Phase II study of **P**re-emptive **O**sci**LL**ation of ER activit**Y** levels through alternation of estradiol/anti-estrogen therapies prior to disease progression in ER+/HER2- metastatic or advanced breast cancer (POLLY)

Principal Investigator: *Mary Chamberlin*
Dartmouth-Hitchcock Medical Center
Dartmouth Cancer Center
One Medical Center Drive
Lebanon, NH 03756
(603) 650-4628

Co-investigator: *Jonathan Marotti, MD (Pathologist)*
Phone: (603) 650-7211

Statistician: *Zhigang Li, PhD*
HB-7927
Phone: (603) 653-6102

Protocol Number: D14122

NCT Number: NCT02188745

Initial version: April 15, 2014
Amended: [July 13, 2015, corrected July 14, 2015]
Amended: October 21, 2015
Amended: February 25, 2016
Amended November 20, 2017
Amended May 14, 2018
Amended August 10, 2018
Amended August 30, 2023

Table of Contents

STUDY SUMMARY **1**

1 INTRODUCTION **4**

1.1 Background 4
1.2 Investigational Agent 5
1.3 Preclinical Data 5
1.4 Clinical Data to Date 6
1.5 Dose Rationale and Risk/Benefits 7

2 STUDY OBJECTIVES **9**

3 STUDY DESIGN **9**

3.1 General Design 9
3.2 Primary Study Endpoints 11
3.3 Secondary Endpoints 11

4 SUBJECT SELECTION AND WITHDRAWAL **11**

4.1 Inclusion Criteria 11
4.2 Exclusion Criteria 12

5 STUDY DRUG **13**

5.1 Description 13
5.2 Treatment Regimen 13
5.3 Method for Assigning Subjects to Treatment Groups 13
5.4 Preparation and Administration of Study Drug 13
5.5 Packaging 13
5.6 Blinding of Study Drug 13

6 STUDY PROCEDURES **14**

6.1 Registration 14
6.2 Schedule 14
6.2.1 *Physical Examination* 14
6.2.2 Laboratory Assessments 14
6.2.3 Research Assessments 14
6.2.4 Treatment Schedule 15
Study Calendar (Table 6.2) 16
6.3 Assessment of Response 18
6.4 Clinical Data Collection. 18
6.5 Retrospective analysis of ESR1 mutation status through circulating tumor DNA 18
6.6 Retrospective genetic analysis of tumors 18
6.7 Retrospective transcriptional analysis of tumors 19

7 STATISTICAL PLAN **19**

7.1 Sample Size Determination 19
7.2 Statistical Methods 19
7.3 Subject Population(s) for Analysis 21

8 SAFETY AND ADVERSE EVENTS **21**

8.1 Adverse Events 21
8.1.1. Methods of Assessment 22
8.2 Definitions 22
8.3 Recording of Adverse Events 23
8.4 Reporting of Serious Adverse Events and Unanticipated Problems 23

8.4.1 Investigator reporting: notifying the Dartmouth IRB 24
8.4.2 Investigator reporting: notifying a non-Dartmouth IRB 25
8.4.3 Expedited Adverse Event Reporting 25

9 DATA HANDLING AND RECORD KEEPING 26

9.1 CONFIDENTIALITY 26
9.2 SOURCE DOCUMENTS 26
9.3 CASE REPORT FORMS 26
9.4 RECORDS RETENTION 26

10 ETHICAL CONSIDERATIONS 26

11 STUDY FINANCES 27

11.1 FUNDING SOURCE 27
11.2 CONFLICT OF INTEREST 27
11.3 SUBJECT STIPENDS OR PAYMENTS 27

12 STUDY MONITORING, AUDITING, AND INSPECTING 28

12.1 SAFETY AND DATA MONITORING 28
12.2 DATA MONITORING 28
12.2.1 Monitoring at DHMC 28
12.2.2 Monitoring at non-DHMC sites 29
12.3 AUDITING AND INSPECTING 29
12.4 INVESTIGATOR COMPLIANCE 29

