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# A Phase I Pharmacokinetic and Randomized Phase II Trial of Neoadjuvant Treatment with Anastrozole plus AZD0530 in Postmenopausal Patients with Hormone Receptor Positive Breast Cancer


**Principal Investigator**
**Joyce M. Slingerland, MD, PhD**
**Braman Family Breast Cancer Institute**
**Division of Hematology Oncology**
**University of Miami Sylvester Comprehensive Cancer Center**
[redacted]

**Sub-Investigators**
**J. Hurley; S. Richman; R. Mahtani; M. Lippman; M. Jahanzeb; C. Calfa; A. Tobon Perez; C. Welsh; K. Hernandez**

**Collaborator**
**Mark Pegram**

**Statistician**
**Isildinha M Reis, PhD**

**Study Sites**
**University of Miami and Stanford University**


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## 1.0 OBJECTIVES

We propose to conduct a Phase I/randomized double-blind Phase II study design in order to test the tolerability and efficacy of AZD0530 when used together with anastrozole in therapy of ER+ and/or PR+ postmenopausal breast cancer. The Phase I pharmacokinetic (PK) cohort of the study (cohort A) will be conducted in postmenopausal women with metastatic disease and will ascertain safety and toxicity. Patients in the randomized Phase II cohort of the study (cohort B) will consist of postmenopausal women with newly diagnosed ER+ and/or PgR+, Her2- breast cancer who are randomized to either neoadjuvant treatment with anastrozole plus placebo, or anastrozole in combination with AZD0530. The Phase II cohort will permit extended assays of tolerability, initial estimates of efficacy, and the investigation of molecular predictors of drug efficacy.

### 1.1 Primary Objectives

**Cohort A (Phase I PK cohort in MBC patients):**
The primary objective of this open label phase I study will be to determine if a well-tolerated dose of AZD0530 can be used in combination with anastrozole for post-menopausal women with ER+ and/or PR+ metastatic breast cancer.

**Cohort B (randomized phase II neoadjuvant cohort):**
The primary objective will be to compare treatment groups (AZD0530 and anastrozole versus anastrozole with placebo) with respect to clinical response, defined as percentage change/reduction in tumor size, by investigator measurements at diagnosis and on completion of neoadjuvant treatment prior to surgery.

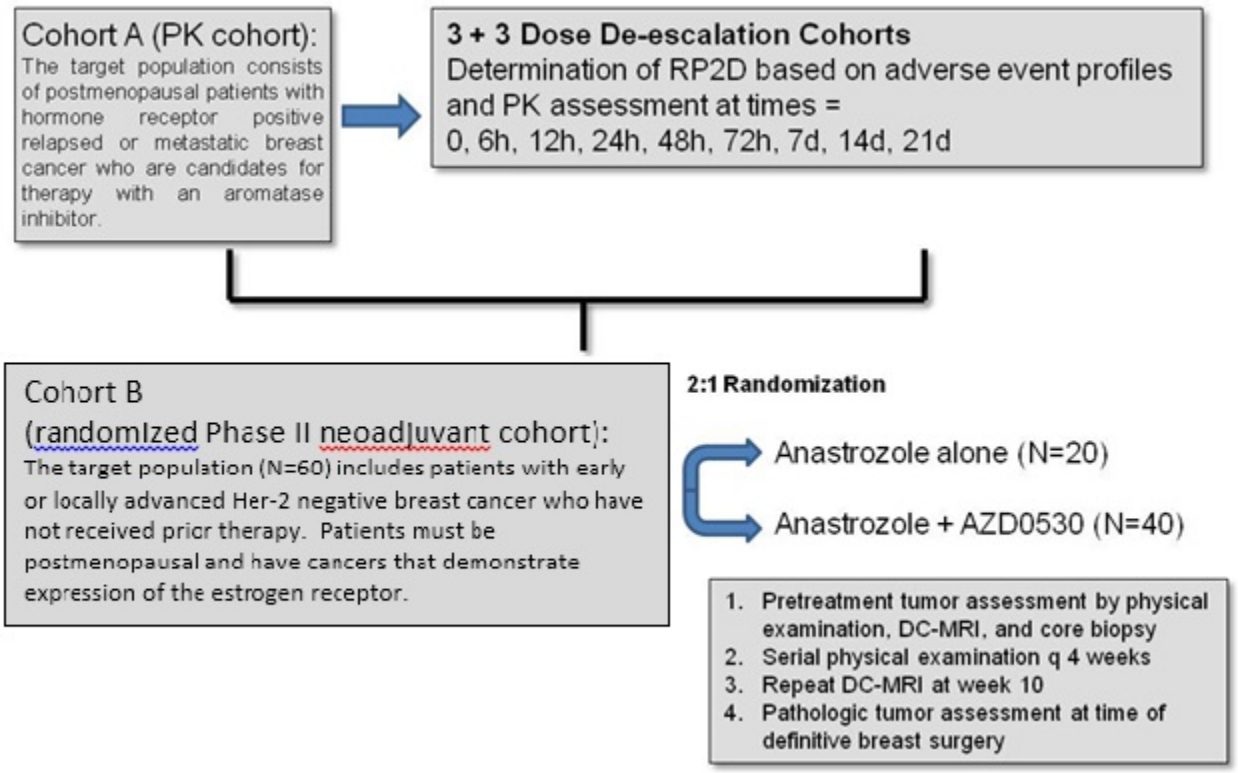
### 1.2 Secondary Objectives

**Cohort A:**
The secondary objective will be to test effects of giving the two drugs together on pharmacokinetics of both drugs.

**Cohort B:**
Secondary objectives will include evaluation of a) clinical response, defined as percentage change/reduction in tumor size, as measured by dynamic contrast-enhanced magnetic resonance imaging (MRI) measurements of tumor volume at diagnosis and on completion of neoadjuvant treatment prior to surgery b)pathological complete response (pCR) c) clinical response (complete

(CR), or partial (PR) responses) and clinical benefit (CR, PR, or stable disease (SD)), as measured by physician measurement of tumor size and by MRI measurements of tumor volume at diagnosis and prior to surgery; d) the qualitative and quantitative toxicities; e) to identify molecular/biologic correlates as indicators of treatment response [redacted]. Other biomarkers may be considered pending initial IHC results.

**1.3 STUDY SCHEMA**




220,000 new cases of invasive breast cancer are diagnosed per year in the United States. About two thirds of these express the estrogen receptor (ER). ER protein, assayed in newly diagnosed breast cancers, is a weak prognostic factor and predicts tamoxifen response. Over 120,000 new patients per year will start adjuvant therapy with an aromatase inhibitor or tamoxifen to prevent disease recurrence. In the USA alone, approximately one million breast cancer patients are on tamoxifen therapy and over 6 million are on this treatment worldwide. Aromatase inhibitors (AI), both reversible, non-steroidal (anastrozole and letrozole) and irreversible steroidal (exemestane) block peripheral conversion of adrenal androstenedione to estradiol and effectively cut off mitogenic estrogen to breast cancer in post-menopausal patients. AIs are now considered to be first line agents in both the metastatic and adjuvant settings for in post-menopausal women (Wong and Ellis, 2004).

## 2.0 BACKGROUND

### 2.1 Aromatase Inhibitors as Endocrine Therapy for Breast Cancer

**Targeting ER + breast cancer by inhibition of estradiol biosynthesis: aromatase inhibition**

Aromatase, product of the *CYP19* gene, is an enzyme of the cytochrome P-450 super family. It is expressed in several tissues, including subcutaneous fat, liver, muscle, brain, normal breast tissues, and mammary adenocarcinoma (Nelson and Bulun, 2001). Aromatase is responsible for the conversion of the adrenal androgen substrate androstenedione to estrogen in peripheral (non-ovarian) tissues (Evans et al., 1986), the predominant source of estrogen in postmenopausal women. Aromatase inhibitors (AIs) can reduce estrogen production by more than 90% (Geisler et al., 1998). Because AIs do not fully inhibit ovarian production of estrogen, only women without functioning ovaries are currently treated with AIs (Altundag and Ibrahim, 2006).

**The aromatase inhibitor anastrozole**

Anastrozole is a potent and highly selective non-steroidal aromatase inhibitor. In postmenopausal women, estradiol is produced primarily from the conversion of androstenedione to estrone through the aromatase enzyme complex in peripheral tissues. Estrone is subsequently converted to estradiol. Reducing circulating estradiol levels has been shown to produce a beneficial effect in women with breast cancer. In postmenopausal women, anastrozole at a daily dose of 1 mg produced estradiol suppression of greater than 80% using a highly sensitive assay. Anastrozole does not possess progestogenic, androgenic or estrogenic activity. Daily doses of anastrozole up to 10 mg do not have any effect on cortisol or aldosterone secretion, measured before or after standard ACTH challenge testing. Corticoid supplements are therefore not needed.

An extensive phase III clinical study program showed that anastrozole is an effective treatment of early breast cancer and advanced breast cancer in postmenopausal women suitable for endocrine therapy.

**Superiority of anastrozole over tamoxifen in metastatic breast cancer**

Several phase III studies have compared the efficacy of the AIs with that of tamoxifen as first-line therapy for metastatic breast cancer (MBC) (Bonneterre et al., 2001a); (Mouridsen et al., 2003); (Paridaens, 2004); (Bonneterre et al., 2000); (Nabholtz et al., 2000). The Tamoxifen and Anastrozole Randomized Group Efficacy and Tolerability (TARGET) trial was a multicenter

randomized trial conducted simultaneously in Europe and in North America (Paridaens, 2004); (Bonneterre et al., 2000)comparing anastrozole with tamoxifen as first-line therapy for MBC. While the n=282 European cohort did not show a significant difference between the two drugs, the322 patient cohort of the North American TARGET showed a significantly higher clinical benefit rate with anastrozole (59.1%) than with tamoxifen (45.6%; $p$ = .0098). The TTP was 11.1 months in the anastrozole group and 5.6 months in the tamoxifen group ($p$ = 0.005). On the basis of these two trials, anastrozole was approved as first-line therapy for MBC (Bonneterre et al., 2000); (Nabholtz et al., 2000). Analysis of the combined patient population in both trials ($n$ = 1,021; median follow-up, 18.2 months) showed that in the subset of patients whose tumors were positive for ER and/or PgR (excluding those with unknown receptor status) anastrozole was *superior* to tamoxifen with respect to TTP (median, 10.7 months for anastrozole and 6.4 months for tamoxifen; $p$ = .022) (Altundag and Ibrahim, 2006).

**Primary adjuvant AI treatment of early breast cancer**

Several trials have shown superiority of AIs over tamoxifen in the adjuvant therapy of breast cancer. The ATAC trial, (Buzdar, 2003) demonstrated superiority of adjuvant anastrozole over tamoxifen to prevent disease recurrence, with reduced time to progression and contralateral disease, and improved survival. Overall, anastrozole was well tolerated. Patients receiving anastrozole had a decrease in hot flushes, vaginal bleeding, vaginal discharge, endometrial cancer, venous thromboembolic events and ischemic cerebrovascular events compared with patients receiving tamoxifen. Patients receiving anastrozole had an increase in joint disorders (including arthritis, arthrosis and arthralgia) and fractures compared with patients receiving tamoxifen. A fracture rate of 22 per 1000 patient years was observed on anastrozole and 15 per 1000 patient years with the tamoxifen group with a median follow up of 68 months. The fracture rate for anastrozole falls within the broad range of the fracture rates reported in an age matched postmenopausal population.

The Breast International Group (BIG) 1-98 trial also indicate superiority of adjuvant letrozole over tamoxifen; indeed the survival benefit appeared to be modestly higher for letrozole over that observed with anastrozole (Thurlimann et al., 2005). The increased risks of thrombosis and endometrial cancer observed with long term tamoxifen are not seen with AIs. AIs are generally well tolerated, with 10-20% patients experiencing hot flushes, and up to 20% with arthralgias and increased menopause related osteopenia. Thus, AI-treated patients should be treated with vitamin D, calcium and if indicated, bisphosphonates to reduce risk of accelerating osteopenia.

In a phase III trial (ABCSG 8) conducted in 2579 postmenopausal women with hormone receptor positive early breast cancer being treated with adjuvant tamoxifen, patients had a superior disease-free survival when switched to anastrozole compared with those continuing on tamoxifen. Two further similar trials (GABG/ARNO 95 and ITA) with anastrozole, as well as a combined analysis of ABCSG 8 and GABG/ARNO 95, supported these results. The anastrozole safety profile in these 3 studies was consistent with the known safety profile established in post-menopausal women with hormone-receptor positive early breast cancer.

**Anastrozole metabolism and drug interactions**

Anastrozole is eliminated slowly with a plasma elimination half-life of 40 to 50 hours. Food slightly decreases the rate but not the extent of absorption. The small change in the rate of absorption is not expected to result in a clinically significant effect on steady-state plasma concentrations during once daily dosing of anastrozole tablets. Approximately 90 to 95% of plasma anastrozole steady-state concentrations are attained after 7 daily doses. There is no evidence of time or dose dependency of anastrozole pharmacokinetic parameters. Anastrozole is extensively metabolized by postmenopausal women with less than 10% of the dose excreted in the urine unchanged within 72 hours of dosing. Metabolism of anastrozole occurs by N dealkylation, hydroxylation and glucuronidation. The metabolites are excreted primarily via the urine.

Antipyrine and cimetidine clinical interaction studies indicate that the co-administration of anastrozole with other drugs is unlikely to result in clinically significant drug interactions mediated by cytochrome P450. A review of the clinical trial safety database did not reveal evidence of clinically significant interaction in patients treated with anastrozole who also received other commonly prescribed drugs. There were no clinically significant interactions with bisphosphonates. Tamoxifen and/or other therapies containing estrogen should not be co-administered with anastrozole as they may diminish its pharmacological action.

## 2.2 Neoadjuvant Endocrine Therapy

**Neoadjuvant therapy of early breast cancer**

A number of observations support the use of neoadjuvant therapy in early breast cancer. First, neoadjuvant therapy provides the earliest possible systemic treatment, potentially eradicating micrometastases prior to surgery through changes in tumor cell growth kinetics or alterations in the

balance of circulating pro- and anti-angiogenic factors (Fisher et al., 1989; Fidler and Ellis, 1994). In addition, neoadjuvant therapy permits an in vivo assessment of drug efficacy (clinical response), which may guide the choice of subsequent treatments. Finally, the likelihood that breast-conserving surgery can be performed is increased with the use of neoadjuvant therapy (Ellis and Smith, 1996).

Several large randomized trials have compared neoadjuvant therapeutic strategies (with cytotoxic chemotherapy regimens) to standard adjuvant treatments (Powles et al., 1995); (Scholl et al., 1994); (Semiglazov et al., 1994); (Mauriac et al., 1991); (Fisher et al., 1997). By far the largest phase III study is the NSABP B-18 trial, which randomized 1,523 patients with operable breast cancer to preoperative or postoperative doxorubicin-cyclophosphamide (AC) chemotherapy (Fisher et al., 1997). There was no significant difference in disease-free or overall survival between the groups and more patients receiving neoadjuvant chemotherapy underwent breast-conserving surgery (68% vs 60%). In light of these data, the concept of neoadjuvant therapy is now well established, with this approach being considered *a standard treatment option* for patients with early breast cancer (Macaskill et al., 2006).

**Neoadjuvant endocrine therapy for breast cancer**

Neoadjuvant antiestrogen therapy for hormone receptor positive early stage breast cancer in postmenopausal women is attractive for a number of reasons. Patients receive a form of systemic therapy that in the adjuvant setting is as good as chemotherapy in providing long term freedom from relapse or death (Abe et al., 2005). The improved potential for breast conserving therapy with neoadjuvant endocrine therapy is as good as with neoadjuvant chemotherapy (Ma and Ellis, 2006a) and there is no indication that deferring surgery during neoadjuvant endocrine treatment is detrimental. Hormonal therapy has a well-tolerated toxicity profile compared to chemotherapy. Neoadjuvant therapy allows immediate assessment of short term efficacy of therapy. Finally, in this context, serial tumor samples can be obtained for ascertainment of biologic endpoints or markers of disease response. Biomarker studies can be done to help define the subgroup of tumors that are likely to respond to therapy. Although pathologic complete remission (pCR) tend to be low in neoadjuvant hormonal therapy studies, clinical response rates of 37-55% are reported for AIs and can be corroborated by imaging studies and molecular markers of cell proliferation and death (Ma and Ellis, 2006b). In addition to providing information about short term efficacy of therapy and identifying predictive biomarkers, *neoadjuvant* therapy also has the potential to predict efficacy of endocrine treatment in the *adjuvant* setting. Powerful results obtained with new targeted

therapies and the identification of predictive biomarkers in the *neoadjuvant* setting would provide strong impetus to initiate more cost and labor intensive phase II-III clinical trials in the adjuvant setting.

**Tamoxifen as neoadjuvant therapy for breast cancer**

Tamoxifen is a competitive antagonist of estradiol binding to the estrogen receptor. Tamoxifen has been used in lieu of surgery in frail and elderly patients, with response rates of 49-68% and stable disease in up to 30% of patients. Median time to maximal tamoxifen response is reported as 14 weeks (Akhtar et al., 1991). Hormonal therapy has been used in postmenopausal patients with locally advanced breast cancer to reduce tumor bulk to allow breast conserving surgery. It is noteworthy that though pathologic complete response rates are low (<10%), lower pCR rates in ER-positive postmenopausal disease are also reported after neoadjuvant chemotherapy. Unfortunately, tamoxifen increases the risk for endometrial cancer by 2.4 times and the risk for thromboembolic disease by 1.9 times (Cuzick et al., 2003). Furthermore, despite 5 years adjuvant tamoxifen relapses continue even beyond 15 years after therapy (Fisher et al., 2001). Therefore, an alternative or additional hormonal therapy may help reduce breast cancer mortality more than tamoxifen does and have a more favorable toxicity profile (Altundag and Ibrahim, 2006).

**AIs versus tamoxifen as neoadjuvant therapy**

Several studies showed efficacy of AIs as neoadjuvant therapy for hormone receptor-positive breast cancers. In a phase II trial, 112 postmenopausal women with locally advanced ER-positive breast cancer received neoadjuvant anastrozole (Milla-Santos et al., 2004). Fifty-five percent of the patients had complete clinical responses, and 29% had partial clinical responses. However, an impressive 23% of the patients had complete pathologic responses. In a phase III randomized trial, 324 postmenopausal patients with stage II or III hormone receptor-positive breast cancer were treated with letrozole or tamoxifen as neoadjuvant therapy (Eiermann et al., 2001). The clinical response rate to letrozole was significantly higher than to tamoxifen (55% vs. 36%; $p < .001$). Patients receiving letrozole also had a higher incidence of breast-conserving surgery (Ellis et al., 2001).

