial Coordinator will be in regular contact with site personnel (by phone/fax/email/letter) to check on progress and answer any queries that they may have. Trial staff will check incoming CRF for compliance with the protocol, consistent data, missing data and timing. Sites may be barred from further recruitment in the event of serious and persistent non-compliance and/or very poor recruitment.

Monitoring will be done according to the CRCTU policy and the NEO-EXCEL Quality Management Plan. Investigators will allow the trial monitors access to source documents as requested. If a monitoring visit is required the NEO-EXCEL Study Office will contact the site to arrange a date for the proposed visit. Data to be verified will include:

- Informed Consent
- Eligibility
- Adverse Events
- Outcome

Any major problems identified during monitoring will be reported to the NEO-EXCEL Steering Committee. All records will be maintained in accordance with local regulations and in a manner that ensures security and confidentiality. The completed original CRF are the sole property of the NEO-EXCEL Steering Committee and should not be made available in any form to third parties (except for authorised representatives of appropriate Health/Regulatory Authorities) without written permission from the NEO-EXCEL Steering Committee.

10.5 Closure of trial – end date

The end of trial will be 6 months after the last patients' last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The Neo Excel Trial Office will notify the Sponsor, the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial. 10.6 Archiving

To enable monitoring, peer review and/or audits from Health Authorities, the Investigator must agree to keep records, including the identity of all participating subjects (sufficient information to link records e.g. CRF and hospital records), all original signed Informed Consent Forms, copies of all CRF and detailed records of drug disposition.

To comply with international regulations these records should be retained by the Investigator for 15 years, including assessments such as CT scans.

10.7 Trial Steering Committee / Trial Management Group

The Trial Steering Committee will provide the overall supervision for the trial, in particular: trial progress, protocol compliance, patient safety and review of updated information.

The Trial Management Group will be responsible for the clinical set-up, on-going management, promotion of the study, and for the interpretation of the results.

This is a clinician-initiated and clinician-led trial, funded through a project grant from Cancer Research UK, and an educational grant from the pharmaceutical industry. The study has been independently peer reviewed and endorsed by Cancer Research UK Clinical Trials Awards & Advisory Committee (CTAAC), and is part of the National Cancer Research Network (NCRN portfolio).

10.8 Independent Data Monitoring Committee

An independent Data Monitoring Committee (DMC) will be responsible for the regular monitoring of trial data. The Committee will consist of two clinicians not entering patients into the trial and an independent statistician. The DMC will assess the progress of the trial and give advice on whether the accumulated data from the trial, together with the results from other relevant trials, justifies the continuing recruitment of further patients. The committee will first meet to review data from the first 50 patients randomised (see section 14.4) and then annually thereafter until the trial closes to recruitment. The DMC may consider discontinuing the trial if the recruitment rate or data quality are unacceptable or if there are cases of excessive toxicity that in the DMC's opinion jeopardise patient safety. In addition the DMC may recommend the trial stop early if the interim analyses showed differences between treatment arms that are sufficient to be deemed convincing to the general clinical community. If a decision is made to continue, the DMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The DMC will make confidential recommendations to the Trial Steering Committee (TSC) as the decision-making Committee for the trial.

11. CONCURRENT STUDIES

Patients entering NEO-EXCEL may be considered for entry into additional trials provided compatibility has been agreed by Trial Management Groups or Steering Committees of both studies.

12. STUDY DRUG SUPPLIES

Full pharmacy details and guidelines for ordering study drug supplies, and labelling requirements are contained within the NEO-EXCEL Pharmacy File which will be sent to the appointed Pharmacist.

13. PHARMACOVIGILANCE

ADVERSE EVENTS: DEFINITIONS AND REPORTING

The collection and reporting of data on Adverse Events and Serious Adverse Events will be in accordance with EU Directive 2001/20/EC and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments.

13.1 Adverse Event definitions

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

Comment
An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an Investigational Medicinal Product (IMP), whether or not considered related to the IMP.