13 REFERENCES 29

List of Abbreviations and Definitions

DCC – Dartmouth Cancer Center
DH IRB – Dartmouth Hitchcock Institutional Review Board
ER- estrogen receptor alpha
HER2- the HER2 (ERBB2) proto-oncogene
PFS- progression-free survival; defined as the time period from start of a given treatment for locally advanced or metastatic breast cancer until the time of cancer progression or death from any cause.
RFS- recurrence-free survival; defined as the time period from initiation of adjuvant anti-estrogen therapy until the time of disease recurrence or death.
TTP- time to disease progression; defined as the time from start of a given treatment until the time of disease progression. Patients who discontinue protocol therapy due to toxicity will be censored.
RECIST- Response Evaluation Criteria in Solid Tumors
OS- overall survival
CR- complete response
PR- partial response
SD- stable disease
CB- clinical benefit- defined as SD at 24 weeks + CR + PR.
CBR- clinical benefit rate; the proportion of evaluable patients experiencing CB.
OR- objective response- defined as CR + PR
ORR- objective response rate; the proportion of evaluable patients experience OR.
DES- diethylstilbestrol
AI- aromatase inhibitor (third-generation AIs include exemestane, letrozole, anastrozole)
E2- 17β-estradiol

Advanced breast cancer- has inconsistent definitions in the field. Herein, refers to disease that is locally recurrent or metastatic (stage IV).

Metastatic breast cancer (stage IV)- refers to disease that has spread to other parts of the body beyond breast and regional lymph nodes, as defined by current American College of Pathology staging criteria.

Study Summary

Title	Phase II study of Pre-emptive OsciLLation of ER activitY levels through alternation of estradiol/anti-estrogen therapies prior to disease progression in ER+/HER2- metastatic or advanced breast cancer (POLLY)
Short Title	POLLY- Alternating estradiol/anti-estrogen therapies in ER+/HER2- breast cancer
Protocol Number	D14122
Phase	2
Methodology	Patients with anti-estrogen-resistant stage IV breast cancer (or patients with locoregional breast cancer recurrence not amenable to treatment for curative intent) will have a biopsy of recurrent or metastatic disease as standard-of-care; additional tumor tissue will be collected for research purposes. Alternatively, archived tumor tissue acquired within the past 4 months may be used for research purposes. A current biopsy is required except in cases of bone-only disease, or in patients in whom a current biopsy cannot be readily and/or safely performed. Patients with ER+/HER2- disease will be treated with 17β-estradiol for 8 weeks, then treated with an aromatase inhibitor for 16 weeks, alternating between 17β-estradiol and aromatase inhibitor until disease progression. Patients may (optional: at patient's and treating physician's discretion) then be switched to the alternate therapy for one last line of continuous therapy on this study, and treated until another instance of disease progression. At this point, the patient will end protocol therapy. Tumor biopsies obtained at/before baseline (required except as noted above) and after 12-14 days of 17β-estradiol therapy (optional), and blood samples obtained at baseline (required), after 12-14 days of 17β-estradiol (optional), and at the first (required) and second (optional) incidences of cancer progression will be analyzed to identify molecular biomarkers predictive of response to 17β-estradiol therapy.
Study Duration	48 months
Study Center(s)	3

Objectives	*Primary Objective:* Determine the rate of clinical benefit from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer. *Secondary Objectives:* 1) Determine the objective response rate of patients treated with 17β-estradiol therapy for 8 weeks. 2) Determine the progression-free survival from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer. 3) Determine the adverse event profiles of 17β-estradiol and aromatase inhibitors in this patient population. 4) Determine whether specific tumor genetic lesions in *ESR1* are predictive of response to 17β-estradiol and/or subsequent aromatase inhibitor therapies. *Exploratory Objectives:* 1) Determine the rate of clinical benefit and interval of progression-free survival from continuous aromatase inhibitor therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy. 2) Determine the rate of clinical benefit and interval of progression-free survival from continuous 17β-estradiol therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy. 3) Identify molecular biomarkers predictive of response to 17β-estradiol and/or alternating 17β-estradiol/aromatase inhibitor therapies. 4) Determine whether genetic lesions in *ESR1* become detectable in circulating DNA in plasma at the time of disease progression on alternating 17β-estradiol/aromatase inhibitor therapies. 5) Identify molecular changes that occur in response to 17β-estradiol therapy in ER+/HER2- breast tumors.
Number of Subjects	29