The randomized, double-blind phase III Preoperative Anastrozole Compared with Tamoxifen (PROACT) trial compared 12 weeks of anastrozole versus tamoxifen as neoadjuvant therapy in 451 postmenopausal women with hormone receptor-positive breast tumors (Cataliotti et al., 2006b). Additional preoperative chemotherapy was optional and was determined before

randomization. In the subset of patients who received only hormonal therapy (*n* = 314), 43% of those treated with anastrozole had improved surgical status in favor of breast-conserving surgery compared with 31% of those treated with tamoxifen (*p* = .04) (Cataliotti et al., 2006c).

In the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) trial, 330 postmenopausal women with ER-positive, invasive, operable breast cancer were randomized in a double-blinded fashion to 3 months of preoperative treatment with anastrozole, tamoxifen, or both. The ORR was 37.2% in the anastrozole group, 36.1% in the tamoxifen group, and 39.4% in the combination group. In patients who were assessed as requiring mastectomy at baseline (*n* = 124), 44% received breast-conserving surgery after anastrozole compared with 31% of those given tamoxifen (*p* = .23); this difference became significant for patients who were deemed candidates for breast-conserving surgery by their surgeons (46% vs. 22%, respectively; *p* = .03). All treatments were well tolerated [43] (Altundag and Ibrahim, 2006).

To date only one trial has compared neoadjuvant chemotherapy with AIs (Semiglazov, 2007). Semiglazov et al randomized 121 patients to four cycles of doxorubicin 60mg/m$^2$ with paclitaxel 200 mg/m$^2$ versus exemestane or anastrozole for 3 months. Clinical response rates were encouraging and did not differ significantly (76% vs 82% vs 76%) nor did the pCR rates (7.4% vs 6.8% vs 4%). While this data suggest potential equivalency in response to neoadjuvant chemo or AI therapy, this small single institution study has not been repeated.

### 2.3 Activation of the Src oncogene: a mediator of antiestrogen therapy resistance

**Src activation is common in human breast cancer**

cSrc is over-expressed in up to 70% of primary human breast cancers. Up to 70% of primary human breast cancers show Src activation, with 40% at high levels. Src is activated by the IGF-1R, EGFR and HER2 and further phosphorylates and activates these receptor tyrosine kinases to stimulate cell proliferation, motility and survival. Estrogen bound ER recruits and activates Src, leading to Shc, and MAPK activation. Recent gene expression profiling has defined a gene signature of Src activation that is observed in up to 40% of human breast cancers (Nevins ref). This Src signature was a powerful predictor of antiproliferative responsiveness to a Src inhibitor drug in vitro. Overexpression of HER2 or Src in ER positive breast cancer cells can cause resistance to antiestrogen therapies by misregulating the cell cycle inhibitor, p27 (see below).

**The cell cycle inhibitor p27 is a key regulator of cell proliferation**

Since this protocol investigates how a Src inhibitor drug may increase responsiveness anastrozole by activating p27, the cell cycle will be briefly reviewed. Cell cycle progression is governed by cyclin dependent kinases (Cdks) (Reed et al., 1994) that coordinate cell cycle transitions. The Cdks are activated by cyclin binding and regulated by the cdk inhibitors, including p27. p27 is a key regulator of proliferation in mammary epithelial cells p27 binds and inhibits cyclin E-Cdk2, an activator of DNA replication and cell proliferation p27 levels are high in growth arrested cells and p27 levels must fall to allow cells to proliferate p27 null animals exhibit hyperproliferation of many organs and p27+/- heterozygous animals are more tumor prone. Oncogenic activation of signaling pathways leads almost invariably to loss of p27 function in human cancers, most commonly through accelerated p27 proteolysis. We and others have shown that p27 levels are frequently reduced in up to 60% of primary human breast cancers (Catzavelos et al., 1997).

**p27 is required for growth arrest by tamoxifen and by estrogen deprivation**

Estrogen stimulates breast cancer proliferation by causing p27 degradation and cyclin E-Cdk2 activation. Antisense inhibition of p27 expression caused cells arrested by tamoxifen, fulvestrant or estrogen deprivation to re-enter cell cycle, thus, p27 is required for the therapeutic antiproliferative effect of tamoxifen and AIs (Cariou et al., 2000). Others have made similar findings (Carol ref).

**Src phosphorylates p27 to promote p27 degradation**

Src plays a critical role to trigger the degradation of p27 (Chu et al., 2007). Since we had shown that estrogen-driven breast cell proliferation requires rapid p27 proteolysis (Cariou et al., 2000) and since Src was known to be activated in breast cancer by growth factors receptors and by estrogen, we investigated whether Src could contribute to p27 proteolysis. We showed that Src phosphorylates p27 and reduces its inhibitory function toward cyclin E-Cdk2, thereby facilitating subsequent p27 degradation (Chu et al., 2007). Data from human cancers also supports a relationship between Src activation and p27 loss. The levels of p27 and of activated cSrc (detected with an anti-pY416-Src antibody) were assayed by immunohistochemistry in 482 primary human breast cancers. Reduced nuclear p27 was seen in 37% of these tumors. Little or no cSrc activation was detected in a minority (23%) and 39% of the breast cancers showed strong cSrc activation. Of 392 ER positive cancers, 127/339 (37%) showed strong Src activation. This proportion is similar to the fraction of ER positive breast cancers that manifest *de novo*

antiestrogen resistance. Reduced p27 was strongly associated with Src activation (p=0.02) (Chu et al., 2007).

### 2.4 Pre-clinical Data Using Src Inhibitor AZD0530 with Anastrozole

**Src inhibitorAZDO530 cooperates with anastrozole to inhibit breast cancer growth in vitro**

Preclinical data indicates that the Src inhibitor drug, AZD0530 can cooperate with anastrozole to inhibit breast cancer cell growth. To make a breast cancer line that could be treated with aromatase, the aromatase gene was introduced into MCF-7 cells The resulting MCF-AROM5 line is estrogen dependent and arrests with a low % of cells S phase (%S) when deprived of estrogen, but resumes proliferation in the presence of androstenedione, reflecting the intracellular conversion of androstenedione to estrone by the aromatase enzyme. Cells were treated with anastrozole, or Src inhibitor (AZD0530) or both. While each drug alone had a partial effect, the two used together more effectively stopped cell proliferation (See Table 1).

**Table 1. Effects of Anastrozole, Src inhibitor or both together on cell cycle**

| | | | | | | |
|---|---|---|---|---|---|---|
| **estrogen** | **+** | **-** | **-** | **-** | **-** | **-** |
| **no estrogen** | **-** | **+** | **+** | **+** | **+** | **+** |
| **androstenedione** | **-** | **-** | **25nM** | **25nM** | **25nM** | **25nM** |
| **anastrozole** | **-** | **-** | **-** | **-** | **100μM** | **100μM** |
| **Src inhibitor** | **-** | **-** | **-** | **1μM** | **-** | **1μM** |
| **S%** | **43** | **16** | **53** | **48** | **27** | **14** |

Furthermore, when these cells were treated with a dose of AZD0530 that on its own had no effect, the dose of anastrozole needed to arrest cell growth was tenfold lower than when anastrozole was used alone. These data indicate synergy between AZD0530 and anastrozole in vitro.

**AZD0530 augments antitumor effects of anastrozole in breast xenografts in vivo**

MCFAROM5 was growth as xenograft tumors in nude mice. While AZD0530 alone had little effect on breast cancer xenograft tumor growth, and anastrozole partially inhibited tumor growth, growth inhibition by anastrozole was augmented by concurrent administration of AZD0530 (Fig 1). These data support clinical trials of Src inhibitors to prevent or delay the development of anastrozole resistance in ER positive breast cancer.

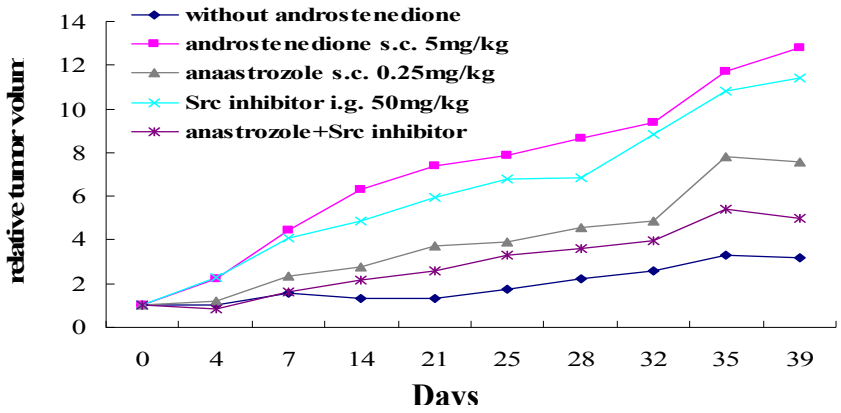



**Fig. 1 Anastrozole and Src inhibitor effects on *in vivo* growth of breast cancer xenograft tumors**. All of the groups except that marked without androstenedione received androstenedione s.c. 5mg/kg/d.

### 2.5 Clinical Development of the Src Inhibitor AZD0530

AZD0530 is a potent, orally available dual inhibitor of Abl and Src family kinases (Ple et al., 2004). It showed potent (<uM IC50) antiproliferative activity in 17 human cancer cell lines and inhibited growth of 6/13 xenograft tumor models. AZD0530 inhibits cell motility and invasion *in vitro* (Hiscox et al., 2005) and inhibits metastasis in animal models in vivo T. Green, ASCO Abstract , 2005).

Four Phase I studies in 163 healthy subjects have been completed and dosing is complete in a Phase I study in 237 patients with advanced solid malignancies (as of March 2008), providing data on pharmacokinetics, pharmacodynamics, bioavailability, safety and tolerability of AZD0530. AZD0530 is relatively slowly absorbed (median tmax of 5 hours) and is cleared moderately slowly (t1/2 of approximately 40 hours). AZD0530 is moderate inhibitor of CYP3A4. At steady state, the amount recovered in urine, (AZD0530 plus the N desmethyl metabolite) ranged from 10% to 18% indicating the relative importance of renal clearance. Food had no effect on the bioavailability of the AZD0530 Phase II formulation.

In a single ascending dose Phase I study, doses between 2.5 and 500 mg were well tolerated by healthy subjects and 1000 mg was declared as the maximum tolerated single dose (as a result of grade 3 diarrhea in 2 of 5 subjects). Multiply dosed healthy subjects showed a dose-related effect of AZD0530 on neutrophil and platelet counts. Neutrophil nadirs were above 1.0 x 109/L in all cases occurring between 10-14 days. For all individuals the counts returned to normal within 4

days of the nadir. A decrease in platelet count was seen in 1 of 9 subjects treated with 125 mg AZD0530 with nadir 118 x 109/L on Day 12, with recovery to within normal range during ongoing treatment. A dose-dependent rise in serum creatinine was noted which did not exceed the upper limit of normal and returned to pre-dose levels within 6 days after the final dose. In total, 163 volunteers and 237 patients have been exposed to AZD0530 as of March 2008. In heavily pre-treated cancer patients (mean # prior systemic therapies = 5; range 1-13) with advanced solid malignancies and high metastatic burden, events of ≥2 CTCAE Grade 2 occurring in greater than 10% of the study population included asthenia, anorexia, anemia, diarrhea, dyspnea, neutropenia, fatigue and anorexia. Many of these events are within the realm of what would be expected in this patient population. [redacted]

Preliminary efficacy analysis showed 10 patients had stable disease for longer than 21 days and 6 patients had stable disease for ≥60 days. These data indicate that Src inhibition in vivo by AZD0530 in human subjects is clinically feasible with oral bioavailability, predictable pharmacokinetics, and manageable toxicities. Please refer to the AZD0530 Investigator Brochure for descriptions of completed Phase I and ongoing phase II trials.

### 2.6 Summary of scientific rationale for targeting Src in combination with aromatase inhibition in ER+ postmenopausal breast cancer

Aromatase inhibition is a highly effective antiestrogen therapy for ER+ post-menopausal breast cancers and is more effective than tamoxifen for metastatic and adjuvant therapy (Wong and Ellis, 2004) . In the aromatase transfected cell line MCF-AROM5, we found that AZD0530 showed a synergistic inhibition of proliferation when added to anastrozole. Anastrozole together with AZD0530 caused a greater rise in the p27 level, a greater increase in p27 binding to its target cyclin E-Cdk2 and a greater proliferative arrest than did either drug alone (data not shown). Our xenograft data also indicate that AZD0530 could cooperate with anastrozole to arrest the growth of orthotopic MCF-

AROM5 tumors in nude mice (Fig 1). Taken together, These data, along with Phase I data indicating safety and tolerability of bio-effective doses of AZD0530 from Phase I trials in humans, provide a strong rationale for the proposed Phase I-randomized Phase II trials to test safety of co-administration of these drugs and then test their short term efficacy in a context in which molecular endpoints of drug effect can be ascertained in serial tissue samples from treated tumors.

**2.7 Scientific Rationale for the Study**

We postulated that Src inhibition would augment p27 to promote the anti-proliferative effects of anastrozole in ER positive breast cancer cells, leading to a greater reduction in tumor volume, and increase in pCR rate. Moreover, it is postulated that responsive cancers can be distinguished from poorly or non-responsive cancers by expression of a Src activated protein or gene expression profiles in the primary tumors and/or by changes in these parameters during treatment. We also postulate that changes in the viability of tumor initiating cells (cancer stem cells) may be critically linked to both immediate responsiveness to neoadjuvant therapy and to long term outcome. We will investigate the hypothesis that Src inhibition will augment p27 to promote the anti-proliferative effects of anastrozole in ER positive breast cancer, leading to a greater reduction in tumor volume, and increase in pCR rate. Moreover, we postulate that responsive cancers can be distinguished from poorly or non-responsive cancers by expression of a Src activated protein or gene expression profile in the primary tumors and/or by changes in these parameters during treatment. We also postulate that changes in the tumor initiating cells (cancer stem cells) may be critically linked to both immediate responsiveness to neoadjuvant therapy and to long term outcome.

**2.8 Rationale for AZD0530 Dosing and Scheduling**

As noted above, four Phase I clinical trials of AZD0530 have been completed, three in healthy volunteers and one in patients with advanced solid tumors. From the cancer patient study, an MTD of AZD0530 of 175mg po od has been established. At this dose no SAEs were experienced and few DLTs. Hemopoetic toxicity, with transient grade 3 neutropenia, was observed in <10% of study patients within the first 10-15 days of continuous oral therapy, returning to baseline with continued drug treatment. A non-physiologically relevant rise in serum creatinine (Cr) (mean 10%, maximum 25%) was not accompanied by a reduction in GFR tested pre- and post-treatment and returned to baseline within 6 days of stopping the drug. Among the 237 cancer patients and 163 healthy volunteers studied in Phase I trials and who have been exposed to AZD0530 prior to March 2008, no permanent change in Cr was noted. The 175 mg dose caused no significant, non-hematologic

toxicities. Diarrhea, significant only above 500 mg, was not above that in placebo treated controls at the 175 mg dose. A papular rash, noted at higher doses, was not observed at 175 mg.

As noted in the background section, AZD0530 is metabolized predominantly via the cytochrome P450 CYP3A4 enzyme. Phase I AZD0530 studies with concurrent medazolam administration indicate that AZD0530 administered concurrently with anastrozole may increase modestly the bioavailability of anastrozole. Anastrozole is well tolerated, with the most common adverse effect, hot flushes, seen in up to 20% patients, but diminishing over the first three months of continuous therapy. Other ADE includes joint pains and stiffness and symptoms related to reduce estrogen: vaginal dryness and hair thinning. Rare effects include rash, GI symptoms (nausea and diarrhea), carpal tunnel syndrome, in <0.1-1%. Earlier studies in women with metastatic breast cancer showed that both 1 mg and 10 mg daily oral dosing levels of anastrozole were effective and non-toxic (Buzdar et al., 1996). Thus, concurrent AZD0530 and anastrozole administration may modestly increase the anastrozole bioavailability but not to levels associated with adverse effects. Moreover, co-administration of anastrozole with AZD0530 is not anticipated to reduce bioavailability of AZD0530, nor is it expected to increase AZD0530 bioavailability, leading to increased drug toxicity. In vitro pharmacokinetic studies have shown that anastrozole can modestly impair CYP3A4 activity, but only when administered at 30 fold higher doses than those planned in the present study (Grimm and Dyroff, 1997). *Thus the serum levels of AZD0530 are not expected to be decreased or augmented by concurrent therapy with anastrozole and vice versa.*

The Phase I trial will test the safety, tolerability, and bioavailability of anastrozole given with AZD0530. Although such studies usually test PK on the new drug starting at 0.5X, then 0.75X and only later 1X the MTD of the new biologic agent, because the MDT of 175 mg AZD0530 has minimal ADE and because pharmacokinetic studies of both drugs indicate that neither should interfere with bioavailability nor augment toxicity of the other, we will start the Phase I study using the MTD of both drugs. Daily oral AZD0530 therapy will be added to anastrozole in patients with ER positive MBC whose disease has progressed after any hormonal therapy and have been on anastrozole for at least one week. *Toxicity* will be closely monitored and both *AZD0530 and anastrozole levels* will be measured before and at intervals after the addition of AZD0530.

**2.9 Rationale for Molecular Investigations**

In addition to assessing the safety of the anastrozole + AZD0530 regimen, another principal aim of this study is to investigate whether changes in gene expression, or the expression of specific

biomarkers, are either predictive of response to AZD0530 or indicative of response. Given the sample size, the molecular data obtained in this trial will be considered hypothesis-generating, with the ultimate goal being to develop additional trials which will identify which patients have a greater likelihood of benefiting from anastrozole + AZD0530 therapy. The ability to identify markers that can predict response, or are indicative of response, will allow more rational use of AZD0530 in breast cancer (and potentially other solid tumors).

We propose to assay molecular targets and upstream regulators of Src in diagnostic samples and resected cancers at mastectomy or lumpectomy after 4-6 months of treatment with anastrozole alone or anastrozole and AZD0530 [redacted] in an effort to define molecular endpoints of treatment response that could be used to guide patient selection and monitor disease response in subsequent trials.

Pre-clinical studies in cell lines and xenograft studies have suggested that tumor cells with the highest Src activation have the greatest anti-proliferative response to AZD0530. These data suggest that cancer cells may manifest “Src addiction”’ when this pathway is oncogenically activated and not tolerate its inhibition. Since Src activation is at least one of the most probable determinants of drug efficacy, we will test whether combined anastrozole/AZD0530 may be most effective in Src activated cancers. We will focus the molecular and cellular correlative assays to accompany this trial on pathways indicative of Src activation. [redacted] Actual protocols used will be updated at the time of molecular analysis on completion of study accrual.