Adverse Reaction
An Adverse Reaction (AR) is defined as all untoward and unintended responses to an IMP related to any dose administered.
Comment
An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected Adverse Reaction
An Unexpected Adverse Reaction (UAR) is defined as an AR, the nature or severity of which is not consistent with the applicable product information (e.g. Summary of Product Characteristics (SmPC)).

Comment
When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.
Severity: The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.

Serious Adverse Event or Serious Adverse Reaction
An SAE or Serious Adverse Reaction (SAR) is defined as any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening*
- Requires inpatient hospitalisation** or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity

- Is a congenital anomaly/birth defect (in offspring of patient regardless of time to diagnosis)
- Or is otherwise considered medically significant by the Investigator***

Comment
The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.
* Life threatening in the definition of an SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE
*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Suspected Unexpected Serious Adverse Reactions
A Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as a SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information.

A SUSAR should meet the definition of an AR, UAR and SAR as detailed above.

13.2 Procedures for collecting Adverse Events

All medical occurrences (which meet any of the above definitions) from the first dose of IMP to 30 days after the last dose of IMP should be reported as adverse events and must be accurately recorded on an AE or SAE form as appropriate and sent to the NEO-EXCEL Study Office.

Toxicities will be reviewed using the National Cancer Institute Common Toxicity Criteria (NCI CTC version 3.0, see Appendix 5). Any toxicities incurred but not categorised by the NCI CTC should be graded by a physician and be recorded on the CRF using a scale of (1) mild, (2) moderate or (3) severe (as defined in Appendix 6). For each sign/symptom, the highest grade observed since the last visit should be recorded.

Exceptions to AE reporting

- A pre-existing condition should not be reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event-reporting period
- Symptoms of the targeted cancer should not be reported as adverse events
- Symptoms relating to disease progression or death unless the investigator deems them related to use of the study drug
- Symptoms related to treatment for disease progression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13.3 Recording and reporting Serious Adverse Events

In the case of a SAE the Investigator (or person delegated that responsibility) must **immediately** on becoming aware of the event:

- **Complete a 'Serious Adverse Event Form'**. This should contain all of the information known at the time of the report
- **Fax** within 24 hours of becoming aware of the event the signed and dated 'Serious Adverse Event Form' to the NEO-EXCEL Study Office at the CRCTU, Birmingham: **UK Fax: 0800 328 6412 or 0121 414 3700**
- In addition, **send by post** the original copy of the SAE form and ensure that the reporting clinician has signed/dated it. Forms can be signed by nurse but must be co-signed by the reporting Investigator.

Investigators should also report SAEs in accordance with their local institutional policy.

Documenting SAEs
The responsible clinician must determine the severity of an event (according to the NCI CTCAE-3, see Appendix 5), and relatedness of the events to the study drugs. Seriousness, relatedness, and expectedness will also be independently assessed by a Clinical Coordinator. A SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a Serious Adverse Reaction (SAR). If the event meets the definition of a Serious Adverse Reaction that is unexpected in nature it will be classified as a SUSAR.

Reporting period for SAEs
Details of all SAEs will be documented from the commencement of treatment until 30 days post-treatment (i.e. 30 days from last administration of the study drug). SAEs occurring thereafter should be reported only if the Investigator believes them to be related and unexpected (i.e. SUSAR).

Reporting of SAEs to Regulatory Authorities
The CRCTU will report all fatal or life threatening SUSARs to the Medicines and Healthcare products Regulatory Agency (MHRA) and MREC within 7 days of receiving initial notification from the study site. Any follow-up information will be provided within an additional 8 days. Non-fatal and non-life threatening SUSARs will be reported within 15 days. The CRCTU will submit an Annual Safety Report to the MHRA and MREC summarising all reported SARs.

The CRCTU, will forward details of SUSARs to all Investigators in the form of an Annual Safety Report. SAEs will be reported to the relevant Pharmaceutical Companies as appropriate.