Main Inclusion Criteria	1) Patients with metastatic, or locally recurrent and not amenable to therapy for curative intent, strongly ER+ (criteria as defined below)/HER2- breast cancer previously treated at some time with anti-estrogen therapy. 2) If most recent therapy was in adjuvant setting, recurrence-free interval must have been ≥2 years since initiation of adjuvant hormonal therapy. 3) If most recent therapy was in advanced/metastatic setting, progression-free interval must have been at least 3 months (except in the case of investigational hormonal therapies). 4) Must not have received fulvestrant (faslodex) within the past 16 weeks. 5) Patients must meet one of the following criteria: A) undergo a clinically indicated biopsy of recurrent or metastatic disease, allowing for additional tumor tissue that will be acquired for research purposes. B) have had a biopsy within the past 4 months that will provide sufficient tumor tissue for research purposes. C) have bone-only metastatic disease for which a biopsy is not required, or not have a site of metastatic disease that can be readily and safely biopsied. 6) ER+ status defined as ER staining by immunohistochemistry in ≥50% of malignant cell nuclei with an intensity ≥2+ on a scale of 0-3+. These criteria are equivalent to an Allred score ≥6.
Study Product, Dose, Route, Regimen	Approved agents: 17β-estradiol, Letrozole, Anastrozole, Exemestane
Duration of administration	Duration of therapeutic benefit
Statistical Methodology	**Sample size calculation:** This study was designed using Simon's two-stage design. The null hypothesis that the true Clinical Benefit Rate (CBR) is 10% will be tested against a one-sided alternative. In the first stage, 10 patients will be accrued. If <1 patient experiences CB among these first 10 patients, the study will be stopped for futility. Otherwise, 19 additional patients will be accrued for a total of 29 patients. The null hypothesis will be rejected if ≥6 patients experience CB among 29 total patients. This design yields a type I error rate of 5% and power of 80% when the true CBR is 30%.

1. Introduction

This document is a protocol for a human research study. This study is to be conducted according to U.S. and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, and institutional research policies and procedures.

1.1 Background

Patients with estrogen receptor alpha (ER)-positive breast cancer are typically treated with anti-estrogen therapies that antagonize ER signaling. These often include the selective ER modulator tamoxifen, the aromatase inhibitors (AIs) exemestane, letrozole, and anastrozole, and the selective ER downregulator fulvestrant. While anti-estrogen therapies have changed the natural history of ER+ breast cancer, many patients exhibit or develop anti-estrogen-resistant locally advanced or metastatic disease, and nearly all patients with metastatic disease are not cured by approved therapies. The only mechanism of endocrine resistance for which there is supporting clinical evidence is overexpression/amplification of the HER2/*ERBB2* proto-oncogene; such tumors are treated with anti-HER2 therapies and are not the subject of this study.

Upon the development of antiestrogen-resistant breast cancer, patients are often treated with subsequent lines of anti-estrogen therapy. It is acceptable to even recycle prior lines of anti-estrogen therapy in the advanced disease setting. Some cancers continue to respond to anti-estrogens; however, nearly all such cancers eventually become resistant to all available anti-estrogens. Upon progressing through treatment with one anti-estrogen, a cancer may respond to a different anti-estrogen; the mechanism underlying this phenomenon is not well-understood, but may involve interpatient variability in drug metabolism, different mechanisms of drug action, and/or non-overlapping mechanisms of resistance. Nonetheless, in patients with locally advanced or metastatic ER+ disease, median time-to-progression (TTP) or progression-free survival (PFS) on first-line anti-estrogen therapy ranges in studies from 6 to 24 months (3-7), and median PFS or TTP on second-line or later anti-estrogens is approximately 3.7 to 5 months (8-10). It is well-established that the PFS and TTP intervals shorten substantially with subsequent lines of anti-estrogen therapy in patients with ER+ breast cancer.