**2.10 Rationale for Imaging Studies**

Dynamic Contrast Enhanced MRI

A considerable literature indicates that breast MRI may provide important advantages in documenting tumor response. It is possible using MRI to calculate tumor volume and gain graphic information about tumor blood flow (Tao et al., 2005) and (Hylton, 2006a). Recent MRI studies indicate that AIs may reduce tumor blood flow (Tao et al., 2005). MRI may provide a greater accuracy in clinical response measurement than ultrasound, that is highly operator dependent, than mammography, since some tumors are not mammographically detected. Dynamic contrast-enhanced MRI was shown to predict neoadjuvant chemotherapy responses (Martincich et al.,

2004c)and not only affords 3-dimensional tumor characterization, but has revealed distinct tumor imaging patterns that may predict treatment response (Esserman et al., 2001a), (Chou et al., 2007), (Yankeelov et al., 2007).. While MRI-measured tumor volume after two cycles of treatment was predictive of neoadjuvant chemotherapy response (Martincich et al., 2004b), (Hylton, 2006b), to date no studies have ascertained the predictive value of MRI after short term neoadjuvant endocrine therapy.

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) performed at high temporal resolution following the administration of gadolinium (Gd)-chelated contrast medium enables non-invasive evaluation of tumor and normal tissue vasculature. DCE-MRI studies of the breast can be performed to be sensitive to the vascular phase of contrast medium delivery which reflect on tissue perfusion and blood volume or sensitive to the presence of contrast medium in the extravascular extracellular space (EES) which reflect on microvessel permeability and extracellular leakage space in addition to perfusion (Padhani, 2003).

A number of studies have attempted to correlate the morphological findings on breast MRI with key histological features (Knopp et al., 1999); (Buadu et al., 1996); (Matsubayashi et al., 2000); (Stomper et al., 1997). For example, early rim enhancement appears to correlate with high microvessel density particularly in the tumor periphery, increased expression of VEGF, relatively small amounts of fibrosis and small cancer cell nests. Other studies have attempted to correlate the kinetic DCE-MRI parameters with immunohistochemical staining in breast cancer. Broad correlations between MRI parameters and MVD have been shown by some studies (Buckley et al., 1997); (Hulka et al., 1997) while others have found no correlation (Su et al., 2003); (Morgan, 2003). It appears that factors other than MVD are important in determining the degree of tissue enhancement (Knopp et al., 1999). For example, Knopp et al used high temporal resolution imaging to quantify contrast enhancement, and were able to calculate the intensity of enhancement (amplitude), redistribution rate constant ($k_{ep}$) and the elimination rate constant ($k_{el}$), in a study comparing the vasculature of benign and malignant breast lesions (Knopp et al., 1999). As a correlate, tissue vascular density was assessed using CD31 and expression of VEGF determined using immunohistochemistry. The results suggested there were significantly faster exchange rates in malignant lesions compared to benign breast tissue, distinct differences between histologic subtypes and a significant dependence of enhancement kinetics on VEGF expression. However, some authors have not found a correlation between VEGF expression and the degree of enhancement in breast cancer (Su et al., 2003).

A number of clinical studies have reported on the usage of DCE-MRI in phase 1 trials of antiangiogenesis cancer drugs (Morgan, 2003); (Jayson et al., 2002); (Galbraith et al., 2002); (Yung, 2003); (Galbraith et al., 2003). The use of DCE-MRI was to provide biological evidence of drug action and to impart confidence and thus accelerate the development of a particular drug or approach (go-no-go decisions). Good examples of the use of DCE-MRI in this way can be found in studies performed by Jayson et al, Morgan et al. and Galbraith et al (Morgan, 2003); (Jayson et al., 2002); (Galbraith et al., 2002); (Knopp, 2007). The value of DCE-MRI as an early predictor of the efficacy of neoadjuvant chemotherapy in patients with breast cancer has not been fully assessed. Both Knopp and Hayes et al. have shown that successful treatment causes decreases in the rate and magnitude of enhancement and that poor response results in persistent abnormal enhancement (Hayes et al., 2002); (Ah-See, 2004). DCE-MRI changes become pronounced after 2 cycles of therapy and DCE-MRI at that stage can be used to identify potential non-responders (Martincich et al., 2004a); (Leach et al., 2003). A small neoadjuvant trial in patients with inflammatory breast cancer utilized DCE-MRI to assess the effect of bevacizumab on the tumor before and after combining with the preoperative chemotherapy (Wedam SB, 2004). The results demonstrated marked changes in the permeability as measured by the $K_{21}$ as well as other measures of tumor blood flow.

The goal of functional imaging in this study is to establish a correlation between DCE-MRI parameters (as outlined above) with clinical/molecular parameters, and to identify whether DCE-MRI can be used as an early predictor of response to endocrine therapy with or without Src perturbation. Thus, in detail the aims are:

1. To assess whether parameters derived from the DCE-MRI study correlate with molecular parameters that reflect the underlying tumor response.
2. To assess whether changes in MRI kinetic parameters following the treatment anastrozole vs. anastrozole + AZD0530 enabling assessments of drug effect.
3. To determine if changes in DCE-MRI parameters after 10 weeks (+/- 5 business days) of combination therapy correlate with or predict final pathological outcome following anastrozole alone or anastrozole plus AZD0530.
4. To determine which MRI parameter is best in being able to predict pathological responsiveness/non-responsiveness to combination therapy.

## 3.0 PATIENT SELECTION

Cohort A (PK cohort): The target population consists of postmenopausal patients with hormone receptor positive relapsed or metastatic breast cancer who are candidates for therapy with an aromatase inhibitor.

Cohort B (randomized double-blind phase II neoadjuvant cohort): The target population includes female patients with invasive, ≥ 2 cm, HER2-negative breast cancer who have not received prior therapy. Patients must be postmenopausal and tumor must demonstrate expression of the estrogen receptor and/or progesterone receptor. Patients with distant metastatic disease are excluded from study. Patients with no distant metastasis but with local metastatic extension of tumor to skin will not be excluded. Patients may not have inflammatory breast cancer. Bilateral or multifocal invasive breast cancer is not an exclusion criteria. The patient may have concurrent DCIS but the DCIS will not be measured as part of the study endpoints.

### 3.1 Inclusion Criteria

**Inclusion Criteria (phase I PK cohort A)**

1. Female patient ≥ 18 years.
2. Patient must be postmenopausal, verified by 1 of the following:
    a. Bilateral surgical oophorectomy
    b. No spontaneous menses > 1 year
    c. No menses for < 1 year with FSH and estradiol levels in postmenopausal range

    If a study subject under the age of 60 reports prior surgery in which the ovaries were removed and if the operative report cannot be obtained to confirm bilateral salpingo-oophorectomy, the subject will have serum estradiol, LH and FSH drawn to confirm menopausal status prior to study entry.
3. Postmenopausal women with primary invasive breast cancer, histologically confirmed by core needle (or incisional biopsy), whose tumors are estrogen (ER) and/or progesterone (PgR) positive. Estrogen- and/or progesterone-receptor positive disease based on 10% or more nuclear staining of the invasive component of the tumor.
4. Stage IV disease (as defined by the AJCC Staging Manual, 6th Edition, 2002); or locally relapsed, unresectable disease.
5. Measurable or evaluable disease according to RECIST criteria (see appendix VI).

6. Both HER2-positive and HER2-negative disease (as defined by IHC or by fluorescence in situ hybridization [FISH]). HER2+ must have had prior treatment with trastuzumab and/or lapatinib.
7. ECOG performance status 0-2 (see appendix V).
8. Patients are suitable candidates for treatment with anastrozole (patients may have had any prior endocrine therapy or prior chemotherapy for treatment of their disease, either as adjuvant therapy, or as treatment for advanced disease). There is no restriction on the number of prior regimens in the phase I cohort A.
9. Patient is accessible and willing to comply with treatment and follow-up.
10. Patient is willing to provide written informed consent prior to the performance of any study-related procedures.
11. Required laboratory values
    a. Absolute neutrophil count $\geq$ 1.5 x $10^9$/L
    b. Hemoglobin $\geq$ 9.0 g/dL
    c. Platelet count $\geq$ 100 x $10^9$/L
    d. Creatinine $\leq$ 1.5 mg/dLTotal bilirubin $\leq$ 1.0 x upper limit of normal (ULN)
    e. Alkaline phosphatase and AST/ALT within the following parameters. In determining eligibility, the more abnormal of the two values (AST or ALT) should be used.

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| **TABLE 2** | **AST or ALT:** | | | |
|---|---|---|---|---|
| **ALK PHOS:** | **≤ ULN** | **>1x but ≤1.5x** | **>1.5x but ≤2.5x** | **>2.5x ULN** |
| **≤ ULN** | Eligible | Eligible | Eligible | Ineligible |
| **>1x but ≤2.5x** | Eligible | Eligible | Ineligible | Ineligible |
| **>2.5x but ≤5x** | Eligible | Ineligible | Ineligible | Ineligible |
| **>5x ULN** | Ineligible | Ineligible | Ineligible | Ineligible |

**Inclusion Criteria (randomized, phase II neoadjuvant cohort B)**

1. Female patient $\geq$ 18 years
2. Patient must be postmenopausal, verified by 1 of the following:
    a. Bilateral surgical oophorectomy
    b. No spontaneous menses $\geq$ 1 year
    c. No menses for < 1 year with FSH and estradiol levels in postmenopausal range

If a study subject under the age of 60 reports prior surgery in which the ovaries were removed and if the operative report cannot be obtained to confirm bilateral salpingo-oophorectomy, the

subject will have serum estradiol, LH and FSH drawn to confirm menopausal status prior to study entry.

3. Postmenopausal women with primary invasive breast cancer, histologically confirmed by core needle (or incisional biopsy), whose tumors are estrogen (ER) and/or progesterone (PgR) positive. Estrogen- and/or progesterone-receptor positive disease based on 10% or more nuclear staining of the invasive component of the tumor. Bilateral or multifocal invasive breast cancer is not an exclusion criteria. The patient may have concurrent DCIS in either breast but the DCIS will not be measured as part of the study endpoints.
4. Tumor size $\geq$ 2 cm
5. Tumor measurable either by clinical examination, mammography, MRI, or ultrasound.
6. HER2-negative disease (as defined by fluorescence in situ hybridization [FISH] or by IHC)
7. ECOG performance status 0-1 (see Appendix V)
8. Patient is accessible and willing to comply with treatment and follow-up.
9. Patient is willing to provide written informed consent prior to the performance of any study-related procedures.
10. Required laboratory values
    a. Absolute neutrophil count $\geq$ 1.5 x $10^9$/L
    b. Hemoglobin $\geq$ 9.0 g/dL
    c. Platelet count $\geq$ 70 x $10^9$/L
    d. Creatinine $\leq$ 1.5 mg/dL
    e. Total bilirubin $\leq$ 1.5 x upper limit of normal (ULN)
    f. Alkaline phosphatase and AST/ALT $\leq$ 1.5 x upper limit of normal (ULN).

### 3.2 Exclusion Criteria

**Exclusion Criteria (cohort A)**

1. Concurrent therapy with any other non-protocol anti-cancer therapy.
2. Any agent with estrogenic or putatively estrogenic properties, including herbal preparations, must be stopped at least one week prior to registration.
3. Ongoing, chronic administration of bisphosphonate therapy is allowed so long as such treatment was ongoing at the time of study entry.
4. Current therapy with hormone replacement therapy, or any hormonal agent such as raloxifene, tamoxifen, or other selective estrogen receptor modulators (agents must be stopped prior to randomization).

5. Presence of neuropathy ≥ grade 2 (NCI-CTC version 3.0) at baseline.
6. History of any other malignancy within the past 5 years, with the exception of non-melanoma skin cancer or carcinoma-in-situ of the cervix.
7. Clinically significant cardiovascular disease (e.g., hypertension [BP > 150/100], history of myocardial infarction or stroke within 6 months, unstable angina), New York Heart Association (NYHA) Grade II or greater congestive heart failure, or serious cardiac arrhythmia requiring medication.
8. Active, uncontrolled infection requiring parenteral antimicrobials.
9. A history of a severe hypersensitivity reaction to anastrozole, or AZD0530 or their excipients.
10. Evidence of bleeding diathesis or coagulopathy.
11. Resting EKG with measurable QTc interval of >480 msec at 2 or more time points within a 24 hr. period.
12. Since AZD0530 is a substrate and inhibitor of CYP3A4, patients requiring medication with drugs listed in Appendix X should be excluded from study.
13. Any evidence of severe or uncontrolled systemic medical or psychiatric conditions (e.g. Severe hepatic impairment, interstitial lung disease [bilateral, diffuse, parenchymal lung disease]) or current unstable or uncompensated respiratory or cardiac conditions which make it undesirable for the patient to participate in the study or which could jeopardize compliance with the protocol.
14. Evidence of underlying pulmonary dysfunction as evidenced by oxygen saturation <90% by pulse oximetry, interstitial pulmonary infiltrates on high resolution CT scan prior to study entry and/or symptomatic pulmonary (pleural or parenchymal) metastasis.

**Exclusion Criteria (cohort B)**

1. Prior chemotherapy, endocrine therapy, or radiotherapy for the presenting breast cancer. Prior incidence and treatment of contralateral invasive or non-invasive breast cancer is not an exclusion criterion.
2. Inflammatory BC, clinically defined as the presence of erythema or induration involving one-third or more of the breast, or pathologically defined as dermal lymphatic invasion.
3. Prior excisional biopsy or complete resection of the primary invasive tumor (prior sentinel node biopsy allowed).
4. Prior ipsilateral radiation therapy for invasive or non-invasive breast cancer.
5. Distant metastatic disease is an exclusion criterion – Isolated ipsilateral supraclavicular node involvement and/or direct invasion of the primary tumor into skin is allowed.

6. Concurrent therapy with any other non-protocol anti-cancer therapy.
7. Any agent with estrogenic or putatively estrogenic properties, including herbal preparations, must be stopped at least one week prior to registration .
8. Current therapy with hormone replacement therapy, or any hormonal agent such as raloxifene, tamoxifen, or other selective estrogen receptor modulators (agents must be stopped at least one week prior to randomization).
9. Presence of neuropathy $\geq$ grade 2 (NCI-CTC AE version 3.0) at baseline.
10. History of any other malignancy within the past 5 years, with the exception of non-melanoma skin cancer or carcinoma-in-situ of the cervix.
11. Clinically significant cardiovascular disease (e.g., history of myocardial infarction or stroke within 6 months, unstable angina), New York Heart Association (NYHA) Grade II or greater congestive heart failure, or serious cardiac arrhythmia requiring medication.
12. Active, uncontrolled infection requiring parenteral antimicrobials.
13. A history of a severe hypersensitivity reaction to anastrozole, or AZD0530 or their excipients.
14. Evidence of bleeding diathesis or coagulopathy.
15. Resting EKG with measurable QTc interval of >480 msec at 2 or more time points within a 24 hr. period.
16. AZD0530 is a substrate and inhibitor of CYP3A4. Since concurrent administration of AZD0530 with other CYP3A4 substrates has been shown to be well tolerated, continuation or initiation of medically indicated drugs that are substrates of CYP3A4 is permitted at MD discretion. Drugs listed in Appendix X that are known to strongly induce or inhibit CYP3A4 activity should be discontinued prior to study entry and should not be initiated during protocol treatment.
17. Any evidence of severe or uncontrolled systemic psychiatric or medical conditions (e.g. Severe hepatic impairment, interstitial lung disease [bilateral, diffuse, parenchymal lung disease]) or current unstable or uncompensated respiratory or cardiac conditions which make it undesirable for the patient to participate in the study or which could jeopardize compliance with the protocol.
18. Evidence of underlying pulmonary dysfunction as evidenced by oxygen saturation <90% by pulse oximetry prior to study entry and/or symptomatic pulmonary (pleural or parenchymal) disease.
19. Subjects unwilling or unable to undergo breast MRI as required by protocol will be excluded from study.

### 3.3 Enrollment procedures

A signed, written informed consent form must be obtained prior to initiation of any study-specific procedures. The screening for metastatic disease is part of routine patient work up and should proceed whether or not the patient has signed consent. Staging work-up is not paid for by the study.

Prior to the performance of any study-specific procedures or assessments, each subject must sign an IRB approved informed consent form. The investigator or member of the research team must explain to each subject the nature of the study, its purposes, the procedures involved, the expected duration, potential risks, and benefits, and any discomfort it may entail. Each subject must be informed that participation is voluntary, and that she may withdraw from the study at any time without prejudicing further medical treatment or her relationship with the treating physician.

The person obtaining consent, and the subject, must sign and date the informed consent form. The subject must receive a copy of the signed and dated consent form, and the original must be retained in the site's records.

The informed consent is considered part of the study protocol, and any changes to the informed consent must be approved by the IRB.

**Enrollment**

All eligible patients must be enrolled prior to the start of the study.

The Phase II portion of this study will be opened at both U of Miami (JMH and Sylvester Comprehensive Cancer Center) and at Stanford University (Dr. Slingerland PI at UM and Dr. Pegram as PI at Stanford). Enrollment procedures below are detailed for both sites (UM and Stanford).

To enter a patient, the investigator or study team member will contact the assigned study coordinator at the respective study site. All eligibility requirements must be reviewed by the study coordinator and the eligibility checklist must be completed and signed by the Principal Investigator or designated Sub-Investigator prior to initiating registration to enter the patient on study. All eligibility documents for Stanford patients must be reviewed and approved by the PI (J.

Slingerland) or her designees at UM prior to patient enrollment and randomization. The following information will be retained in the patient's study chart:

1) Completed and signed protocol-specific eligibility checklist;
2) All pages of the original signed informed consent forms (ICFs), including HIPAA Form B.
3) Relevant source documents such as: subject medical history and physical exam, admission or discharge notes, diagnostic reports, pathologic confirmation of diagnosis, and relevant subject-specific written communication.

The study coordinator will enter a patient on this study once eligibility requirements have been verified.

**Cancellation Guidelines**

If a patient does not receive protocol therapy, the patient may withdraw. Contact the assigned study coordinator at the respective study site or e-mail the information including the reasons for withdrawal within 10 working days. Patients who are enrolled on study but not treated will be excluded from all analyses.

**Randomization and Blinding in Cohort B**

For cohort B, after a signed informed consent has been obtained and eligibility has been established, patients will be randomized using a stratified permuted block design to ensure allocation 2:1 ratio between combined treatment of AZD0530 plus anastrozole (40 patients) or anastrozole plus placebo (20 patients). Patients will be stratified by study site (UM/SCCC or Stanford Cancer Institute), and baseline tumor size (≥ 2 and < 6 cm; ≥ 6 cm).

In order to preserve the double-blind nature of this study, the investigators, study team, and patients will be blinded to assignment of AZD0530 or placebo. The UM/SCCC CRS office will be responsible for centralized treatment assignment for all registered study patients. Patients will not be unblinded until accrual of all patients is completed. However, if a patient should experience a serious adverse event that is unexpected and possibly or likely drug related, unblinding will be permitted to assess the study drug related SAE as medically indicated. The investigator or sub-investigator will contact the sponsor who will arrange for unblinding of the individual.

Randomization lists for each site will be prepared by SCCC Biostatistics and provided to the SCCC CRS-Informatics office, where they will be maintained independent of the study team and implemented by CRS-Informatics at the UM site. Apart from designated unblinded research Pharmacists and monitors at each study site, members of the study team, including those responsible for patient enrollment, will not have access to the randomization lists.