13.4 Follow-up of AEs/SAEs

All AEs will be recorded on the CRF until 30 days after the last treatment dose on study or until the start of other anti-cancer treatment, whichever occurs first. Additionally, all AEs deemed possibly related to the trial medication will be followed until resolution, or the Investigator assesses them to be chronic or stable, or initiation of other anti-cancer therapy, whichever occurs first.

In the case of SAEs, the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until disease has stabilised. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information will be collected on a new SAE form. Extra annotated information and/or copies of test results should also be provided where available.

Receipt of all SAE forms will be acknowledged via fax.

13.5 Codebreaks

Codebreaks should be avoided whenever possible. Approval must be given from one of the NEO-EXCEL Clinical Coordinators before a codebreak is undertaken. This will be checked before the code is broken (except in emergency situations). Unblinding should only be undertaken for medical reasons. When assessing SAEs both the Investigator and Clinical Coordinator must assume that the patient received the IMP (i.e. celecoxib). If the event is thought to be related, unblinding will be performed by the CRCTU.

To break the randomisation code for a patient:

During office hours (9:00am-5:00pm Monday to Friday):
Call the NEO-EXCEL Study Office on 0121 414 2535 or 0121 414 3792

Outside of office hours:
Codebreak envelopes will be provided with the Celecoxib/Placebo Treatment Packs delivered to site pharmacies and will contain the unblinding information. Codebreak envelopes should be kept by the site pharmacy for availability 24 hours per day.

Refer to ISF/Pharmacy File for additional information.

14. STATISTICAL CONSIDERATIONS

14.1 Definition of Outcome Measures

The treatment arms will be compared in terms of the following outcome measures:

Primary Outcome Measure

- Objective clinical response (CR, PR) to neoadjuvant treatment

Objective clinical response as measured by callipers is defined as either a partial or complete response. A complete clinical response (CR) is defined as no palpaple lesion from which to take calliper measurements. A partial clinical response (PR) is defined as a decrease in the longest uni-dimensional measurement by callipers of at least 30%.

Patients who achieve a CR or a PR will be defined as achieving an objective clinical response to neoadjuvant treatment.

Secondary Outcome Measures

- Objective ultrasound-determined response (CR, PR) to neoadjuvant treatment

Objective ultrasound-determined response is defined as either a partial or complete response.

A partial response (PR) is defined as at least a 30% decrease in the longest dimension of the lesion in accordance with RECIST target lesion classification. A complete response (CR) is defined as complete disappearance of the radiological (USS) lesion.

Patients who achieve a CR or a PR will be defined as achieving an objective ultrasound-determined response to neoadjuvant treatment.

Type of surgery
This is a binary outcome recorded as either breast conserving surgery or mastectomy.

Axillary lymph node involvement at surgery
This is a binary outcome recorded as either lymph node involvement or not.

Complete pathological response
This determined by the pathologist and defined as when all detectable tumours have disappeared.

Local recurrence-free survival
Local recurrence-free survival is defined as time from date of trial entry to date when local recurrence is first observed and is censored at date last seen free of local recurrence in those patients who have not experienced the event.

Progression-free survival
Progressive disease is defined by the tumour growing significantly or new tumours appearing.

Progression-free survival is defined as the time from date of trial entry to date when progression is first observed and is censored at date last seen free of local recurrence in those patients who have not experienced the event.

Overall survival
Overall survival is defined as the time from date of trial entry to date of deaths from any cause and is censored at date last seen alive in those patients who have not experienced the event.

14.2 Statistical analysis

The main analysis will be carried out when all patients have completed their neoadjuvant therapy and had their assessment of response. The main analyses comparing the objective clinical response rates will be carried out with and without adjustment for the additional randomisation; in the 128 patients receiving celecoxib vs 128 receiving celecoxib-placebo, objective clinical response rates will be assessed using the chi-squared test and repeated using the Mantel-Haenszel test to allow for the adjustment of exemestane or letrozole.
The secondary outcomes of survival and local control will be assessed using Kaplan-Meier survival curves and treatments will be compared using the log rank test. The effect of prognostic factors in addition to treatment will also be assessed using Cox regression models. All analyses will be carried out on an intention to treat basis.