Several decades ago, synthetic estrogens such as diethylstilbestrol (DES) were used for the treatment of breast cancer. 17β-estradiol also became a moderately utilized therapy after disease progression on anti-estrogens. This seems counterintuitive since we now rely on anti-estrogens for disease management, but response rates to “high-dose estrogens” are similar to those of anti-estrogens, particularly in the advanced disease setting (1, 11-14). In 1944, Haddow *et al*. found that longer time intervals after menopause were associated with increased clinical benefit from DES therapy (15). This phenomenon is thought to occur because the post-menopausal period creates a low-estrogen environment, and tumors growing under low-estrogen conditions may be sensitized to estrogen therapy. Therapeutic suppression of estrogen signaling (via modern anti-estrogens) may similarly sensitize cancers to estrogen therapy. Indeed, some ER+ breast cancers respond to withdrawal/cessation of anti-estrogen therapy (16-21), which may be caused by A) return of ER signaling to physiological levels induced by endogenous estrogens, and/or B) cessation of agonistic effects on ER in the case of tamoxifen. The primary reason that anti-estrogens (primarily Tamoxifen) replaced estrogens has to do with toxicity, which was more frequent and more severe with DES than Tamoxifen (11, 12). DES is no longer available in the U.S. However, 17β-estradiol is a therapeutic option for treatment of advanced ER+ breast cancer worthy of further study. However, the mechanism(s) underlying response to estrogens or anti-estrogen withdrawal, and biomarkers predicting response, are unknown.

Given that ER+ breast cancers are often responsive to anti-estrogens and estrogens, the concept of alternating anti-estrogen/estrogen therapies has been discussed for several years but not yet formally explored. Anecdotal evidence indicates that such a strategy of alternating therapies is effective in some patients (1). Preclinical evidence suggests that anti-estrogen-resistant ER+ breast cancers are sensitized to the anti-tumor effects of estrogens (22-27). Such cells harbor subpopulations that can ultimately regain the ability to grow in the presence of estrogens, and revert to their anti-estrogen-sensitive state. We thus

propose a clinical study to formally test whether alternating 17β-estradiol/anti-estrogen therapies is effective for the management of metastatic ER+/HER2- disease, and to identify molecular biomarkers that predict tumor response to 1) 17β-estradiol and 2) alternating 17β-estradiol/anti-estrogen therapies. If successful, this study would present a novel strategy to manage metastatic ER+/HER2- breast cancer by switching therapies before disease progression.

1.2 Investigational Agent

This study will use approved therapies. Aromatase inhibitors (AIs) are clinically indicated and widely used for treatment of advanced ER+ breast cancer. 17β-estradiol is approved for hormone replacement therapy (HRT) in post-menopausal women, and as noted above is used therapeutically for advanced breast cancer as well.

1.3 Preclinical Data

Work in preclinical models demonstrates that ER+ breast cancer cells that become anti-estrogen-resistant are sensitized to the anti-tumor effects of estrogens (22-27). Such cells harbor subpopulations that can ultimately regain the ability to grow in the presence of estrogens, and revert to their anti-estrogen-sensitive state. Anti-estrogen-resistant breast cancer cells often exhibit upregulated levels of ER, which may contribute to their apoptotic response to 17β-estradiol (E2).

The human ER+ breast cancer cell line MCF-7 is a staple for laboratory research. MCF-7 cell growth is stimulated by estrogens and inhibited by anti-estrogens, or by RNA interference-mediated knock-down of ER expression. Chronic exposure to anti-estrogens induces the outgrowth of anti-estrogen-resistant cells. Fulvestrant-resistant MCF-7 cells (MCF-7/FR) are maintained in the presence of 1 μM fulvestrant, which effectively neutralizes ER transcriptional activity. Similarly, cells maintained in hormone-depleted conditions ('long-term estrogen-deprived', LTED) model resistance to AIs by suppressing estrogen-induced ER activity. Withdrawal of fulvestrant from MCF-7/FR cells, or re-introduction of 17β-estradiol to MCF-7/LTED cells allows re-engagement of ER signaling and re-expression of markers of ER activity, followed by growth arrest and cell death after 1-2 weeks. Eventually, a minority of cells regain the ability to grow in the presence of estrogens, and revert to an anti-estrogen-sensitive state. The theory underlying such estrogen/ER-induced cell death is that hyperactivation of ER, or activation of ER in an inappropriate transcription cofactor context, has deleterious effects in breast cancer cells. Since MCF-7/LTED and MCF-7/FR cells exhibit increased ER levels and estrogen-independent ER activity compared to parental MCF-7 cells (28, 29), re-introduction of estrogen can drive excessive ER signaling. Cells that survive the onslaught of ER signaling may eventually adapt and grow, reverting to an anti-estrogen-sensitive state.