After all eligibility requirements are met and signed off, UM CRS study coordinators will register the patient by entering the required information into an informatics database. The CRS coordinators at each site will not directly receive the designated study arm information. The automated patient registration and randomization software used for this study will automatically determine the patient's treatment assignment, and this information will be sent by e-mail to the "unblinded" Research Pharmacist at the respective site, in charge of providing study drug to patients at the particular site (UM/SCCC and Stanford Cancer Institute). After randomization is completed the CRS coordinator will provide a printed copy of the randomization confirmation to the Research Pharmacist at the particular site (UM/SCCC and Stanford Cancer Institute). All registration documents and randomization confirmations will be printed by the study coordinator and placed in the patient's research chart at each study site.

**Additional procedures**

All study procedures/treatments must occur within the following time periods (also refer to the Study Schema):

- No more than 28 days must elapse between registration and the start of protocol treatment. All screening studies and procedures must be performed in this window.
- In cohort B, repeat dynamic MRI should be performed on week 10 (+/- 5 business days) after the start of AZD0530. The final pre-surgical MRI study must be performed prior to definitive surgery.
- For cohort B, definitive surgery, should occur within 28 days after the final dose of AZD0530.
- The post-surgery visit should occur between 4-6 weeks after surgery.

### 3.4 Regulatory Authority Approval

The principal investigator will be responsible for obtaining approval, in accordance with all specific regulatory requirements, to conduct the study under appropriate regulatory agencies.

### 3.5 Ethical Conduct of the Study

This study will be conducted in accordance with Good Clinical Practice (GCP) and ICH guidelines, the Declaration of Helsinki (Edinburgh, Scotland Amendment, 2000), and all applicable local regulatory requirements.

### 3.6 Institutional Review Board/Ethics Committee

The protocol, informed consent form, and all other required documents will be reviewed and approved by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the IRB has approved the study must be forwarded to the sponsor prior to the initiation of the trial. Any amendments to the protocol must also be approved by the IRB, according to applicable regulations.

The Investigator is responsible for filing required progress reports with the IRB and with reporting serious adverse events, life-threatening events, and deaths. The Investigator is also responsible for filing with the IRB reports of serious adverse events occurring with study treatment in other trials, as reported by the sponsor. The Investigator is responsible for informing the IRB of the termination of the study.

## 4.0 TREATMENT PLAN

### 4.1 Cohort A (Phase I PK cohort) Study Treatment

**AZD0530 Dose Finding for Combined Treatment with Anastrozole**

We will begin by testing the combination of AZD0530 at 175mg with anastrozole 1 mg (both drugs administered once daily P.O.). Since the steady state anastrozole is reached in one week, study patients will be treated with anastrozole alone for one week, followed by three weeks of combined daily treatment of AZD0530 plus anastrozole. When given alone, steady state AZD0530 concentrations are reached in 11 days. Thus, we propose to evaluate dose limiting toxicity (DLT) during the first 21 days of combined treatment.

In this study, DLT is defined as any grade 3 or higher non-hematologic toxicity or a grade 4 hematologic toxicity as per NCI CTCAE v3.0, that is of clinical significance (as defined by the investigator as an event that results in an alteration to the planned routine management which would not normally be necessary), and occurs within the first 21 days of combined treatment and is

considered to be related to the combination of AZD0530 and anastrozole. Combined treatment will be continued for as long as the disease is stable or responding to treatment, provided the patient tolerates the treatment.

We will use the standard design (cohorts of 3-6 patients), testing initial dose of AZD530 at 175 mg/day (weeks 2-4) in combination with fixed 1 mg/day anastrozole (in weeks 1-4). In this study, simultaneous enrollment is only permitted under the following circumstances: if the first patient to receive a dose level does not experience DLT within the first 21 days of combined treatment, patients 2 and 3 may be enrolled simultaneously at the same dose level; if no DLTs occur in the first 3 patients evaluated for minimum 21 days of combined treatment, patients 4 and 5 may be enrolled simultaneously at the same dose level; and if no DLTs in first 4 patients, patients 5 and 6 may be enrolled simultaneously at the same dose level. This procedure ensures no more than two patients will experience DLTs among the first 6 patients treated.

Dose de-escalation of AZD0530: If one patient with DLT is observed in the first cohort of 3 patients, additional 1-3 patients will be enrolled at the 175 mg AZD0530 dose. If 2 out of 2-6 patients experience a DLT, we will dose de-escalate to accrue a further 2-6 patients at the dose of 125mg AZD0530 together with anastrozole 1 mg daily. (AZD0530 is available in pills of 50 mg and 125 mg). If 2 out of 2-6 of this de-escalated cohort have DLTs at 125mg AZD0530 and 1 mg anastrozole, this will lead to further dose reduction of AZD0530 at 100 mg, which will be tested in the same manner in cohorts of 2-6 patients.

**Table 3 AZD0530 Dose Reduction Guidelines**

| Drug | Starting dose | Dose Level -1 | Dose Level -2 |
|---|---|---|---|
| Anastrozole | 1 mg | 1 mg | 1 mg |
| AZD0530 | 175 mg | 125 mg | 100 mg |

No dose re-escalations will be permitted.

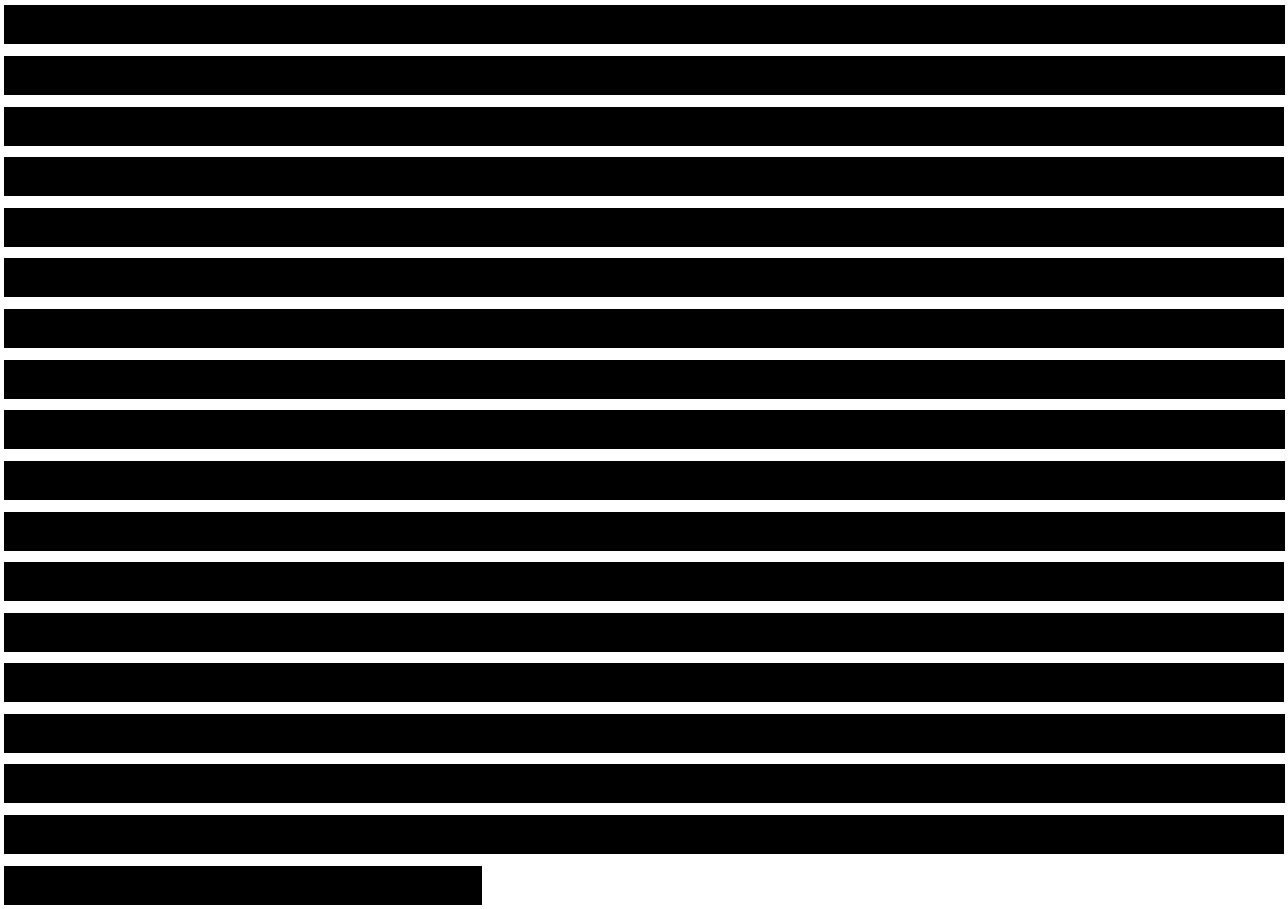
The MTD (maximum tolerated dose) of AZD0530 combined with fixed anastrozole 1 mg is defined as the highest dose of AZD0530 for which the incidence of DLT is less than 33% in 6 patients. We will enroll additional patients at the determined MTD of AZD0530 (in combination with anastrozole

1mg daily), to evaluate pharmacokinetics in a total of 12 patients. The determined MTD of AZD0530 will be then be used in the phase II portion of study.

**Pharmacokinetic evaluation**

At intervals after initiation of AZD0530 on day one at 6, 12, 24, 48, and 72 hours after the first dose of AZD0530; and on days 8, 15 and 22 blood will be drawn and sent to AstraZeneca for pharmacokinetic measurement of AZD0530 and anastrozole. Because of potential inter-patient variability in drug clearance, once drug doses that allow effective bioavailability, without undue toxicity are determined, we will accrue a total of twelve patients at those doses for complete PK determinations. Samples will be taken before the patients ingested the drug doses on the day of the sample to make sure the concentrations were actually at a minimum. Anastrozole concentrations during AZD0530 treatment would be compared with those measured prior to initiating AZD0530; AZD0530 concentrations would be compared with data on file at AstraZeneca.

**Summary of Phase I Data**



**4.2 Treatment Plan Phase II Cohort B (randomized neoadjuvant cohort)**

The phase I cohort of the study established a dose of AZD0530 that can be used together with anastrozole that is safe and potentially efficacious for subsequent Phase II and III studies in patients with locally advanced or metastatic breast cancer. The neoadjuvant context offers a number of advantages for Phase II drug development: 1. it allows a rapid in vivo response assessment; 2 the disease context allows comparison of diagnostic biopsy and surgical specimen to determine biologic indicators of treatment response; 3. promising new adjuvant endocrine strategies can be tested short term against standard treatment; 4. greater response rates, surgical outcomes and evidence of enhanced efficacy at the cellular level provide a sound basis for taking this therapy forward and committing the resources required for Phase III trials.

When a combination of non-toxic doses of both drugs is found that allows good bioavailability of both, we will proceed to a randomized *Phase II trial of anastrozole and AZD0530* for neoadjuvant first line treatment of postmenopausal patients with ER positive with newly diagnosed disease given for 4-6 months prior to surgery. There is considerable prior data on anastrozole given alone as neoadjuvant endocrine therapy (Smith et al., 2005; Cataliotti et al., 2006a) . We postulate that AZD0530 may reverse or delay development of resistance to AIs such as anastrozole and more effectively treat early breast cancers. Patients will be accrued in a 2:1 AZD0530 plus anastrozole vs. anastrozole plus placebo randomization protocol. The randomization modestly reduces our power to detect differences, but will give the greatest information about the potential safety and efficacy of the new drug combination. Patients will be treated with anastrozole and AZD0530 or placebo starting on day 1.Treatment efficacy will be indicated by clinical measurement and magnetic resonance imaging (MRI) of the primary tumor and the rate of pathologic complete response observed at the time of definitive surgery (lumpectomy or mastectomy). The randomization not only permits an indication of early efficacy, it will allow the cleanest ascertainment of molecular and cellular endpoints specific to the combined therapy that are not observed with anastrozole alone.

Patients with newly diagnosed ER and/or PR positive breast cancer (tumors ≥2 cm) will be randomized to receive anastrozole (1 mg po/day) plus placebo; or combined anastrozole 1 mg po/day and the dose of AZD0530 determined in the phase I cohort of study above (175mg), for up to 6 months prior to definitive lumpectomy or mastectomy. Informed consent must be signed before randomization and study drugs will be initiated within 10 business days of randomization. For study subjects at Stanford University, randomization will be done at U of Miami. Response to

AIs is typically slower than with neoadjuvant chemotherapy. Moreover clinical response with neoadjuvant AI at 4 months has been reported as greater than that at 3 months, suggesting that some may benefit from a greater duration of pre-operative therapy (Ma and Ellis, 2006). Responding patients therefore will be treated for up to 6 months. Progression on neoadjuvant anastrozole alone has been reported in as few as <11% (Smith, et al., 2005). Patients with primarily non-responsive disease or a clinically palpable tumor that responds initially then shows stability over the three subsequent 4-week cycles will have their preoperative MRI and proceed to surgery directly.

Inclusion criteria include a tumor that may be measurable either by clinical examination, mammography, MRI, or ultrasound at diagnosis.

For non-palpable tumors: If a tumor is not clinically palpable at diagnosis and is measurable only by radiologic means (MRI, mammography or ultrasound), treatment response will be evaluated by both physical exam and radiologic tumor evaluation. The first on treatment radiologic evaluation will be the planned MRI at 10 weeks. If the week 10 MRI shows the tumor size unchanged compared to pre-treatment, further radiologic evaluation will be performed 4 weeks later (14 weeks) (See Appendix I). Radiologic imaging must show decrease in tumor size within 14 weeks. If the tumor shows no size reduction at that time, the patient will be sent to surgery. Non-palpable tumors that increase in size radiologically will be sent for surgery. If the week 10 MRI shows size reduction, the next planned radiologic evaluation will be at 18 weeks for subjects completing 20-24 weeks or at end of study for subjects who stop therapy after 4 cycles. Responding tumors that decrease or remain stable on imaging between 10 and 18 weeks will complete planned therapy provided there is no sign of tumor progression on physical exam.

**Pharmacokinetic evaluation**

The day on which study drugs are first taken for Phase II Cohort B will be designated day zero. To extend PK data obtained from Cohort A, Cohort B patients will have PK draws on days 28 and at the follow-up visit of the next 2 cycles thereafter (days 56 & 84+/- 3 business days)).

### 4.3 Supportive Care Guidelines

**Use of Hematopoietic Growth Factors**

**i. G-CSF**

The use of G-CSF as primary prophylaxis is **not permitted**. G-CSF is permitted at the discretion of the treating investigator for patients with febrile neutropenia, or grade 4 neutropenia lasting >7 days. Patients receiving Neupogen® or Neulasta® should receive the following doses and schedules:

- Neupogen®: 300 ug or 480 ug subcutaneously for 7-10 days
- Neulasta®: 6 mg subcutaneously

**ii. Erythropoietic agents**

Erythropoietic agents may be used at the discretion of the treating investigator for patients with an Hgb ≤ 11 g/dL. Epoietin-alpha, epoietin-beta, or darbepoietin may be used, and each should be administered according to standard clinical practice guidelines for the Phase I trial only.

iii. **Prophylactic Antibiotics**

The use of prophylactic antibiotics is not permitted.

**4.4 Duration of Therapy**

Protocol treatment will continue until one of the following criteria is met:

- Completion of all prescribed protocol therapy.
- Disease progression (Phase I and II) and in the Phase II trial (Cohort B patients) disease stability over three successive 4-week cycles. In the Phase II trial, patients with palpable tumor size unchanged after 3 successive 4-week cycles will be sent for surgery. See criteria for evaluation of non-palpable tumors on page 36.
- Unacceptable toxicity (defined as toxicity necessitating the discontinuation of study drug, as outlined in Section 4.5 below).
- Withdrawal of patient consent.
- Continuation would, in the judgment of the investigator, not be in the best interests of the subject.

Clinical review for toxicity will be performed weekly for four weeks at the time of PK draws, and tumor assessments by physical examinations will then be monthly until evidence of disease

progression or removal from protocol for Cohort A. Patients in Cohort B will be assessed at baseline and then every 4 weeks (+/- 3 business days). Imaging studies will also be obtained serially as described in Section 5.2 below.

### 4.5 DOSE DELAYS/DOSE MODIFICATIONS

In the event of severe toxicities or failure of hematologic recovery, AZD0530 may be delayed up to 3 weeks. Any toxicity necessitating a treatment delay greater than 3 weeks will result in the discontinuation of study drug treatment. Patients will remain on study (but off AZD0530) during the period required to assure resolution of possible or actual AZD0530 related AEs. Since anastrozole has been in use for decades and is very well tolerated on its own, and since anastrozole is of known significant efficacy in the neoadjuvant setting for patients being treated with curative intent, patients are to remain on study-supplied anastrozole during evaluation of potential AZD0530 toxicity.

Dose reductions should be made according to the organ system demonstrating the greatest degree of toxicity. Dose reductions should be made according to Table 4.

No dose re-escalations will be permitted.

**Dose Delays for Neutropenia**

The following modifications should be instituted for neutropenia:

**Table 4 Delayed ANC Recovery Guidelines**

| **Neutrophil count (x $10^9$/L)** | **Action to be taken** |
|---|---|
| $\geq$ 1.5 | Treat on time |
| < 1.5 | Delay AZD0530 for 1 week and repeat CBC<br>1. If ANC $\geq$ 1.5, proceed with full-dose AZD0530<br><br>2. If ANC < 1.5, hold AZD0530 for one additional week<br>If no recovery by day 14, patient should discontinue AZD0530 |

**Dose Reductions for Febrile Neutropenia**

Febrile neutropenia is defined as a fever $\geq$ 38.5 in the presence of neutropenia (ANC < 1.0 x $10^9$/L).

Febrile neutropenia should be immediately treated according to institutional guidelines. Such treatment should include the use of empiric antibiotics and hospital admission if indicated.

In-hospital care for neutropenic fever will be per standard of care.

After a first episode of febrile neutropenia, AZD0530 should be reduced one dose level per table 4, and use of g-CSF considered.

Patients who develop two episodes of febrile neutropenia will not receive further study drug treatment and will be discontinued from the study.

**Dose Reductions for Infection**

In the event a patient develops grade 3 or grade 4 infection during treatment with AZD0530, the drug will be reduced one dose level.

Patients who develop two episodes of grade 3 or grade 4 infection despite G-CSF treatment will not receive further study drug treatment.

**Thrombocytopenia**

The following dose modifications will occur for thrombocytopenia, based on the platelet count:

**Table 5 Dose Modifications for Thrombocytopenia**

| Platelet Count (x $10^9$/L) | Dose Modification |
|---|---|
| > 50 | No dose modification. Treat as planned. |
| < 50 | Hold AZD0530 and repeat platelet count weekly.<br>If count > 50 after delay, reduce AZD0530 one level for current and all subsequent cycles.<br>If count < 50 after 2 weeks, discontinue study drug treatment. |

**Anemia**

The use of erythropoietic agents is permitted in all patients who develop an Hgb < 11 g/dL while receiving AZD0530 in the Phase I study. Erythropoietic agents should be administered according to institutional guidelines. Blood transfusions and/or an erythropoietic agent should be administered for all patients developing grade 3 or greater anemia. For patients developing grade 3 or grade 4 anemia, AZD0530 should be reduced one dose level and the patient should be considered for treatment with an erythropoietic agent (Phase I only).