14.3 Sample size

The objective clinical response rate for patients receiving letrozole is reported to be approximately 55%. Randomising 256 patients into the study will allow detection of absolute differences in excess of 15% between the celecoxib and placebo arms with 80% power at the 10% (two-sided) level of significance.

14.4 Timing of interim and final analyses

The first interim analysis will be carried out and presented to the independent DMC after 50 patients have been recruited into the trial. This analysis will assess recruitment, toxicity, compliance, number of adverse/treatment related events and response. It is anticipated that interim analyses will be carried out and presented to the DMC annually thereafter. See Section 10.8 for further details. The final analysis and publication of results for all outcomes except time-to-event measures will be carried out once all randomised patients have completed surgery. The final analyses and publication for time-to-event outcome measures will occur when all randomised patients have a minimum of 2 years follow-up and again at a minimum of 5 years follow-up.

15. ETHICAL AND REGULATORY STANDARDS

15.1 Ethical / regulatory conduct of the study

This study will be conducted in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association (WMA) Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989), and South Africa (1996) (Appendix 3).

This study will be carried out under a CTA and conducted in accordance with EU Directive 2001/20/EC and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments.

The protocol will be submitted for ethical approval prior to circulation, in accordance with the new guidance in force from March 1st 2004. Before enrolling patients into the study, each site must apply for Site Specific Assessment from their Local Research Ethics Committee and must also obtain Trust Research & Development (R&D) approval. Sites will not be permitted to enrol patients until written confirmation of ethical and R&D approval is received by the NEO-EXCEL Study Office. It is the responsibility of the Investigator to ensure that all subsequent amendments gain the necessary approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

15.2 Patient confidentiality

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation and the Data Protection Act (2018).

With the patient's consent, their full name, date of birth, National Health Service (NHS) number, or in Scotland the Community Health Index (CHI), hospital number and GP details will be collected at trial entry to allow tracing through the Cancer Registries and The NHS Information Centre for Health and Social Care (service formally provided by the Office of National Statistics) and to assist with long-term follow-up via other health care professionals (e.g. patient's GP). Patients will be identified using only their unique trial number, initials, hospital number and date of birth on the CRF and correspondence between the NEO-EXCEL Study Office and the participating site. However, patients are asked to give permission for the CRCTU to be sent a copy of their signed Informed Consent Form which will not be anonymised. This will be used to perform in-house monitoring of the consent process and may also be forwarded to other health care professionals involved in the treatment of the patient's breast cancer (e.g. patient's GP).

The Investigator must maintain documents not for submission to the Study Office (e.g. Screening/Enrolment Logs) in strict confidence. In the case of special problems and/or governmental queries, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

The CRCTU will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party, other than those directly involved in the treatment of the patient's breast cancer and organisations for which the patient has given explicit consent for data transfer (e.g. Cancer Registries). Representatives of the trial team may be required to have access to patient notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

16. TRANSLATIONAL SCIENCE

Whilst there is extensive in vivo and in vitro data to suggest that inhibition of the COX 2 mediated synthesis of prostaglandins may promote tumour response and prevent tumour spread, there is no clear consensus as to the relevant mechanisms and their relative importance. Early experimental evidence suggests that prostaglandin E2 (PGE2) expression is elevated in breast cancer [52] and that this is associated with tumour invasion[53]. Prostaglandins are implicated in a wide range of molecular actions including: the induction of interleukin6 (IL-6) and other inflammatory cytokines[54], neo-vascularisation and tumour invasion/proliferation. The rationale for linking COX 2 inhibitors with aromatase inhibitors in endocrine-responsive disease is due to the action of PGE2, mediated via the 1.3 promoter of the aromatase gene itself and leading to enhanced aromatase expression as a direct consequence of COX 2 activity. Given the increasingly widespread use of celecoxib as a novel anti-tumour agent there is a strong need for a clearer understanding of the mechanisms by which COX 2 mediates its actions in vivo. NEO-EXCEL represents an ideal opportunity to investigate this question and to provide strong evidence for predictive factors that may be used to inform future treatment decisions.