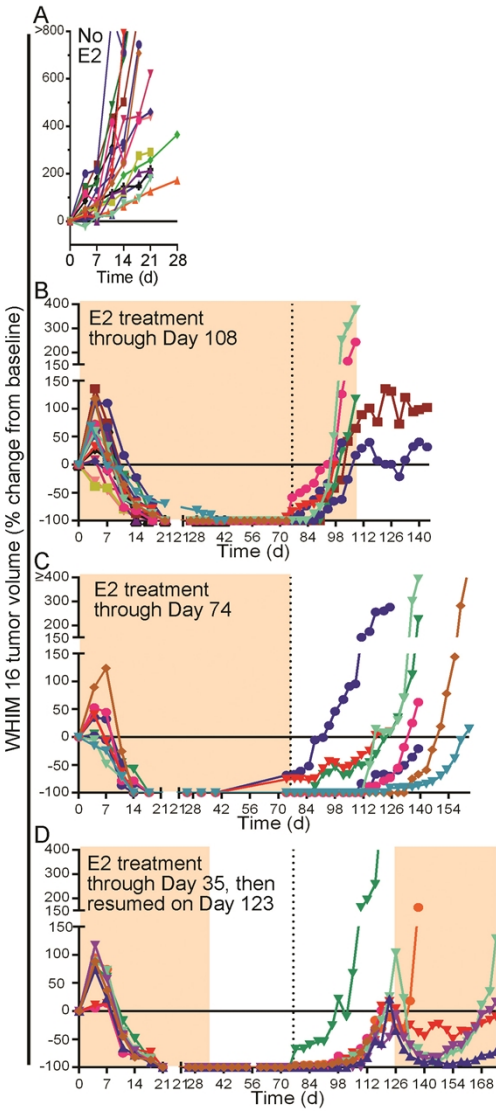


Fig. 1- E2 treatment induces WHIM16 tumor regression. Ovariectomized mice were implanted s.c. with WHIM16 tumor fragments. Tumor volumes were measured twice weekly. Each line represents an individual mouse. Mice bearing WHIM16 tumors ~200 mm^3 were implanted s.c. with a wax pellet containing 1 mg E2 (2) on Day 0 where indicated by orange shading, and pellets were removed as indicated by white background.

The WHIM16 xenograft model was derived from a patient with AI-resistant ER+/HER2- metastatic breast cancer that responded to E2 therapy (1, 30). WHIM16 tumors harbor *ESR1* amplification and high ER levels (30). Ovariectomized NOD/SCID/IL2R$\gamma^{-/-}$ (NSG) mice bearing WHIM16 subcutaneous tumors were treated +/- E2 via subcutaneous pellet. Control mice showed continued tumor growth (Fig. 1A), while tumors in mice treated with E2 (orange shading in Fig. 1B-D) completely regressed. In mice with continuous E2 treatment, tumors eventually regrew and became palpable around Day 74 (dotted vertical line in Fig. 1B).

Most patients treated with estrogen therapy eventually experience disease progression, and tumors often exhibit restored sensitivity to anti-estrogens (1, 11, 31-39). Since regressed WHIM16 tumors may recur due to E2 stimulation of a residual cell population (*i.e.*, E2-induced growth), we treated tumor-bearing mice with E2 for 74 d or 35 d, then withdrew E2 (Fig. 1C/D). Earlier E2 withdrawal (Fig. 1D) delayed tumor regrowth compared to continuous E2 therapy (Fig. 1B), suggesting that residual cancer cells following E2 treatment are resensitized to estrogen deprivation. Indeed, two tumors that recurred on E2 therapy were growth-inhibited by E2 withdrawal (Fig. 1B).