**Other Toxicities**

All toxicities should be treated symptomatically whenever possible.

For grade 3 toxicities, AZD0530 will be held for a maximum of 3 weeks, until resolution to ≤ grade 1, and then reinstituted if medically appropriate. AZD0530 should be reduced one dose level for all subsequent cycles. If the toxicity does not resolve to ≤ grade 1, study drug treatment will be discontinued.

The Phase I part of this study will exclude patients with interstitial lung disease as diagnosed by baseline high resolution CT scan. Further evaluation for development of interstitial pneumonitis will include a second and third high resolution CT scan, at 8 and 16 weeks respectively, during the Phase I study only. Decreased O2 saturation by pulse oximetry, or new pulmonary symptoms (dyspnea and/or cough) shall prompt further pulmonary studies using high resolution CT to look for evidence of pulmonary interstitial pneumonitis. If interstitial lung disease (bilateral, diffuse, parenchymal lung disease) is observed during these evaluations AZD0530 therapy should be permanently discontinued and appropriate management should be initiated as per local practice.

Study drug treatment will be discontinued for all grade 4 non-hematologic toxicities.

**Discontinuation of AZD0530 Alone**

Patients requiring discontinuation of AZD0530 for AZD0530-specific toxicity will be removed from the study after the appropriate follow-up to monitor toxicity resolution (see section 9.8).

**Dose Reductions and Delays for Anastrozole**

No dose reductions will be permitted for anastrozole.

**Concomitant Treatment**

Patients should be maintained on the same medications throughout the study, as medically feasible. All concomitant medications will be recorded in the CRF with indication, dose information, and dates of administration up until the date of surgery or end of study biopsy for the Phase II Cohort B patients. Please refer to the “Appendix X List of medications which strongly inhibit or induce the CYP3A metabolic pathway”. Since concurrent administration of AZD0530 with other CYP3A4 substrates has been shown to be well tolerated, continuation of medically indicated drugs that are substrates of CYP3A4 is permitted at MD discretion. AZD0530 administered concurrently with CYP3A4 substrates may increase modestly drug bioavailability and careful review of concurrent medications and AEs is required at each follow up visit while Phase II Cohort B patients are on therapy.

The following will not be permitted:

- Prophylactic antibiotics
- Any other investigational agent.

## 5.0 CLINICAL AND LABORATORY EVALUATIONS

### 5.1 Baseline/Pretreatment Assessments

The following will be obtained at screening:

**Table 6 Screening Assessments (both Phase I and II)**

| Assessment/Procedure | Explanation | Timing |
|---|---|---|
| Informed consent | Signed | Prior to registration |
| Medical history | General medical history including prior and concomitant conditions and medications (phase II only); tumor history including date of diagnosis, histology, stage, all prior therapy, hormone receptor status, and HER2 status by FISH or IHC; history of all baseline toxicities and ECOG performance status | Within 28 days prior to start of protocol treatment |
| Physical examination | Physical exam performed by an ARNP or physician, including vital signs and clinical tumor status by physical examination (bi-dimensional) if palpable | Within 28 days prior to start of protocol treatment |
| Electrocardiogram | 12-lead ECG | Within 28 days prior to start of protocol treatment |
| Dynamic MRI (cohort B only) | MRI of breasts | Within 28 days prior to start of protocol treatment. |
| Hematology | Complete blood count including differential | Within 28 days prior to start of protocol treatment |
| Standard chemistry evaluation | Sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, alkaline phosphatase | Within 28 days prior to start of protocol treatment |
| HER2 status | By FISH or IHC | Prior to start of protocol treatment |
| Hormone Receptor Status | ER and PgR status by IHC | Prior to study enrollment |
| Tumor status | Documentation of measurable and non-measurable disease for Phase I. For Phase II: clinical tumor status by physical examination (bi-dimensional measurements) at time of entry or by radiologic imaging if non-palpable. | Within 28 days prior to study entry |
| Radiologic assessment to exclude metastases in | Will include bone scan and CT abdomen with and without contrast and a CT scan of | Prior to study entry |

| | | |
|---|---|---|
| cohort B | lungs that may be done without contrast. | |
| Tumor tissue collection (snap frozen and paraffin-embedded) | See Appendix II for collection requirements | Prior to start of protocol therapy |
| O2 saturation | Pulse oximetry (room air) | Within 28 days prior to start of therapy |

## 5.2 Assessments during AZD0530 treatment

**Table 7A Phase I Cohort A**

| **Assessment/ Procedure** | **Explanation** | **Timing** |
|---|---|---|
| Toxicity Evaluation | History of all toxicities or adverse events (AE) and ECOG performance status | Every 28 days ± 3 business days |
| Physical examination | Physical exam, including vital signs, and bi-dimensional tumor measurement if measurable clinically | Every 28 days ± 3 business days |
| Hematology | Complete blood count including differential | Every 28 days ± 3 business days |
| Standard chemistry evaluation | Sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, alkaline phosphatase | Every 28 days ± 3 business days |
| Tumor status | Documentation of clinical tumor status by physical examination if palpable and by radiologic investigations CT scans of chest, abdomen, and pelvis (and bone scan, CT brain scan and/or PET scan if clinically indicated) | Every 8 weeks ± 3 business days |
| High resolution lung CT scan | [redacted] | Week 8 and 16 if still on protocol ±2 weeks |

**Table 7B Phase II Cohort B**

| Assessment/ Procedure | Explanation | Timing |
|---|---|---|
| Toxicity Evaluation | History of all toxicities or AE and concomitant medication and ECOG performance status | Every 28 days ± 3 business days |
| Physical examination | Physical exam, including vital signs, and bi-dimensional breast tumor measurement* | Every 28 days ± 3 business days |
| Hematology | Complete blood count including differential | Every 28 days ± 3 business days |
| Standard chemistry evaluation | Sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, alkaline phosphatase | Every 28 days ± 3 business days |
| Dynamic breast MRI | To monitor tumor size | Week 10 ± 5 business days, and prior to definitive surgery (within 4 weeks ± 3 business days of stopping study drug) |

** **For non-palpable tumors:** If a tumor is not clinically palpable at diagnosis and is measurable only radiologically (MRI, mammography or ultrasound), treatment response will be evaluated by both physical exam and radiologic tumor evaluation. The first on treatment radiologic evaluation will be the planned MRI at 10 weeks. If the week 10 MRI shows the tumor size unchanged compared to pre-treatment, further radiologic evaluation will be performed 4 weeks later (14 weeks). If tumor size is not reduced at that time, the patient will be sent to surgery. Non-palpable tumors that increase in size radiologically will be sent for surgery. If the week 10 MRI shows size reduction, the next planned radiologic evaluation will be at 18 weeks for subjects completing 20-24 weeks or at end of study for subjects who stop therapy after 4 cycles. Responding patients whose tumors decrease or remain stable on imaging between 10 and 18 weeks will complete planned therapy provided there is no sign of tumor progression on physical exam.

### 5.3 Cohort B Assessments at End of Anastrozole + AZD0530/Presurgical Visit

The following assessments and procedures will be performed at the conclusion of neoadjuvant therapy, prior to definitive surgery (if surgery is to be performed) or radiotherapy:

**Table 8 Assessments at End of Neoadjuvant Therapy (Pre-surgical Visit or Early Study Withdrawal)**

| Assessment/Procedure | Explanation | Timing |
|---|---|---|
| Toxicity evaluation | History since prior exam, including history of all toxicities or AE, concomitant medication and ECOG performance status | Within 4 weeks prior to surgery or at withdrawal from study |
| Physical examination | Physical exam, including vital signs, weight | Within 4 weeks prior to surgery or at withdrawal from study |

| | | |
|---|---|---|
| Hematology | Complete blood count including differential | Within 4 weeks prior to surgery or at withdrawal from study |
| Standard chemistry evaluation | Sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, alkaline phosphatase | Within 4 weeks prior to surgery or at withdrawal from study |
| Tumor status | Documentation of bi-dimensional clinical tumor size by physical examination | Within 4 weeks prior to surgery or at withdrawal from study |
| Tumor tissue (snap frozen)* | See Appendix II for collection requirements | At the time of definitive surgery or at withdrawal from study* |
| Pathologic assessment | Evaluation of tumor specimen in patients undergoing mastectomy or breast-conserving procedure to determine response (pCR). | After definitive surgery or at withdrawal from study |
| Dynamic breast MRI | To monitor tumor size | Prior to surgery (if operable) and within 4 weeks of stopping study drugs |

*Per study amendment June 2014, patients that withdraw from study for any reason will have an end of study MRI within 4 weeks of stopping study drug and every effort will be made to obtain an end of study biopsy or tissue sample at the time of surgical tumor removal. For patients withdrawing from study due to SAE, it will not be considered a study violation if they fail to have a final study biopsy. For patients whose tumors remain inoperable, repeat core biopsies or an incisional biopsy may be obtained after the completion of neoadjuvant study therapy, prior to the administration of additional therapy.

### 5.4 Assessments at Post-Surgical Visit

The following assessments will be performed at a mandatory follow-up visit 4-6 weeks after surgery (if surgery is performed).

**Table 9 Assessments at Post-Surgical Visit**

| Assessment/Procedure | Explanation |
|---|---|
| Toxicity evaluation | History since prior exam, including history of all toxicities or AEs and ECOG performance status |
| Physical examination | Physical exam, including vital signs, weight, wound assessment. |
| Hematology | Complete blood count only as clinically indicated |
| Standard chemistry evaluation | Sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, alkaline phosphatase only if abnormal pre-operatively or if clinically indicated |

| | |
|---|---|
| Pathology report | Document pathologic tumor size and lymph node involvement |

## 6.0 AGENT FORMULATION AND PROCUREMENT

### 6.1 Anastrozole

Anastrozole will be obtained from AstraZeneca, Inc., for the purposes of this study.

The drug should be stored, prepared, and administered according to the manufacturer's guidelines and institutional practice.

The dose administered will be 1 mg PO daily.

### 6.2 AZD0530

AZD0530 will be obtained from AstraZeneca, Inc. for the purposes of this study.

The drug should be stored, prepared, and administered according to the manufacturer's guidelines.

The dose administered will be 175 mg PO daily or as otherwise specified in Section 4 above. Further details on the administration of AZD0530 are found in Section 4 of this protocol.

### 6.3 Study Drug Storage

Upon receipt of AZD0530 or placebo, study drug is to be stored according to the manufacturer's specifications.

## 7.0 CORRELATIVE/SPECIAL STUDIES

### 7.1 Tissue Collection and Molecular Studies

A principal aim of this study is to explore whether changes in gene expression, or the expression of specific biomarkers, are either predictive of response to AZD0530 or indicative of response. Given the sample size, the molecular data obtained in this trial will be considered hypothesis-generating, with the ultimate goal being to develop additional trials that will identify which patients have a greater likelihood of benefiting from AZD0530 therapy.

To perform the molecular studies on tumor cells, tumor tissue will be obtained serially at the following time points:

**Core Biopsies (Baseline)**
Tumor samples must be collected at the time of the initial diagnosis (or in a subsequent procedure) either by core needle or incisional biopsy. **Excisional biopsy will not be allowed**. Adequate core biopsies, (or the equivalent amount of tissue with an incisional biopsy), are required to perform the molecular analyses identified below.

Once the core biopsies have been removed, a minimum of one sample must be immediately snap-frozen using the procedure outlined in Appendix II or an equivalent technique, then stored in liquid nitrogen or at -70 to -80 °C on site. A minimum of one biopsy sample should undergo formalin fixation and embedding in paraffin according to standard institutional guidelines.

**Definitive Surgery**
A second tumor sample must be collected by biopsy before surgery using the same SOP for pre-treatment sample if tumor size permits or at the time of the patient's definitive surgery in the same manner as above. Please refer to Appendix II for further details on the procedure.

In patients whose tumors remain inoperable after the completion of chemotherapy, repeat core biopsies or an incisional biopsy will be obtained and prepared as described in Appendix II or using an equivalent technique.

In the event that a sentinel node biopsy procedure is performed, the dye can be injected into the tumor bed after tumor removal. The dye should not be injected into the tumor itself as this may alter the tissue and affect the molecular analyses. In the event of tumor response with no evidence of tumor at surgery, tissue surrounding the marker clip will be recovered for study evaluations, if feasible.

## 8.0 MEASUREMENT OF EFFECT

### 8.1 Functional Imaging Studies

All patients will have a dynamic contrast-enhanced MRI obtained at the study center at baseline, at 10 weeks ± 5 business days after initiating study drugs, and prior to surgery. The radiologist carrying out the DCE-MRI will be blinded to the patient treatment arm. Contrast enhanced MRI will be carried our according to institutional protocol. Patients will be imaged in the prone position with IV access established prior to onset of imaging. High resolution 3-dimensional, fat-suppressing images will be performed immediately before, after, and at a delayed time point following injection of gadolinium-DTPA. Gd-DTPA will be given at a dose of 0.1 mmol/kg body weight over 15 sec, followed by a saline flush over 15 sec. Pulse sequence parameters will be as described (Esserman et al., 2001b). One three-dimensional acquisition of 64 slices, covering the entire tumor affected breast will be acquired in a scan time of 5 minutes. Tumor diameter, blood flow and volumes will a calculated using computer assisted diagnostic software as described (Hylton, 2006c). Statistical considerations for tumor size measurement will be as above.

We anticipate that MRI evaluation will not only provide a more rigorous evaluation of tumor size (diameter), but will also enhance the accuracy of volumetric calculations and provide data on effects of hormonal therapy on tumor blood flow that have not been systematically studied previously in the context of endocrine therapy. The blood flow information may be of biologic interest and may stimulate further analysis of angiogenic factors that may be revealed in the molecular endpoint studies. Response to neoadjuvant chemotherapy can be predicted for some regimens by MRI response after two treatment cycles. It will be of interest to ascertain if MRI response is predictive of clinical and pathologic response to an AI and the novel biologic agent AZD0530 in this study.

The following 4 dynamic MRI modeling parameters will result from the dynamic MRI analyses:

- Transfer constant ($K^{trans}$)
- Tissue leakage space ($v_e$)
- Rate constant ($k_{ep}$)
- Area under the Gd-DTPA curve at 60-seconds ($AUGC_{60}$)

All imaging studies will be reviewed and scored by radiologists blinded to treatment assignment or

outcome.

### 8.2 Clinical and Molecular Efficacy Assessment

In patients with assessable disease, the response to treatment will be determined by evaluating both the clinical objective response rate and, in those who undergo mastectomy or a breast-conserving procedure, the pathologic complete response rate. Efficacy will also be evaluated by determining the percentage of patients in each group who undergo breast-conserving surgery.

### 8.3 Clinical Response Assessment

#### Assessment of Response by Physical Examination and MRI Evaluation

The primary objective of the phase II part of the study will be to compare treatment groups (AZD0530 and anastrozole versus anastrozole alone) with respect to relative change in tumor size, as documented by the greatest tumor diameter on bi-dimensional measurement as measured by MD. A secondary objective is to assess size change by MRI volumetric assays at diagnosis and after neoadjuvant treatment (prior to surgery). Since clinical improvement is anticipated in both treatment groups, we expect to compare the two treatment groups with respect to relative reduction in tumor size. Tumors will be measured by the attending physician each 4 week cycle and an interim MRI evaluation is planned at 10 weeks after treatment initiation and within 4 weeks after stopping study drug. We chose relative reduction/change in tumor size as the primary endpoint of the phase II part of our study, because relative reduction/change in tumor size leads to a more statistically sensitive comparison of treatments groups compared to RECIST categories. Detailed methodology is discussed in the statistical section of the protocol (cf. below).

#### Assessment of Pathologic Response

A pathologic complete response will be defined as the absence of viable tumor cells in the resected specimen, as determined by standard histologic examination. All specimens will be reviewed by a central pathologist to determine pathologic response.

#### Breast-conserving Surgery

Efficacy will also be evaluated by determining the percentage of patients in each group who undergo breast-conserving surgery.

## 9.0 SAFETY

The investigator is responsible for the detection and documentation of events meeting the criteria and definitions of an Adverse Event (AE) or Serious Adverse Event (SAE).

### 9.1 Definition of an Adverse Event (AE)

An AE is any undesirable sign, symptom, or medical condition occurring after starting study treatment, even if the event is not considered to be related to study treatment. This can include any physical or clinical change experienced by the patient whether or not considered related to the use of the study drug. An adverse event can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease (including the onset of new illness and the exacerbation of pre-existing conditions) temporally associated with the use of the study treatment. In this protocol, study treatment refers to AZD0530 (or placebo) or anastrozole plus AZD0530 (or placebo).

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency and/or intensity of the condition.

- New conditions diagnosed or detected after study treatment administration, even though it may have been present prior to the start of the study.

- Signs, symptoms, or the clinical sequelae of a suspected interaction.

- Signs, symptoms, or the clinical sequelae of a suspected overdose.

Examples of an AE <u>do not</u> include:

- medical or surgical procedures (the condition that leads to the procedure is an AE).

- Anticipated day-to-day fluctuations of pre-existing diseases or conditions present or detected at the start of the study that do not worsen.

- Progression of the disease/disorder being studied, unless more severe than expected for the subject's condition.

### 9.2 Reporting of Adverse Events

All adverse events must be recorded in the patient's medical records and on the Case Report Form. The onset and end dates, severity, duration, effect on study drug administration (e.g., discontinuation), relationship to study drug, and administration of any other drug(s) to treat this event will be recorded for each AE. The severity of the AE and relationship to study drug will be assessed according to specific guidelines. Patients will be questioned and/or examined by the Investigator or his/her designee for evidence of AEs. The presence or absence of specific AEs may also be solicited from patients by review of systems, as clinically indicated.

### 9.3 Grading of Adverse Events

Adverse events will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0 (Appendix III).

For AEs which are not covered by the NCI CTCAE grading system, the following terminology will be used:

- Mild – awareness of sign, symptom, or event, but easily tolerated.
- Moderate – discomfort severe enough to cause interference with usual activity and which may warrant intervention.
- Severe - incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention such as hospitalization.
- Life-threatening – immediate risk of death.

### 9.4 MONITORING OF ADVERSE EVENTS

Patients having adverse events will be monitored with relevant clinical assessments and laboratory tests as determined by the Investigator. All adverse events must be followed to satisfactory resolution or stabilization of the event(s). Any actions taken and follow-up results must be recorded on the appropriate page of the Case Report Form, as well as in the patient’s source documentation. Follow-up laboratory results should be filed with the patient's source documentation.