As a secondary objective, we intend to exploit the tissue collected by the NEO-EXCEL study to investigate expression profiles relating to tumour response to steroidal (exemestane) versus non-steroidal (anastrozole/letrozole) aromatase inhibitors. Patients with metastatic disease who have progressed after treatment with non-steroidal inhibitors (anastrozole and letrozole) have been treated with exemestane. Of 241 patients treated, 24% experienced clinical benefit (CR + PR + SD) lasting for a median time of 37 weeks[55]. This suggests that there is a degree of non cross-resistance between these classes of aromatase inhibitors. Secondly, there appear to be differences between the molecular profiles of tumours responsive to steroidal irreversible (exemestane) and non-steroidal reversible inhibitors of aromatase, as suggested by data presented at the San Antonio breast cancer symposium[56]. We therefore propose, within NEO-EXCEL, to establish a tissue and serum bank for future gene expression profiling and proteomic analysis to answer two key questions. Firstly and most importantly, we hypothesise the expression profiling of sequential tumour biopsies will identify molecular mechanisms which underpin the effects of the COX 2 inhibitor celecoxib in the context of neoadjuvant therapy of breast cancer. Secondly, we further hypothesise that markers of differential response to aromatase inhibitors will be identified in a linked analysis.

Our aim is to collect three sequential tumour samples (pre-treatment, 2 weeks after therapy initiation and at surgery) from patients randomised between the different aromatase

inhibitors and also between celecoxib and placebo; these timepoints have been chosen to link to previous studies (Dixon et al, Dowsett et al) of early response to hormone therapy. We also aim to analyse, in a subset of patients (participating in the frozen tissue sub-protocol), sequential tumour biopsies and the changes in Gene Expression Profiles (GEPs) of breast tumours from each treatment arm to determine, at the level of gene expression, the molecular differences induced in tumours during treatment with exemestane and letrozole +/-celecoxib combination therapy. We predict that a number of the identified differentially expressed genes will serve as useful response markers for adjuvant studies (e.g. TEAM/ATAC). A significant change post treatment may provide useful candidate response markers in the context of neoadjuvant treatment, which may be profiled in the wider tumour databank (tissue microarrays) from the NEO-EXCEL trial.

It is now clear that only a small number of genes are up-regulated in all breast tumours[57]. Furthermore, while single genetic markers in clinical specimens have often failed to be predictive of prognosis, recent studies have demonstrated that the GEP of multiple genes in combination can predict the response to tumours in terms of outcome[58] and can provide prognostic[59] and response information[60].

Affymetrix human GeneChip microarrays will be used to undertake this analysis, each containing oligonucleotides representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes. Such a set will include the majority of genes whose expression changes have been shown in the above studies to be relevant to the development or progression of different types of breast cancer. We propose to select 100 patients (50 patients from each treatment group) to yield 300 samples for expression array analysis. Our aim is to collect this material during the course of recruitment and to bank this material in a central laboratory prior to submission of a TRICC translational science project or CRUK project grant for the expression profiling of this material in conjunction with the CRUK Genome Profiling Laboratory at the Paterson Institute. Subsequently the predictive value of genes shown to be linked to tumour response from the study will be tested using material collected as formalin fixed tissue from the remainder (900 cases + 100 entered into the profiling sub-study). These profiles may also inform the analysis of samples in TRANS-REACT and TRANS-MA27.