1.4 Clinical Data

Ingle et al. performed a study with 143 post-menopausal patients with advanced ER+ breast cancer randomized to treatment with tamoxifen or the synthetic estrogen diethylstilbestrol (DES) (11). Therein, 99 patients had received no prior systemic therapy, and 44 patients had received prior chemotherapy. Response rates were 41% vs. 33% in patients treated with DES vs. tamoxifen, and TTP was similar in both arms (142 days vs. 171 days). An updated analysis of this study showed that median overall survival (OS) was 3.0 years for DES vs. 2.4 years for tamoxifen (adjusted p=0.039). Rates of 5-year survival were 35% for DES vs. 16% for tamoxifen (13). Of note, none of these patients received prior endocrine therapy.

In a study with 32 post-menopausal patients with advanced breast cancer previously exposed to multiple lines of endocrine therapy (median of 4 lines, range of 2-10 lines), Lonning *et al*. observed that treatment with DES yielded clinical benefit (CB) in ~41% of cases (31). Four patients terminated therapy within 2 weeks on start of DES therapy due to side effects; another 2 patients terminated therapy before disease progression for similar reasons [1 patient with stable disease (SD) after 15 weeks, and 1 patient with a partial response (PR) at 39 weeks]. Four patients achieved a complete response (CR), 6 patients had PR, and 2 patients had SD for at least 6 months. Five patients had an objective response, and one patient had SD lasting over a year.

Gockerman *et al*. conducted a randomized study of 115 post-menopausal women with advanced ER+ or ER-unknown breast cancer (12) (note: ~75% of unselected breast cancer cases are ER+). Patients were treated with tamoxifen or DES. Response frequencies were similar between the 2 arms (complete response rates of 2% vs. 2%; partial response rates of 4% vs. 8%). Stable disease was observed in 78% (tamoxifen) vs. 73% (DES) of patients. Median time-to-progression (5 months vs. 6 months) and median survival (34 months vs. 35 months) were similar for tamoxifen and DES. Upon withdrawal of each agent and crossover to the alternative agent, responses were frequently observed, suggesting non-overlapping mechanisms of resistance.

A recent study by Ellis *et al.* (1) compared treatment with 6 or 30 mg/day of 17β-estradiol (E2) in women with advanced, anti-estrogen resistant, ER+/HER2- breast cancer. Patients had either received AI therapy in the advanced disease setting with ≥24 weeks of progression-free-survival (PFS), or had received AI therapy in the adjuvant setting without disease recurrence for ≥2 years. One line of chemotherapy for advanced disease was allowed, and any number of prior lines of anti-estrogen therapy was allowed. Similar rates of CB were observed between the two doses of 17β-estradiol, but the higher dose was associated with more adverse events; thus, we will use the lower dose of 6 mg/day 17β-estradiol in the study described herein. In the combined analysis of 66 patients treated with 17β-estradiol, 4 had a PR, and 15 experienced SD, providing a clinical benefit rate of 29%. Seven patients entered the study who had relapsed while receiving adjuvant AI therapy; first-line treatment for advanced disease with 6 mg/d 17β-estradiol induced a PR in one patient, and SD in another. In addition, 7 patients who benefited from

17β-estradiol therapy were re-treated with an AI after disease progression on 17β-estradiol; among these 7 patients, 3 experienced clinical benefit from AI re-treatment (2 had SD; 1 had PR). These results were adapted from ref. (1) and are presented in the following table:

Adapted from ref. (1)
Supplementary Table. Outcomes for Patients Receiving Repeat Treatment With an Aromatase Inhibitor After Experiencing an Initial Response to Estradiol.

Pt. #	Dose and # of weeks on estradiol (E2) therapy	Aromatase inhibitor used for re-treatment	Weeks on repeat AI; Disease Site; Response Category
1019	E2 6-mg 72 weeks Best response SD	Exemestane	28+ weeks; Bone; Best response SD
1024	E2 30-mg to 6-mg 40 weeks Best response SD	Anastrozole	36 weeks; Bone, lymph nodes; Best response PR
1025	E2 6-mg 56 weeks Best response SD	Letrozole	12 weeks; Bone, lymph node; Best response PD chest wall
2010	E2 6-mg 48 weeks Best response PR	Exemestane	36 weeks; Lymph node; Best response PR Liver
2011	E2 30-mg to 6-mg 44 weeks Best response SD	Exemestane	8 weeks; Liver; Best response PD
5001	E2 30-mg arm 84 weeks Best response SD	Letrozole	24 weeks; Bone; Best response PD Breast recurrence
5003	E2 6-mg 32 weeks Best response SD	Exemestane	12 weeks; Bone, lymph node; Best response PD pleura, chest wall