### 9.5 Serious Adverse Events

In the event of a serious adverse event (SAE) the first concern will be for the safety of the subject.

An SAE or STEAE is any sign, symptom or medical condition that emerges during study drug treatment or during a post-treatment follow-up period that (1) was not present at the start of study drug treatment and it is not a chronic condition that is part of the patient's medical history, **OR** (2) was present at the start of study drug treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, **AND** that meets any of the following regulatory serious criteria:

- Results in death.
- Is life-threatening (subject was at immediate risk of death at the time of the event).
- Requires in-patient hospitalization or prolongation of an existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Constitutes a congenital anomaly or birth defect.
- Is medically significant, in that is may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

In the event of an SAE that is felt to be possibly or probably linked to study drug, the subject may be unblinded if it is felt to be in the medical interest of the subject to do so, that is, if knowledge of use of placebo or saracatinib might assist in medical management of the SAE. This will be done at the discretion of the PI in consultation with the Sponsor-Investigator, Dr. Slingerland. The PI involved will obtain study drug allocation information from the Research Pharmacist and may disclose this to the patient and relevant medical personnel involved in this patient's management. The date, circumstances and the reason for unblinding of the subject will be documented in the patient's study chart in a note to file by the CRC and by the Investigator or Sub-Investigator in the physician clinic note. The Research Pharmacist involved will also record the study subject's unblinding in a note to file in the Pharmacy records.

### 9.6 Reporting of Serious Adverse Events

Reports of all **serious adverse events**, as well as all Alert Safety Reports sent by Astra Zeneca, Inc. to the Investigators (an IND Safety Report is an adverse event determined by the sponsor to require immediate distribution to the Investigators and appropriate Health Regulatory Authorities), must be reported to the appropriate Institutional Review Board/Ethics Committee and/or reported

in accordance with local law/regulations. The Investigator must keep documentation of such notifications at the trial site.

The causality of SAEs (their relationship to study treatment) will be assessed by the investigator(s).All serious adverse events, whether or not deemed drug-related or expected, must be reported by the Investigator or sub-Investigator immediately or within 24 hours (one working day) of when the investigator is notified of the event by telephone to the Sponsor. A written report to the IRB must follow within 14 days, which includes a full description of the event and any sequelae. This includes serious adverse events that occur anytime while enrolled in the study or 30 days past the end of therapy or withdrawal visit.

All SAE's must be recorded on a MedWatch 3500 form and faxed or emailed to the Sponsor. The Sponsor will submit to the IRB. Participating sites should follow their IRB policies and guidelines.

Investigators and other site personnel must inform the FDA, via a Medwatch form, of any unexpected and possibly study drug related SAE that occurs according to the FDA reporting requirement timelines. A copy of the MedWatch report must be faxed to AstraZeneca at the time the event is reported to the FDA. It is the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

A cover page should accompany the Medwatch form indicating the following:

- Arimidex/AZD0530 Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name and AstraZeneca D5390L00104

***Send by way of fax to:*** [redacted]

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

## 9.7 Reporting for Investigational Agents

The FDA MedWatch 3500 Reporting Form that can be obtained electronically at http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM163919.pdf for FDA IND agents.

Expedited reporting requirements for adverse events experienced by patients on study arm(s) who have received at least one dose of an investigational agent

| **Attribution** | **Grade 2** | **Grade3** | | **Grade 4** | | **Grade 5**b | | **Protocol-specific Require-ments/ Exceptions** |
|---|---|---|---|---|---|---|---|---|
| | Unexpected | Unexpected | Expected | Unexpected | Expected | Unexpected | Expected | See footnote (c) for special requirements. See footnote (d) for special exceptions. |
| Unrelated or Unlikely | | *MedWatch 3500* if Hospitalized | *MedWatch 3500* if Hospitalized | *MedWatch 3500* | *MedWatch 3500* | *MedWatch 3500* | *MedWatch 3500* | |
| Possible, Probable, or Definite | *MedWatch 3500* a | *MedWatch 3500* a | *MedWatch 3500* if Hospitalized | | | | | |

**a** *MedWatch 3500A* reporting is only required if the event is related to the investigational agent(s); it is not required if the event is related only to the commercial agent(s) included in the protocol treatment.

**b** This includes all deaths within 30 days of the last dose of treatment with an investigational agent(s), regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with an investigational agent(s) and is attributed (possibly, probably, or definitely) to the agent(s) and is not due to cancer recurrence must be reported via *MedWatch 3500A*.

**c** **Protocol-specific expedited reporting requirements:** The adverse events listed below also require expedited monitoring for this trial:

**Hospitalization:** Any grade 1 or 2 adverse event which precipitates a hospitalization lasting ≥ 24 hours or prolongs hospitalization must be submitted.

**d** The adverse events listed below do **not** require reporting:

- Grade 4 myelosuppression

## 9.8 Reports of Patient Death

The death of any patient during the study or follow-up phase, regardless of the cause, must be reported within 24 hours by telephone to the principal investigator and to the IRB in accordance with local law/regulations. The Investigator must keep documentation of such notifications at the trial site. A written report must follow as soon as possible. If an autopsy is performed, the report must be provided.

## 9.9 Period of Observation

The period of observation for collection and reporting of adverse events for this study is the interval between when the subject starts study treatment and the date of surgical tumor removal or end-of-study biopsy, within 28 days after discontinuing study drug. During the Phase II trial, if a patient has an AE or SAE that precipitates study withdrawal, the patient should remain on study until complete resolution of the AE. Thus, the saracatinib (AZD0530) may be withheld for maximum 3 weeks, before the decision is made to stop saracatinib entirely. After this 3 weeks in

which saracatinib is stopped, the patient will be monitored for at least an additional for 30 days, or until full resolution of the SAE. During the time of monitoring for resolution of the adverse event, the patient should continue to take anastrozole (provided by the study) unless this exceeds the six 4 week cycles of treatment stipulated by protocol. The patient will be considered off study and no longer monitored once the AE is resolved.

If an SAE occurs after the period of observation expires, the Investigator should be notified to determine how the SAE should be reported. In general, late fatal complications or conditions of medical importance should be documented and reported as an SAE.

## 10.0 DATA REPORTING

Data will be collected using designated Case Report Forms. Subject data necessary for analysis and reporting the results of the study will be entered into a validated, secure database. Clinical data management and data validation will be performed in accordance with defined procedures. Access to the data will be strictly controlled.

The investigator must complete the Case Report Forms and transmit the data as instructed, and must store copies of the Case Report Forms with other study documents in a secure location in accordance with all regulatory requirements. All entries to the Case Report Forms must be made as described in the completion guidelines.

Essential documents must be retained by the investigator for as long as necessary to comply with national and international regulations. Essential documents must be retained for 15 years. Essential documents include:

- IRB/EC approvals for the protocol, informed consent, and all amendments
- All source documents and laboratory records
- CRF paper copies
- Informed consent forms
- FDA Form 1572 (as required)
- Any other pertinent study document

## 10.1 STUDY MONITORING

The study will be monitored by the UM Data Safety and Monitoring Committee. The extent, nature, and frequency of review will be based on enrollment rate and other relevant considerations.

**Data and Safety Monitoring Plan**

The Research Team will continuously monitor study accruals, toxicities, and response to treatment at both study sites. Patients will be monitored closely throughout the study for any toxicity or adverse reaction to treatment. All toxicities, regardless their grade, will be recorded in the patient case report form using NCI/CTEP Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (Appendix A). Dose escalation/de-escalation and stopping rules described in section 5.0 will be followed.

The UM/Sylvester Comprehensive Cancer Center's Data and Safety Monitoring Committee (DSMC) will monitor all patients entered on study at all sites for this protocol according to the Cancer Center's DSM Plan (Appendix IV). Data and Safety monitoring for Stanford study site patients will be monitored by the UM DSMC. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study. In the event of a major AE resulting in death, the Sponsor will be informed, make the decision to unblind the study case affected and make further decisions in consultation with the UM DSMC.

DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event data. In addition, the DSMC will review reports from all audits or study reviews pertaining to this clinical trial and take appropriate action.

### 10.2 Auditing

In addition to routine monitoring procedures, the IRB, principal investigator and/or the industry collaborators may conduct audit for the purposes of quality assurance, and to ensure compliance with GCP, ICH, and other applicable guidelines. Regulatory authorities may also conduct audits at any time during or after the completion of the study.

### 10.3 Modification of the Protocol

Any substantive modifications of the protocol that may affect the conduct of the study, patient safety, or scientific validity of the study require a protocol amendment. Such amendments must be approved by the IRB/EC prior to implementation at a site.

### 10.4 Information Disclosure and Publications

All information provided by and/or generated relating to this study or the investigational agent, and not previously published, is considered confidential and proprietary. This confidential information shall not be disclosed or used (except in the performance of this study) without prior written authorization.

No publication, abstract, or presentation of any aspect of the study shall be made without the approval of the principal investigators and Astra Zeneca, Inc. A pre-specified procedure will be used to determine the authorship list of any publication or presentation, taking into account participation in the leadership, design, and conduct of the trial as well as patient accrual.

## 11.0 CRITERIA FOR DISCONTINUATION OF THERAPY

Criteria for removal from the study include the following:

- Completion of all prescribed protocol therapy.
- Disease progression.
- Unacceptable toxicity (defined as toxicity necessitating the discontinuation of study drug, as outlined in Section 4).
- Withdrawal of patient consent .
- Continuation would, in the judgment of the investigator, not be in the best interests of the subject.

## 12.0 STATISTICAL METHODS AND CONSIDERATIONS

### 12.1 Phase I PK cohort: Statistical Considerations

Study patient demographics (age and race) and disease characteristics will be recorded, including previous therapy. The safety data, including toxicities, number of treatment cycles initiated and completed, reasons for study withdrawal, laboratory data, and concomitant medications, will be summarized descriptively. Toxicities will be tabulated by type, grade, duration, attribution to treatment, and administered dose of study drug in accordance with Data Safety Monitoring Committee guidelines.

The effect of AZD0530 on pharmacokinetics of anastrozole will be summarized as geometric means and standard deviations for peak concentration (PC) of study drug in plasma and corresponding areas under the curve (AUC).

**12.2 Randomized double-blind phase II cohort: Statistical Considerations**

Study size of 60 patients is based on patient availability and the known efficacy of anastrozole as a single agent. Subject accrual will continue until sixty subjects have completed at least 4 of the planned 6 months of therapy. For data analysis, subjects treated for at least 4 months will be considered to have completed therapy. Patients removed from treatment due to disease progression have completed treatment, but disease progression will be recorded in the CRF. Patients will be randomized in a 2:1 ratio; 40 patients will receive combined treatment of AZD0530 and anastrozole and 20 patients will receive anastrozole alone. Patients will be stratified by study site (UM/SCCC or Stanford Cancer Institute), and baseline tumor size (≥2 and <6 cm; ≥6 cm). In order to prevent selection bias and protect the assignment sequence, the investigators, study team, and patients will be blinded to assignment of AZD0530 or placebo. This study size will allow an ongoing evaluation of toxicity and initial assessment of efficacy. The primary objective of the phase II part of the study will be to compare treatment groups (AZD0530 and anastrozole versus anastrozole alone) with respect to relative change in tumor size, as documented by the greatest tumor diameter on bi-dimensional measurement. Since clinical improvement is anticipated in both treatment groups, we expect to compare the two treatment groups with respect to relative reduction in tumor size. Tumors will be measured each 4 week cycle. We chose relative reduction/change in tumor size as the primary endpoint of the phase II part of our study, because relative reduction/change in tumor size leads to a more statistically sensitive comparison of treatments groups compared to RECIST categories.

Secondary endpoints include tumor size evaluation by MRI, pathologic complete response (pCR), clinical response (complete (CR), or partial (PR) responses), and clinical benefit (CR, PR, or stable disease (SD)) by RECIST criteria; toxicities; and identification of biologic correlates as indicators of treatment response. Because tumors are assayed by MRI at fewer time points (pre-treatment, 10 weeks and at end of neoadjuvant treatment), the MRI-based tumor size will be analyzed only at the end of the study and will not be part of the interim analysis (See Section 12.4.1.2).

We will use clinical improvement as the main study endpoint, defined as relative reduction (percentage change) in tumor size = $100\times(ts1 - ts2)/ (ts1)$, where ts1 and ts2 are respectively the tumor sizes (largest diameter measured by MD) at baseline and after completing neoadjuvant treatment.

With 60 patients allocated 2:1 to the combined treatment, our study has 83.8% power to detect an absolute mean difference of 20%, corresponding to an anticipated mean relative reduction in tumor size of 60% in the combined AZD0530 + anastrozole group (40 patients) and 40% in patients receiving anastrozole alone. Furthermore, a smaller absolute difference of 15% between treatment arms, i.e. 55% vs. 40%, can be detected with 59.4% power. Power calculations assume common standard deviation of 24% and comparison based on a two-sided t-test with 0.045 significance level at final analysis, accommodating an interim analysis at 0.005 significance. (See section 12.4.1.2) Note that assuming a slightly smaller standard deviation of 20% increases power to 94.3% to detect a difference 60% vs. 40%, and to 75.4% to detect a difference 55% vs. 40%.

Analysis of Phase II data will include a description of baseline characteristics and summary of safety data by treatment arm. We will characterize tumor size (measured by MD and by MRI) in study patients at baseline and post neoadjuvant treatment (prior to surgery) by descriptive statistics (minimum, maximum, median, average, standard deviation). A similar description will be given for relative reduction in tumor size = $100\times(ts1 - ts2)/ (ts1)$, where ts1 and ts2 are respectively the tumor sizes at baseline and post neoadjuvant treatment. The mean relative reduction in tumor size between the two treatments will be compared by a two-sample t-test. The pathologic complete response rate (pCR) and overall clinical response rate (CR or PR), clinical benefit (CR, PR, or SD), and individual response categories (CR, PR, and SD), will be estimated by the exact binomial method.

### 12.3 Correlative Molecular Studies

For the purposes of this protocol, tumor tissue samples will be considered evaluable if:

- Patient meets eligibility criteria.
- Patient has received AZD0530 or placebo, together with anastrozole.
- Frozen and paraffin-embedded tumor tissues are collected before and after protocol treatment as specified in Appendix II and are obtained by the principal investigator in the UM Sylvester research laboratory.
- Tumor tissue specimens are adequate for molecular assessment. The adequacy will be evaluated by the UM Sylvester laboratory according to the following criteria:
    - Sufficient tissue to perform immunohistochemistry.
    - RNA available for microarrays.
    - Acceptable RNA quality confirmed by Oncogenomics Core, with a rRNA ratio (28S/18S) of 1.8-2.4.

Given these assumptions, it is expected that approximately 40-50 matched specimens (pre- and post-treatment) will be obtained for molecular analysis.

### 12.4 Randomization and Blinding in Cohort B

After a signed informed consent has been obtained and eligibility has been established, patients will be randomized using a stratified permuted block design to ensure allocation 2:1 ratio between combined treatment of AZD0530 + anastrozole (40 patients) and anastrozole alone (20 patients). In order to prevent selection bias and protect the assignment sequence, the investigators, study team, and patients will be blinded to assignment of AZD0530 or placebo. See more details in section 3.3.

### 12.5 Safety Evaluation

The safety analysis will be conducted on all patients who received all or any portion of one pill of any study drug.

Descriptive statistics will be used to summarize the number and types of adverse events, serious adverse events, abnormal laboratory data, and number of patients in whom study treatment had to be stopped, dose-reduced, or delayed. In the phase II part of the trial (Cohort B), adverse events in the two study arms will be compared using two-tailed $\chi^2$ tests or, when expected counts are low, Fisher's exact test.

**Interim monitoring of phase II component of study**

The Research Team will continuously monitor study accruals, toxicities, and clinical outcome. The Sylvester Comprehensive Cancer Center's Data and Safety Monitoring Committee (DSMC) will monitor this protocol according to the Cancer Center's DSM Plan. DSMC oversight of the conduct of this trial includes ongoing review of adverse event data and periodic review of trial outcomes. The DSMC also reviews reports from internal monitoring of protocol compliance and data integrity conducted by the University of Miami, Office of Clinical Research Operations, and Regulatory Support. Additionally, the Sylvester Protocol Review Committee will monitor study progress with respect to patient accrual.

**In order to preserve the blinding of the phase II part of the study, all analysis needed for DMSC review will be done by an independent biostatistician and reported directly to the**

**Sylvester DSMC. These reports will not be provided to the PI or any study team member until the trial is completed.**

**Guidelines for early stopping due to toxicity**

We propose the following guidelines for the DSMC in its review of accumulating data on toxicity. The proposed guidelines were developed using Bayesian methods, which can be applied at any stage of enrollment without advance specification of the number of interim analyses to be performed, or the number of patients evaluable for toxicity, at the time such assessments are made (Spiegelhalter, et al., 1994; Fayers, et. al, 1997).

Under the Bayesian method, we assign a prior probability (level of belief at the start of the trial) to a range of possible values for the true toxicity rate. As data on treated patients become available, the prior probability distribution is revised and the resulting posterior probability becomes the basis for recommending either early termination or continuation of the study. Specific stopping guidelines based on posterior probabilities for both acute and late occurring toxicity are given below along with underlying assumptions for the prior distributions.

Toxicity will be tabulated by arm and if the stopping guideline is exceeded in either arm, the DSMC will consider study termination.

If a treatment-related (possible, probable, or definite) death occurs, enrollment will be suspended and continuation of the study will be reassessed by the DSMC.

For the purposes of safety monitoring, we define unacceptable toxicity to be any of the following **treatment-related (possible, probable, or definite)** adverse events:

- Grade 3 or higher non-hematologic toxicity, excluding rash and fatigue,
- Grade 4 or higher hematologic toxicity, excluding lymphopenia, leucopenia, and neutropenia lasting ≤ 7 days, and anemia.

Unacceptable toxicity is expected to occur in no more than 10% of patients in either treatment arm during the phase II component of study. If there is evidence that the true rate of this toxicity exceeds 20% in either arm, then the study should be suspended or possibly terminated early. Specifically, we suggest as a guideline for early termination a posterior probability of 90% or higher

that the true rate exceeds 20%. Table 10 shows specific instances where this guideline is met, suggesting early termination due to evidence of excessive toxicity.

**Table 10. Early Stopping Guidelines due to toxicity to be applied to each arm in phase II.**

| Patients with unacceptable toxicity | Patients evaluated for toxicity | Observed toxicity rate ≥ |
|---|---|---|
| 3 | 3 to 5 | 60% |
| 4 | 6 to 9 | 44% |
| 5 | 10 to 12 | 42% |
| 6 | 13 to 16 | 38% |
| 7 | 17 to 20 | 35% |
| 8 | 21 to 24 | 33% |
| 9 | 25 to 28 | 32% |
| 10 | 29 to 32 | 31% |
| 11 | 33 to 36 | 31% |
| 12 | 37 to 40 | 30% |

Unacceptable toxicity: any of the following t**reatment-related (possible, probable, or definite)** adverse events:
Grade 3 or higher non-hematologic toxicity, excluding rash and fatigue.
Grade 4 or higher hematologic toxicity, excluding lymphopenia, leucopenia, and neutropenia lasting ≤ 7 days, and anemia.