Fixed tissue bank and serum bank

Patients consenting into the optional TRANS NEO-EXCEL sub-study will have primary core biopsies (taken prior to treatment) in order to provide material for diagnosis. Additional core biopsies will be taken for formalin fixation to provide material for the TRANS NEO-EXCEL research tissue bank. This will ensure that sufficient tissues, since core biopsies are not suitable for modifying into a tissue micro-array, will be available to test the hypotheses outlined below and those which may arise from the expression profiling described above.

In addition, we will retrieve tumour resection specimens to construct a tissue microarray in which to evaluate markers of clinical response identified by tissue expression profiling. Tissue will be collected at the central research laboratory (Endocrine Cancer Group) where material will be sectioned for further study/made into tissue microarrays.

The key hypothesis to be tested prospectively within TRANS NEO-EXCEL relates to the observed interaction between COX 2 and aromatase expression as a model to explain the efficacy of COX 2 inhibitors, such as celecoxib, in the treatment of invasive breast cancer.

The product of the COX 2 enzyme, PGE2, induces expression of the transcription factors Snail/Slug which co-operatively modulate the I.3 promoter region of the aromatase gene. Paradoxically Snail/Slug are also closely involved in the regulation of E-Cadherin expression. Loss of E-Cadherin has been associated with increased tumour metastatic potential. Using paired pre/post treatment samples from TRANS NEO-EXCEL we will test the hypothesis that reduction of PGE2 production in breast cancer, via celecoxib treatment, reduces the metastatic potential by up-regulation of E-Cadherin expression. We will further test the hypothesis that down-regulation of Snail/Slug expression is linked both to alterations in E-Cadherin expression and to a reduction in aromatase expression (each of these markers will be measured by immunohistochemistry). There has been significant recent progress towards the identification of an appropriate antibody for aromatase analysis in paraffin embedded tissues (presented at the AROMATASE 2004 meeting in Edinburgh). We recognise however that this latter hypothesis may be also influenced by the ability of aromatase inhibitors to initiate a positive feedback regulation pathway to increase aromatase expression. Thus we will explore both the relationship between changes within COX 2 treated and placebo treated tumours exposed to the two aromatase inhibitors selected for this trial. The secondary hypothesis which we will test in the context of NEO-EXCEL is that resistance to anastrozole, a competitive inhibitor of aromatase, may be related to up-regulation of aromatase, a mechanism which is less likely to impact exemestane, which is an enzyme poison.

It is likely that 10 sections will be available for most patients from the pre-treatment core biopsies. Using IHC ER, PgR, HER2 and COX 2 will be measured on all primary specimens. All subsequent results will be correlated with HER2 expression, COX 2 expression and also the degree of ER and PgR positivity (Allred scale) and any changes in these during aromatase inhibitor therapy. There will also be comparison with basic histology: nodal positivity, presence of LVI and grade of tumour as well as apoptosis (tunel assay) characteristics at the time of definitive surgery.

Serum response markers

Whilst tumour markers may be of value in predicting early response (de novo resistance/sensitivity) we recognise the value of dynamic markers of response which, it is widely accepted, are likely to be those detected in the serum of patients with cancer. Both serum and plasma will be collected. We aim to utilise known and identify novel response markers for dynamic assessment of response in the serum of breast cancer patients collected at routine clinical assessment/treatment visits. Serum samples will be collected at baseline, 2 and 16 weeks. All patients will, as appropriate, have a sample at the time of surgery or relapse. Serum HER2/EGFr, CA125, CA15-3 and CRP will be assessed as each has been implicated in the monitoring of disease response in breast cancer. In addition we aim to use a novel approach, protein expression profiling (www.raybio.com), to simultaneously profile 64 cytokines in the serum of patients to prospectively identify potential novel markers of response. Markers which show significant expression levels in pre-treatment sera, and a significant decline post treatment, may provide useful candidate response markers.

17. SPONSORSHIP AND INDEMNITY

This trial is a clinician-initiated and clinician-led study with a grant provided by Cancer Research UK and an educational grant from the pharmaceutical industry.

The University of Birmingham will be responsible for sponsorship of the trial.

The trial is being coordinated by the CRCTU. These offices do not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. As this is a clinician-initiated study, ABPI guidelines for patient compensation by the pharmaceutical industry will not apply. There are no specific arrangements for compensation made in respect of any serious adverse events occurring though participation in the study, whether from the side effects listed, or others yet unforeseen.

In terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation is only available via NHS indemnity in the event of clinical negligence being proven.

18. PUBLICATION POLICY

The results of the analysis will be published in the name of the NEO-EXCEL trial in a peer reviewed journal, on behalf of all collaborators. All presentations and publications, including abstracts, relating to the main trial must be authorised by the NEO-EXCEL Steering Committee.

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APPENDIX 1: ECOG PERFORMANCE STATUS

Status	Description
0	Asymptomatic, fully active and able to carry out all pre-disease performance without restriction.
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature e.g. light housework, office work.
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bed-ridden.
4	Completely disabled. Cannot undertake any self-care. Totally bed-ridden.

APPENDIX 2: ROYAL COLLEGE OF PATHOLOGISTS GUIDELINES

National recommendations for assessment of steroid hormone positivity by immunostaining.

Suggested scoring system

Score for proportion staining		Score for staining intensity	
0	No nuclear staining	0	No staining
1	<1% nuclei staining	1	Weak staining
2	1–10% nuclei staining	2	Moderate staining
3	11–33% nuclei staining	3	Strong staining
4	34–66% nuclei staining		
5	67–100% nuclei staining		

Adding the two scores together gives a maximum score of 8.

(J Clin Pathol 2000; 53:634-635)

APPENDIX 3: DECLARATION OF HELSINKI

1996 Version

Declaration of Helsinki:

**Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964
and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975, 35th World Medical Assembly, Venice, Italy, October 1983, 41st World Medical Assembly, Hong Kong, September 1989, and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996**

A. INTRODUCTION

1. It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated the fulfillment of this mission. The Declaration of Geneva of the World Medical Assembly binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

2. The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

3. In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

4. In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

5. Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

6. Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civic and ethical responsibilities under the laws of their own countries.

B. Basic Principles

7. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

8. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

9. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

10. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

11. Every biomedical research involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interest of the subject must always prevail over the interests of science and society.

12. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

13. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

14. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

15. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is a liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

16, When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

17. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

18. Whenever the minor child is in fact able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

19. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

C. Medical research combined with clinical care
(Clinical research)

20. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

21. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

22. In any medical study, every patient -- including those of a control group, if any -- should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or threapeutic method exists.

23. The refusal of the patient to participate in a study must never interfere with the physician- patient relationship.

24. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).

25. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-therapeutic biomedical research involving human subjects
(Non-clinical biomedical research)

26. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

27. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

28. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well being of the subject.

APPENDIX 4: RECIST CRITERIA

Response Evaluation Criteria In Solid Tumours (RECIST) Quick Reference:

ELIGIBILITY

Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

Measurable disease: the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions: lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

RESPONSE CRITERIA

Evaluation of target lesions

* Complete Response (CR):	Disappearance of all target lesions.
* Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
* Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
* Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

REPORTING OF RESULTS

- All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

- All conclusions should be based on all eligible patients.

- Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

- The 95% confidence intervals should be provided

APPENDIX 5: COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

Toxicities will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. The full CTCAE document is available on the National Cancer Institute (NCI) website, at the following address:
http://ctep.cancer.gov/reporting/ctc.html

APPENDIX 6:

CLASSIFICATIONS OF SEVERITY AND RELATIONSHIP TO THERAPY FOR ADVERSE EVENTS

Relatedness
A determination of relatedness to trial medication, concomitant trial specific and other medication is required for all SAEs reported in clinical trials.

The criteria applied are a determination of whether there is a reasonable possibility that the event is related to the investigational product. Note that a "reasonable possibility" does not include cases where there is only a remote or unlikely possibility that the SAE may have been caused by the product.