Posterior probabilities used to derive guidelines for toxicity were calculated under a prior beta distribution with parameters $\beta_1 = 0.2$ and $\beta_2 = 1.8$, which corresponds to an expected rate of 10% based on prior information roughly equivalent to having studied 2 patients. Furthermore, this prior distribution assigns a small a priori chance (17.5%) to the possibility that the true rate of unacceptable toxicity is 20% or greater.

**Guideline for early stopping for futility**

One interim analysis will be conducted during Phase II when 20 patients in the anastrozole/AZD00530 arm and 10 patients in the anastrozole only arm have been evaluated for the primary efficacy endpoint, 'relative reduction in tumor size' as defined in the second paragraph of section 17. The interim statistical review of disease response/toxicity requested by the DSMC will use clinical size determination alone. A two-sample t-test will be used to compare treatment arms with respect to the mean relative reduction in tumor size from baseline to post neoadjuvant therapy. The interim analysis will use a two-sided significance level of 0.005, and the final analysis will be done at the 0.045 significance level.

Early termination for futility will be based on the significance of the t-test and the direction of the difference as follows. If $p \leq 0.005$ and the mean relative reduction in tumor size is greater for anastrozole alone than for anastrozole/AZD0530, the DSMC should consider stopping the study

early. However, if p≤0.005 and the mean relative reduction is greater for anastrozole/AZD0530 than for anastrozole alone, the study will continue to the full planned accrual of 60 patients to permit the analysis of molecular endpoints of the study and the evaluation of MRI measurement as surrogate for MD measurement as planned. Continuation to full accrual if the direction of difference favors combined treatment will allow confirmation of the interim finding and better precision for estimating the tumor size reduction and other secondary endpoints in both arms.

Since reduction of tumor size is expected with anastrozole alone, accrual should not be suspended during the interim analysis.

## REFERENCES

Abe,O.et al. (2005). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet *365*, 1687-1717.

Ah-See, M. W. Does vascular imaging with MRI predict response to neoadjuvant chemotherapy in primary breast cancer Proc Am Soc Clin Oncol , 22. 2004.
Ref Type: Abstract

Akhtar, S.S., Allan, S.G., Rodger, A., Chetty, U.D., Smyth, J.F., and Leonard, R.C. (1991). A 10-year experience of tamoxifen as primary treatment of breast cancer in 100 elderly and frail patients. Eur. J Surg. Oncol. *17*, 30-35.

Altundag, K., and Ibrahim, N.K. (2006). Aromatase inhibitors in breast cancer: An overview. Oncologist *11*, 553-562.

Bonneterre,J., Buzdar,A., Nabholtz,J.M., Robertson,J.F., Thurlimann,B., von Euler,M., Sahmoud,T., Webster,A., and Steinberg,M. (2001b). Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma. Cancer *92*, 2247-2258.

Bonneterre,J., Thurlimann,B., Robertson,J.F., Krzakowski,M., Mauriac,L., Koralewski,P., Vergote,I., Webster,A., Steinberg,M., and von Euler,M. (2000). Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study. J Clin Oncol. *18*, 3748-3757.

Buadu, L.D., Murakami, J., Murayama, S., Hashiguchi, N., Sakai, S., Masuda, K., Toyoshima, S., Kuroki, S., and Ohno, S. (1996). Breast lesions: correlation of contrast medium enhancement patterns on MR images with histopathologic findings and tumor angiogenesis. Radiology *200*, 639-649.
Buckley, D.L., Drew, P.J., Mussurakis, S., Monson, J.R., and Horsman, A. (1997). Microvessel density of invasive breast cancer assessed by dynamic Gd-DTPA enhanced MRI. J Magn Reson. Imaging *7*, 461-464.

Buzdar,A., Jonat,W., Howell, A., Jones,S.E., Blomqvist,C., Vogel,C.L., Eiermann,W., Wolter,J.M., Azab,M., Webster, A., and Plourde,P.V. (1996). Anastrozole, a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: results of overview analysis of two phase III trials. Arimidex Study Group. J Clin Oncol. *14*, 2000-2011.

Buzdar, A.U. (2003). 'Arimidex' (anastrozole) versus tamoxifen as adjuvant therapy in postmenopausal women with early breast cancer--efficacy overview. J. Steroid Biochem. Mol. Biol. *86*, 399-403.

Cariou, S., Donovan, J.C., Flanagan, W.M., Milic, A., Bhattacharya, N., and Slingerland, J.M. (2000). Down-regulation of p21WAF1/CIP1 or p27Kip1 abrogates antiestrogen-mediated cell cycle arrest in human breast cancer cells. Proc. Natl. Acad. Sci. U. S. A *97*, 9042-9046.

Cataliotti, L., Buzdar,A.U., Noguchi,S., Bines,J., Takatsuka,Y., Petrakova,K., Dube,P., and de Oliveira,C.T. (2006c). Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. Cancer *106*, 2095-2103.

Catzavelos,C., Bhattacharya,N., Ung,Y.C., Wilson,J.A., Roncari,L., Sandhu,C., Shaw,P., Yeger,H., Morava-Protzner,I., Kapusta,L., Franssen,E., Pritchard,K.I., and Slingerland,J.M. (1997). Decreased levels of the cell-cycle inhibitor p27Kip1 protein: prognostic implications in primary breast cancer. Nat. Med. *3*, 227-230.

Chou,C.P., Wu,M.T., Chang,H.T., Lo,Y.S., Pan,H.B., Degani,H., and Furman-Haran,E. (2007). Monitoring breast cancer response to neoadjuvant systemic chemotherapy using parametric contrast-enhanced MRI: a pilot study.. Acad. Radiol. *14*, 561-573.

Chu,I., Blackwell,K., Chen,S., and Slingerland,J. (2005). The dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), cooperates with tamoxifen to inhibit both cell proliferation- and estrogen-dependent gene expression in antiestrogen-resistant breast cancer. Cancer Res. *65*, 18-25.

Chu,I., Sun,J., Arnaout,A., Kahn,H., Hanna,W., Narod,S., Sun,P., Tan,C.K., Hengst,L., and Slingerland,J. (2007). p27 phosphorylation by Src regulates inhibition of cyclin E-Cdk2. Cell *128*, 281-294.

Cuzick,J., Powles,T., Veronesi,U., Forbes,J., Edwards,R., Ashley,S., and Boyle,P. (2003). Overview of the main outcomes in breast-cancer prevention trials. Lancet *361*, 296-300.

Donovan,J.C., Milic,A., and Slingerland,J.M. (2001). Constitutive MEK/MAPK activation leads to p27(Kip1) deregulation and antiestrogen resistance in human breast cancer cells. J. Biol. Chem. *276*, 40888-40895.

Donovan,J.C., Rothenstein,J.M., and Slingerland,J.M. (2002). Non-malignant and tumor-derived cells differ in their requirement for p27Kip1 in transforming growth factor-beta-mediated G1 arrest. J. Biol. Chem. *277*, 41686-41692.

Eiermann,W., Paepke,S., Appfelstaedt,J., Llombart-Cussac,A., Eremin,J., Vinholes,J., Mauriac,L., Ellis,M., Lassus,M., Chaudri-Ross,H.A., Dugan,M., and Borgs,M. (2001). Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized double-blind multicenter study. Ann. Oncol. *12*, 1527-1532.

Ellis,M.J., Coop,A., Singh,B., Mauriac,L., Llombert-Cussac,A., Janicke,F., Miller,W.R., Evans,D.B., Dugan,M., Brady,C., Quebe-Fehling,E., and Borgs,M. (2001). Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J. Clin Oncol *19*, 3808-3816.
Ellis,P.A. and Smith,I.E. (1996). Primary chemotherapy for early breast cancer. Cancer Treat Rev. *22*, 437-450.

Esserman,L., Kaplan,E., Partridge,S., Tripathy,D., Rugo,H., Park,J., Hwang,S., Kuerer,H., Sudilovsky,D., Lu,Y., and Hylton,N. (2001a). MRI phenotype is associated with response to doxorubicin and cyclophosphamide neoadjuvant chemotherapy in stage III breast cancer. Ann. Surg. Oncol. *8*, 549-559.

Evans,C.T., Ledesma,D.B., Schulz,T.Z., Simpson,E.R., and Mendelson,C.R. (1986). Isolation and characterization of a complementary DNA specific for human aromatase-system cytochrome P-450 mRNA. Proc Natl. Acad. Sci. U. S. A *83*, 6387-6391.

Fidler,I.J. and Ellis,L.M. (1994). The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell *79*, 185-188.

Fisher,B., Brown,A., Mamounas,E., Wieand,S., Robidoux,A., Margolese,R.G., Cruz,A.B., Jr., Fisher,E.R., Wickerham,D.L., Wolmark,N., DeCillis,A., Hoehn,J.L., Lees,A.W., and Dimitrov,N.V. (1997). Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol *15*, 2483-2493.

Fisher,B., Dignam,J., Bryant,J., and Wolmark,N. (2001). Five versus more than five years of tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial. J Natl. Cancer Inst. *93*, 684-690.

Fisher,B., Saffer,E., Rudock,C., Coyle,J., and Gunduz,N. (1989). Effect of local or systemic treatment prior to primary tumor removal on the production and response to a serum growth-stimulating factor in mice. Cancer Res *49*, 2002-2004.

Galbraith,S.M., Maxwell,R.J., Lodge,M.A., Tozer,G.M., Wilson,J., Taylor,N.J., Stirling,J.J., Sena,L., Padhani,A.R., and Rustin,G.J. (2003). Combretastatin A4 phosphate has tumor antivascular activity in rat and man as demonstrated by dynamic magnetic resonance imaging. J Clin Oncol *21*, 2831-2842.
Galbraith,S.M., Rustin,G.J., Lodge,M.A., Taylor,N.J., Stirling,J.J., Jameson,M., Thompson,P., Hough,D., Gumbrell,L., and Padhani,A.R. (2002). Effects of 5,6-dimethylxanthenone-4-acetic acid on human tumor microcirculation assessed by dynamic contrast-enhanced magnetic resonance imaging. J Clin Oncol *20*, 3826-3840.

Geisler,J., King,N., Anker,G., Ornati,G., Di Salle,E., Lonning,P.E., and Dowsett,M. (1998). In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients. Clin Cancer Res *4*, 2089-2093.

Grimm,S.W. and Dyroff,M.C. (1997). Inhibition of human drug metabolizing cytochromes P450 by anastrozole, a potent and selective inhibitor of aromatase. Drug Metabolism and Disposition *25*, 598-602.

Hayes,C., Padhani,A.R., and Leach,M.O. (2002). Assessing changes in tumour vascular function using dynamic contrast-enhanced magnetic resonance imaging. NMR Biomed. *15*, 154-163.
Hiscox,S., Morgan,L., Green,T.P., Barrow,D., Gee,J., and Nicholson,R.I. (2005). Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. Breast Cancer Res. Treat. 1-12.

Hulka,C.A., Edmister,W.B., Smith,B.L., Tan,L., Sgroi,D.C., Campbell,T., Kopans,D.B., and Weisskoff,R.M. (1997). Dynamic echo-planar imaging of the breast: experience in diagnosing breast carcinoma and correlation with tumor angiogenesis. Radiology *205*, 837-842.
Hylton,N. (2006c). MR imaging for assessment of breast cancer response to neoadjuvant chemotherapy. Magn Reson. Imaging Clin N. Am. *14*, 383-9, vii.

Hylton,N. (2006b). MR imaging for assessment of breast cancer response to neoadjuvant chemotherapy. Magn Reson. Imaging Clin N. Am. *14*, 383-9, vii.

Jayson,G.C., Zweit,J., Jackson,A., Mulatero,C., Julyan,P., Ranson,M., Broughton,L., Wagstaff,J., Hakannson,L., Groenewegen,G., Bailey,J., Smith,N., Hastings,D., Lawrance,J., Haroon,H., Ward,T., McGown,A.T., Tang,M., Levitt,D., Marreaud,S., Lehmann,F.F., Herold,M., and Zwierzina,H. (2002). Molecular imaging and biological evaluation of HuMV833 anti-VEGF antibody: implications for trial design of antiangiogenic antibodies. J Natl. Cancer Inst. *94*, 1484-1493.

Knopp,M.V. (2007). MR mammography with pharmacokinetic mapping for monitoring of breast cancer treatment during neoadjuvant therapy. Magn Reson. Imaging *2*, 633-658.

Knopp,M.V., Weiss,E., Sinn,H.P., Mattern,J., Junkermann,H., Radeleff,J., Magener,A., Brix,G., Delorme,S., Zuna,I., and van Kaick,G. (1999). Pathophysiologic basis of contrast enhancement in breast tumors. J Magn Reson. Imaging *10*, 260-266.

Leach,M.O., Brindle,K.M., Evelhoch,J.L., Griffiths,J.R., Horsman,M.R., Jackson,A., Jayson,G., Judson,I.R., Knopp,M.V., Maxwell,R.J., McIntyre,D., Padhani,A.R., Price,P., Rathbone,R., Rustin,G., Tofts,P.S., Tozer,G.M., Vennart,W., Waterton,J.C., Williams,S.R., and Workman,P. (2003). Assessment of antiangiogenic and antivascular therapeutics using MRI: recommendations for appropriate methodology for clinical trials. Br. J Radiol. *76 Spec No 1*, S87-S91.

Ma,C.X. and Ellis,M.J. (2006a). Neoadjuvant endocrine therapy for locally advanced breast cancer. Semin. Oncol. *33*, 650-656.

Macaskill,E.J., Renshaw,L., and Dixon,J.M. (2006). Neoadjuvant use of hormonal therapy in elderly patients with early or locally advanced hormone receptor-positive breast cancer. Oncologist. *11*, 1081-1088.

Martincich,L., Montemurro,F., De Rosa,G., Marra,V., Ponzone,R., Cirillo,S., Gatti,M., Biglia,N., Sarotto,I., Sismondi,P., Regge,D., and Aglietta,M. (2004a). Monitoring response to primary chemotherapy in breast cancer using dynamic contrast-enhanced magnetic resonance imaging. Breast Cancer Res. Treat. *83*, 67-76.

Matsubayashi,R., Matsuo,Y., Edakuni,G., Satoh,T., Tokunaga,O., and Kudo,S. (2000). Breast masses with peripheral rim enhancement on dynamic contrast-enhanced MR images: correlation of MR findings with histologic features and expression of growth factors. Radiology *217*, 841-848.

Mauriac,L., Durand,M., Avril,A., and Dilhuydy,J.M. (1991). Effects of primary chemotherapy in conservative treatment of breast cancer patients with operable tumors larger than 3 cm. Results of a randomized trial in a single centre. Ann. Oncol. *2*, 347-354.

Milla-Santos,A., Milla,L., Calvo,N., Portella,J., Rallo,L., Casanovas,J.M., Pons,M., and Rodes,J. (2004). Anastrozole as neoadjuvant therapy for patients with hormone-dependent, locally-advanced breast cancer. Anticancer Res *24*, 1315-1318.

Morgan,B. (2003). Dynamic Contrast-Enhanced Magnetic Resonance Imaging as a Biomarker for the Pharmacological Response of PTK787/ZK 222584, an Inhibitor of the Vascular Endothelial Growth Factor Receptor Tyrosine Kinases, in Patients with Advanced Colorectal Cancer and Liver Metastases: Results from Two Phase I Studies. J Clin Oncol *21*, 3955-3964.

Mouridsen,H., Gershanovich,M., Sun,Y., Perez-Carrion,R., Boni,C., Monnier,A., Apffelstaedt,J., Smith,R., Sleeboom,H.P., Jaenicke,F., Pluzanska,A., Dank,M., Becquart,D., Bapsy,P.P., Salminen,E., Snyder,R., Chaudri-Ross,H., Lang,R., Wyld,P., and Bhatnagar,A. (2003). Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. J Clin Oncol. *21*, 2101-2109.

Nabholtz,J.M., Buzdar,A., Pollak,M., Harwin,W., Burton,G., Mangalik,A., Steinberg,M., Webster,A., and von Euler,M. (2000). Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. J Clin Oncol. *18*, 3758-3767.

Nelson,L.R. and Bulun,S.E. (2001). Estrogen production and action. J Am Acad. Dermatol. *45*, S116-S124.

Padhani,A.R. (2003). MRI for assessing antivascular cancer treatments. Br J Radiol *76*, S60-S80.

Paridaens,R. (2004). First line hormonal treatment (HT) for metastatic breast cancer (MBC) with exemestane (E) or tamoxifen (T) in postmenopausal patients (pts) - A randomized phase III trial of the EORTC Breast Group. Proc Am Soc Clin Oncol *23*.

Ple,P.A., Green,T.P., Hennequin,L.F., Curwen,J., Fennell,M., Allen,J., Lambert-Van Der Brempt,C., and Costello,G. (2004). Discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of c-Src. J. Med. Chem. *47*, 871-887.

Powles,T.J., Hickish,T.F., Makris,A., Ashley,S.E., O'Brien,M.E., Tidy,V.A., Casey,S., Nash,A.G., Sacks,N., Cosgrove,D., and . (1995). Randomized trial of chemoendocrine therapy started before or after surgery for treatment of primary breast cancer. J Clin Oncol *13*, 547-552.

Reed,S.I., Bailly,E., Dulic,V., Hengst,L., Resnitzky,D., and Slingerland,J. (1994). G1 control in mammalian cells. J Cell Sci Suppl *18*, 69-73.

Scholl,S.M., Fourquet,A., Asselain,B., Pierga,J.Y., Vilcoq,J.R., Durand,J.C., Dorval,T., Palangie,T., Jouve,M., Beuzeboc,P., and . (1994). Neoadjuvant versus adjuvant chemotherapy in premenopausal patients with tumours considered too large for breast conserving surgery: preliminary results of a randomised trial: S6. Eur. J Cancer *30A*, 645-652.

Semiglazov,V.F., Topuzov,E.E., Bavli,J.L., Moiseyenko,V.M., Ivanova,O.A., Seleznev,I.K., Orlov,A.A., Barash,N.Y., Golubeva,O.M., and Chepic,O.F. (1994). Primary (neoadjuvant) chemotherapy and radiotherapy compared with primary radiotherapy alone in stage IIb-IIIa breast cancer. Ann. Oncol *5*, 591-595.

Semiglazov, VF. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. Cancer 110[2], 244-254. 2007.

Smith,I.E., Dowsett,M., Ebbs,S.R., Dixon,J.M., Skene,A., Blohmer,J.U., Ashley,S.E., Francis,S., Boeddinghaus,I., and Walsh,G. (2005). Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol. *23*, 5108-5116.

Stomper,P.C., Winston,J.S., Herman,S., Klippenstein,D.L., Arredondo,M.A., and Blumenson,L.E. (1997). Angiogenesis and dynamic MR imaging gadolinium enhancement of malignant and benign breast lesions. Breast Cancer Res Treat *45*, 39-46.

Su,M.Y., Cheung,Y.C., Fruehauf,J.P., Yu,H., Nalcioglu,O., Mechetner,E., Kyshtoobayeva,A., Chen,S.C., Hsueh,S., McLaren,C.E., and Wan,Y.L. (2003). Correlation of dynamic contrast enhancement MRI parameters with microvessel density and VEGF for assessment of angiogenesis in breast cancer. J Magn Reson. Imaging *18*, 467-477.

Tao,Y., Klause,A., Vickers,A., Bae,K., and Ellis,M. (2005). Clinical and biomarker endpoint analysis in neoadjuvant endocrine therapy trials. J Steroid Biochem. Mol. Biol. *95*, 91-95.

Thurlimann,B., Keshaviah,A., Coates,A.S., Mouridsen,H., Mauriac,L., Forbes,J.F., Paridaens,R., Castiglione-Gertsch,M., Gelber,R.D., Rabaglio,M., Smith,I., Wardley,A., Price,K.N., and Goldhirsch,A. (2005). A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N. Engl. J. Med. *353*, 2747-2757.

Wedam SB. A pilot study to evaluate response and angiogenesis after treatment with bevacizumab in patients with inflammatory breast cancer. Low JA and Yang X. Proc Am Soc Clin Oncol 22. 2004.

Wong,Z.W. and Ellis,M.J. (2004). First-line endocrine treatment of breast cancer: aromatase inhibitor or antioestrogen? Br. J. Cancer *90*, 20-25.

Yankeelov,T.E., Lepage,M., Chakravarthy,A., Broome,E.E., Niermann,K.J., Kelley,M.C., Meszoely,I., Mayer,I.A., Herman,C.R., McManus,K., Price,R.R., and Gore,J.C. (2007). Integration of quantitative DCE-MRI and ADC mapping to monitor treatment response in human breast cancer: initial results. Magn Reson. Imaging *25*, 1-13.

Yung, W. K. A phase I trial of single-agent PTK 787/ZK 222584 (PTK/ZK), an oral VEGFR tyrosine kinase inhibitor, in patients with recurrent glioblastoma multiforme. Proc Am Soc Clin Oncol 22, 99. 2003. Abstract

*S*piegelhalter DJ, Freedman LS, Parmar MK. Bayesian approaches to randomized trials (with discussion). J Royal Stat Soc Ser A 1994; 157:357-416.

Fayers PM, Ashby D, Parmar MKB. Tutorial in biostatistics: Bayesian data monitoring in clinical trials. Stat Med 1997;16:1413 - 30.

**APPENDIX I SCHEDULE OF EVENTS PHASE II STUDY**

| **Parameter** | **Screening/ Baseline** | **On study evaluations (q 28 days)** | **End of AZD0530/ Presurg Visit[e]** | **Post-Surgical Visit** | |
|---|---|---|---|---|---|
| Informed Consent | x | | | | |
| Medical History – up to 28 days | x | x | x | x | |
| Physical examination – up to 28 days prior to study entry | x | x | x | x | |
| Performance Status | x | x | x | | |
| Toxicity Assessment | | x | x | | |
| Clinical tumor status – up to 28 days prior to study entry | x | X** | x | | |
| Electrocardiogram (ECG) | x | | | | |
| Dynamic MRI[a] | x | x[f] | x[f] | | |
| CBC, differential, platelet count | x | x | x | x[g] | |
| Serum Chemistries (electrolytes, creatinine, total bilirubin, alkaline phosphatase, AST, ALT) | x | x | x | x[h] | |
| HER2 status (by FISH or IHC) | x | | | | |
| Hormone Receptor status (IHC; local lab) | x | | | | |
| Radiologic Assessment to exclude distant metastases[b] | x | | | | |
| PK Draws[c] | | x [c] | | | |
| O2 saturation by pulse oximetry | x | | | | |
| Core biopsy/tumor tissue collection (snap frozen and paraffin-embedded) | x | | x [d] | | |
| Adverse Events | | x | x | x | |

[a] The baseline Dynamic MRI must be within 28 days of study entry and should, if possible, precede core biopsy collection.
[b] Are mandatory and will include bone scan and CT abdomen with and without contrast and a CT scan of lungs that may be done without contrast.
[c] Blood will be collected for PK evaluation of drug levels at day 28 and every 28 days for the next two cycles (+/- 3 business days).
[d] If surgery is to be performed, tumor tissue collection (See Appendix II) will occur at the time of definitive surgery. For patients whose tumors remain inoperable after neoadjuvant therapy, repeat core biopsies or an incisional biopsy may be obtained after the completion of study drugs and prior to the administration of additional therapy. Patients who withdraw from study due to toxicity will be asked but are not required to have repeat study biopsy.
[e] Procedures/studies do not need to be performed if previously performed within 28 days.
[f] MRI scan at week 10 ± 5 business days and prior to surgery within 4 weeks of stopping study drug
[g,h] Only if clinically indicated or abnormal pre-operatively
**In the event that a tumor is not clinically palpable at diagnosis and is measurable only by radiologic means (MRI, mammography or ultrasound), serial radiologic evaluations will include the week 10 MRI and evaluation at week 18 if subject is responding, or at end of study if therapy is stopped after cycle 4, see page 43.

## APPENDIX II TUMOR TISSUE COLLECTION AND PREPARATION AZD0530/ANASTROZOLE CLINICAL TRIAL TISSUE COLLECTION

### STANDARD OPERATING PROCEDURE

**PURPOSE OF STUDY RELATED CORE BIOPSY:**
Patients will usually have been identified as study candidates based on imaging and page or on a prior diagnostic biopsy which identifies them as having invasive ER+ and/or PR+ (HR+), HER2 negative breast cancer in the post-menopausal setting. If the patient has not had a prior diagnostic biopsy, the patient will be consented for the Tumor Bank Core Facility and during the diagnostic biopsy, sufficient material will be banked to permit retrieval for study related molecular assays, should be patient subsequently consent to trial. If the patient has already had a diagnostic biopsy either at UM or JMH, but no sample was banked, and the patient subsequently consents to the AZD0530/anastrozole Neoadjuvant trial (SCCC20080325), a study related biopsy will be performed to permit molecular correlative studies on the pre-treatment specimen associated with this clinical trial. Verification of the receptor positive and HER2 negative status must be done before the patient is formally entered on the trial.

In this Neoadjuvant clinical trial of anastrozole +/- AZD0530, patients will receive daily oral therapy with anastrozole 1 mg po, together with either placebo or AZD0530 175 mg po for up to 6 months prior to definitive surgery. All patients must have an adequate core biopsy prior to study entry for the purpose of acquisition of samples for molecular correlative studies per the protocol. The procedure will consist of an image guided cores biopsies taken for:

1. At least one but if possible two cores (depending on the tumor size) will be snap frozen in OCT per protocol below and stored at -70-80$^0$C), in a manner that permits subsequent histopathologic verification of the presence of tumor in acquired specimen prior to RNA extraction for gene expression profiling. The SOP detailed below provides the methodology for acquisition of biopsy samples for subsequent RNA extraction.
2. One core will be placed in a cryo-vial without OCT and immediately frozen in liquid nitrogen for subsequent RPPA proteomics analysis
3. Cores will be formalin fixed (two if possible, but at least one) and embedded in paraffin for study related IHC analysis. Biopsy samples will be immediately placed in formalin per clinical pathology SOP for subsequent embedding and slide preparation.

**PRINCIPLE:**
The ability to obtain high quality RNA from fresh tissue is highly dependent on the tissue procurement. Delays in tissue processing usually lead to RNA degradation. Inadequate freezing can be a major source of pre-analytic variance in downstream applications. In addition, estimating the amount of tissue needed to generate a sufficient quantity of RNA can be difficult. The following procedure addresses these issues.

**SPECIMEN:**
Fresh, unfixed clinical tissue.

**MATERIALS**:
Dry Ice
Disposable Gloves
Nunc cryovial for the RPPA specimen
Liquid Nitrogen dewar with sufficient liquid nitrogen to cover samples for RPPA
Formalin obtained from the pathology department for tissue fixation for IHC studies
Cryomold© Embedding Molds (VWR, Cat# 25608-924, 1-800-932-5000)
Cold Plate, 4x4 Stainless Steel sheet (VWR, Cat# 25608-942, 1-800-932-5000)
OCT Embedding Compound (VWR, Cat#25608-930, 1-800-932-5000)
BD 10mL Syringe (VWR, Cat# BD309604, 1-800-932-5000)
Forceps (VWR, Cat#82027-386, 1-800-932-5000)
Reclosable Zip Bag (VWR, Cat#89005-302, 1-800-932-5000)

**STORAGE REQUIREMENTS:**
Tissue samples for RNA extraction must be kept frozen on dry ice or at -80 C.
The RPPA sample will be kept in Liquid Nitrogen after placement in a cryovial

**PROCEDURE:**
1. Obtain dry ice.
2. Place the dry ice into the styrofoam container; fill 1/3 with dry ice and replace lid.
3. (3) Cryomolds are provided for potential cores and labeled with the study participant's unique ID number using a permanent marker or barcode label.
4. Place the cold plate on the dry ice so that it is level and will be cold prior to the actual removal of tissue from the individual.
5. Upon arriving at the site, place the Cryomold containers onto the cold plate.
6. Using the syringe of OCT embedding compound, fill the depth of each Cryomold ½ - ¾ full with OCT. The OCT should freeze within minutes. Do NOT transport the syringe of OCT or OCT bottle on dry ice. These are to be carried separately from the rest of the kit.
7. If diagnostic tissue is being obtained for the clinical management of the patient, the attending Pathologist or Pathology designee must release the tissue for research. This can only be done if sufficient tumor is obtained for diagnosis.
8. If diagnosis has previously been established, the tissue is released for research.
9. Place the provided tissue immediately onto the frozen layer of OCT using forceps.
10. With its Cryomold on the cold plate once again, place a top layer of OCT on the specimen. For optimal quality, the sample should be acquired and frozen within 5 minutes.
11. Replace the lid on the container so that the top layer of OCT will freeze.
12. After the samples are frozen, place them into the provided plastic bag and seal it.
13. Samples will be transported in Liquid Nitrogen or in dry ice to the Slingerland Lab for storage to maintain T of - 70°C -80°C until samples are batched for RNA extraction and gene expression analysis.
14. RNA extraction will be carried out after for frozen section analysis verifies the presence of tumor in the cryopreserved tissue.
15. For patients on clinical trial the submitting physician, phone, pager, email, time, date, trial ID number, [redacted]

## APPENDIX III WEB ADDRESS NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

The NCI Common Terminology Criteria for Adverse Events, version 3.0, can be accessed at the following website:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf

## APPENDIX IV DATA AND SAFETY MONITORING PLAN

The Sylvester Comprehensive Cancer Center (SCCC) Data and Safety Monitoring Committee (DSMC) will monitor this clinical trial according to the Cancer Center's DSM Plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study.

DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event data, and periodic review of response. The guidelines appearing in Section 11 are offered for DSMC consideration in assessing adverse events and response to study treatment. In addition, the DSMC will review reports from all audits, site visits, or study reviews pertaining to this clinical trial and take appropriate action.

## APPENDIX V ECOG PERFORMANCE SCALE

| Point | Description |
|---|---|
| 0 | Normal activity |
| 1 | Symptoms of disease, but ambulatory and able to carry out activities of daily living |
| 2 | Out of bed more than 50% of the time; occasionally needs assistance |
| 3 | In bed more than 50% of the time; needs nursing care |
| 4 | Bedridden; may need hospitalization |

## APPENDIX VI RECIST CRITERIA
### Definitions

- **Measurable disease** – the presence of at least one measurable lesion.
- **Measurable lesions** – lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm using spiral CT.
- **Non-measurable lesions** – all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and abdominal masses that are not confirmed and followed by imaging techniques.

### Baseline Documentation of Target and Non-Target Lesions

All measurable lesions, up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **Target Lesions** and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as a reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as **Non-Target Lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence or worsening of each should be noted throughout follow-up.

**Response Criteria**

Tables 1 and 2 summarize the criteria to be used to evaluate response.

**Table 1 Response Criteria for Target Lesions**

| 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 recorded since the treatment started |

**Table 2 Response Criteria for Non-Target Lesions**

| Response Criteria | Evaluation of Non-Target Lesions |
|---|---|
| Complete response (CR) | Disappearance of all non-target lesions and normalization of tumor marker level |
| Incomplete response or stable disease (SD) | Persistence of one or more non-target lesions and/or maintenance of tumor marker level above normal limits |
| Progressive disease (PD) | Appearance of one or more new lesions and/or unequivocal progression of non-target lesions |
| Unknown (UNK) | Progression has not been documented and one or more non-target lesions have not been assessed or have been assessed using a less sensitive technique than at baseline |

Table 3 summarizes the assessment of overall response, which is a composite of target lesion response, non-target lesion response, and presence of new lesions.

**Table 3 Evaluation of Overall Response**

| Target lesions | Non-target lesions | New lesions | Overall response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Incomplete response/SD | No | PR |
| CR, PR, SD | UNK | No | UNK |
| PR | Non-PD and not UNK | No | PR |
| SD | Non-PD and not UNK | No | SD |
| UNK | Non-PD and/or UNK | No | UNK |
| PD | Any | Yes or No | PD |
| Any | PD | Yes or No | PD |
| Any | Any | Yes | PD |

Note:
Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document objective progression after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

## APPENDIX VII REVERSE PHASE PROTEIN LYSATE ARRAYS (RPPA)

Combinatorial effects of the multiple genomic aberrations in breast cancer may be most clearly manifest at the proteomic level. Thus, functional proteomic analysis has the potential to assist in identification of pathways whose activation may account for heterogeneity in response to AZD0530.

Reverse phase protein lysate arrays (RPPA) is a novel, high throughput, quantitative and cost effective antibody-based approach to the study of functional proteomic patterns in tumors. RPPA offers an approach to quantitative profiling of the levels and activation of multiple proteins in breast cancer patient samples. It is a moderate throughput, quantitative, inexpensive, multiplexed ELISA with the potential to map protein levels as well as function in different intracellular pathways in a comprehensive, convenient, and sensitive manner. Thus, RPPA provides a practical approach to identification and validation of biomarkers for prognosis and prediction of response to Anastrozole /AZD0530. Although RPPA is fully validated for analysis of cell lines1-9, additional questions remain for its efficient application to breast cancer patient samples and eventually to patient management. [redacted]

RPPA allows high throughput quantitation (up to 1000 samples concurrently), low cost (~$0.50 per sample/antibody), sensitivity (fg of target) and amount of sample material required (ng of lysate). RPPA currently costs about 20 times less than ELISA-based approaches and about 10 times less than Luminex (BD). As tiny amounts of protein are required, RPPA is applicable to needle biopsies. These characteristics allow the simultaneous quantitative assessment of multiple different proteins in a single sample giving a comprehensive picture of the activation status of the tumor. The major disadvantage of RPPA is that it does not provide spatial organization, but this is resolved with complementary IHC. [redacted]

## APPENDIX VIII GENE EXPRESSION ANALYSIS BY MICROARRAY

Matched pre- and post-treatment tumor samples will be collected from up to 60 patients. A direct comparison of pre and post-treatment samples will be done to characterize the molecular response to AZD0530 or placebo. [redacted] details will be updated at the time of molecular analysis at trial completion. The following provides a procedural guideline only. Fluor reversals will be done on each sample pair to minimize the effects of dye bias, and each fluor reversal pair will be combined to create an experiment. In addition, a reference sample design will be used to compare each pretreatment sample to a reference sample. Additional microarray experiments will be performed on those patients from whom tumor tissue (and usable RNA) can be obtained at the time of definitive surgery.

[redacted]

[redacted]

[redacted]

A significant gene expression change will be defined as a 1.50-fold change with a p-value of 0.01. Histograms of the frequency of gene changes per patient will be generated. The number of groups defined will depend on the distribution of the samples across the sum of gene changes, with a minimum of two and a maximum of three groups defined.

In addition to treatment response, analysis will also be performed on tumors grouped on the basis of the other molecular markers being measured.

### Data Normalization

Normalization for dye bias and background on a single array will be calculated using appropriate software. This software flags outliers, computes statistics on inlier pixels and local background, does a background subtraction on features, performs dye normalization, and calculates a p-value that is a confidence measure of gene differential expression. Dye and background normalization are performed on a channel basis rather than on intensity ratios, so the corrected intensities for the channel corresponding to the tumor sample can be used to compare tumor samples across arrays. For instance, a direct comparison between pre- and post-treatment could be made by extracting the normalized intensity value for the appropriate channel in each array.

[redacted]

[redacted]

[redacted]

**Supervised Classification Analysis**

Linear discrimination analysis and nearest-neighbor classifiers will be used on filtered gene lists to predict tumor response. Cross validation procedures will be used to assess the repeatability of the results on new data.

**Selecting Significant Genes**

Both clustering and supervised classification algorithms are sensitive to the genes selected for analysis. We propose to use several selection methods to determine significant genes, and then run the analysis on each of these gene sets.

[redacted]

**Detecting a gene expression signature of Src activation**

[redacted]

## APPENDIX IX PHARMACOKINETIC EVALUATION – PHASE I ONLY

| Drug substance | 6 hours | 12 hours | 24 hours | 48 hours | 72 hours | Day 8 | Day 15 | Day 22 |
|---|---|---|---|---|---|---|---|---|
| anastrozole | x | x | x | x | x | x | x | X |
| AZD0530 | x | x | x | x | x | x | x | x |

At intervals after initiation of AZD0530 at 6, 12, 24, 48, and 72 hours; and on days 8, 15 and 22 blood will be drawn and sent to AstraZeneca for pharmacokinetic measurement of AZD0530 and anastrozole.

## APPENDIX X SUMMARY OF STRONG CYP3A INDUCERS AND INHIBITORS PROHIBITED ON STUDY

The listed strong CYP3A4 inducers and inhibitors should not be used while on protocol medications. They are prohibited.

CYP3A4 inhibitors including:

- Antibiotics (clarithromycin, telithromycin)
- Antifungals (ketoconazole, itraconazole, voriconazole)
- Antiretroviral protease inhibitors (atazanavir, indinavir, nelfinavir)
- Grapefruit

CYP3A4 inducers including:

- Rifampin, rifabutin, rifapentin
- Phenytoin, barbiturates (Phenobarbital)
- Carbamazepine
- Other (dexamethasone, St. John's Wort)

Since concurrent administration of AZD0530 with other CYP3A4 substrates that are not strong inducers or inhibitors of CYP3A4 is well tolerated, continuation of medically indicated CYP3A4 substrate drugs is permitted at physician discretion. AZD0530 administered concurrently with CYP3A4 substrates may increase modestly drug bioavailability and careful review of concurrent medications and AEs is required at each follow up visit while Phase II Cohort B patients are on therapy. Consult http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm for list of known CYP3A4 substrates.