Severity
Adverse events will be reviewed using the National Cancer Institute Common Toxicity Criteria (NCI CTC) (Appendix 5). Any adverse events incurred but not categorised by the NCI CTC should be graded by the physician and be recorded using a scale of (1) mild, (2) moderate, (3) severe or (4) life threatening on the CRF, as defined below:

MILD	Does not interfere with subject's usual function
MODERATE	Interferes to some extent with subject's usual function
SEVERE	Interferes significantly with subject's usual function
LIFE THREATENING	Resulting in risk of death, organ damage or disability. Note the distinction between the gravity and the intensity of an adverse event. **Severe** is a measure of intensity; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria that define serious events.

ABBREVIATIONS AND DEFINITION OF TERMS

ABPI	Association of the British Pharmaceutical Industry
ACE	Angiotensin converting enzyme
AE	Adverse Event
AG	Aminoglutethimide
AI	Aromatase inhibitor
ALT	Alanine aminotransferase
APC	Adenoma Prevention with Celecoxib
AST	Aspartate transaminase
ASCO	American Society of Clinical Oncology
AR	Adverse Reaction
AUC	Area Under the Curve
BC	Breast Cancer
BUN	Blood Urea Nitrogen
CHF	Chronic Heart Failure
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CR	Complete Response
CRCTU	Cancer Research UK Clinical Trials Unit
CRF	Case Report Form
CT	Computed tomography
CTA	Clinical Trial Authorisation
CTAAC	Cancer Research UK Clinical Trials Awards & Advisory Committee
COX	Cyclo-oxygenase
DHT	Dihydroxytestosterone
DMC	Data Monitoring Committee
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
ECOG	Eastern cooperative Oncology Group
EORTC	European Organisation for Research and Treatment of Cancer
ER	Oestrogen Receptor
FCE	17- Hydro-exemestane (exemestane metabolite)
FDA	Food and Drug Administration
FISH	Fluorescent In-Situ Hybridization
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GEP	Gene Expression Profile
GI	Gastrointestinal
Hb	Haemoglobin
HPLC	High Performance Liquid chromatography
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
IBCSG	International Breast Cancer Study Group
IDSMB	Independent Data Safety Monitoring Board
ICCG	The International Collaborative Cancer Group
IES	Intergroup Exemestane Study
IMP	Ivestigational Medicinal Product
IRB	Institutional Review Board
ICH	International Conference of Harmonization
IHC	Immunohistochemistry
ISF	Investigator Site File
LFTs	Liver Function Tests
LH	Luteinizing hormone
LREC	Local Research Ethics Committee
MHRA	Medicines and Healthcare products Regulatory Agency
MREC	Multicentre Research Ethics Committee
MTD	Maximum Tolerated Dose
NCRN	National Cancer Research Network
NCI-CTC	National Cancer Institute Common Toxicity Criteria
NSABP	The National Surgical Adjuvant Breast and Bowel Project
NSAID	Non Steroidal Anti Inflammatory Drug

NYHA	New York Heart Association
OS	Overall Survival
PgR	Progesterone Receptor
PGE	Prostaglandin
PLT	Platelets
PR	Partial Response
RBA	Relative Binding Affinity
R&D	Research & Development
RECIST	Response Evaluation Criteria In Solid Tumors
RFS	Relapse (Recurrence)-Free Survival
RIA	Radio immuno-assay
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNO	Trial Number
TRICC	Translational Science Project
TSC	Trial Steering Committee
TTF	Time to Treatment Failure
TTP	Time To Progression
TWIST	Time Without Symptoms or Toxicity
UAR	Unexpected Adverse Reaction
UNL	Upper Normal Limit
USS	Ultrasound scanning
WBC	White Blood Cell Count
WMA	World Medical Association

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Serious Adverse Event Reporting:

🖷 **0121 414 8392 or 0121 414 3700**