1.5 Rationale and Risk/Benefits

Synthetic estrogens and 17β-estradiol have been used for decades to treat patients with ER+ breast cancer. The above-mentioned studies (Section 1.4) are a sampling of evidence supporting the use of such agents. While such agents showed similar anti-tumor efficacy compared to Tamoxifen, estrogens were ultimately relegated to use in very late-stage disease, if at all, due to increased frequencies of adverse effects compared to newer anti-estrogens. In the advanced setting, patients with ER+ disease are often treated with several lines of anti-estrogen therapy, experiencing disease control for several months before disease progresses with each line. Nearly all advanced ER+ breast cancers ultimately develop resistance to all available anti-estrogens, and treatment options for such patients are either traditional chemotherapy, the combination of the mTORC1 inhibitor everolimus plus the AI exemestane (upon which essentially all ER+ cancers eventually progress), the combination of a CDK4/6 inhibitor (*e.g.*, palbociclib or ribociclib) plus an anti-estrogen [upon which essentially all ER+ cancers eventually progress; data are still maturing (40, 41)], or experimental agents. At present however, essentially all these patients develop disease that is ultimately refractory to all hormonal-based regimens, with or without combination biologic or targeted therapies. While some anti-estrogen-resistant cancers respond to a next-line anti-estrogen, the biology underlying such sensitivity is unclear, and is somewhat counter-intuitive: if a cancer is resistant to one anti-estrogen, it is more likely to be cross-resistant to all anti-estrogens than to be sensitive to another anti-estrogen. Such reasoning may partially explain the increasingly shorter PFS intervals observed in patients treated with subsequent lines of anti-estrogens. In contrast, use of estrogen therapy for anti-estrogen-resistant disease makes mechanistic sense (as described above): if an ER+ cancer is resistant to an anti-estrogen, it may be hypersensitive to estrogen therapy.

Given the evidence supporting the anti-tumor effects of estrogen/ER signaling, particularly in breast cancer cells and xenografts following estrogen deprivation, estrogens may be beneficial for the treatment of anti-estrogen-resistant disease. Identification of a biomarker(s) that is predictive of response to 17β-

estradiol would allow selection of future patients likely to benefit from this treatment. Determination of whether consecutive 17β-estradiol/anti-estrogen therapy is beneficial for disease control, and whether a biomarker is predictive of benefit from such consecutive therapies, would provide a foundation for a larger study to develop this strategy as a treatment plan for advanced ER+ breast cancer.

We will evaluate post-menopausal patients with advanced ER+/HER2- breast cancer that received prior treatment with an anti-estrogen (in any setting). Baseline (pre-17β-estradiol) tumor tissue for research purposes is required for patients with safely biopsiable non-bone metastatic disease (*i.e.*, any site except bone); such tumor tissue may either be obtained A) from an archived tissue specimen obtained within the past 4 months (providing the specimen will provide sufficient material for research use), or B) through a clinically indicated biopsy performed as standard of care in which additional tumor tissue will be obtained for research use. Patients will then be treated with 17β-estradiol (6 mg/day, taken as 2 mg PO t.i.d.) for 8 weeks, then switched to an AI for 16 weeks, alternating between these therapies on a defined schedule. An optional research tumor biopsy after 12-14 days of 17β-estradiol therapy will be requested in patients for whom this procedure is considered safe. This 17β-estradiol/AI treatment schedule was selected based on findings that A) AI therapy provides a median PFS of ~4 months in patients with advanced disease previously treated with an anti-estrogen (8, 42, 43), B) time to maximal tumor response to neoadjuvant AI therapy is ~4 months (44, 45), C) 17β-estradiol induces apoptosis within 2-3 weeks in preclinical models [preliminary data and refs. (22-27)], D) 17β-estradiol therapy is accompanied by more adverse events than anti-estrogens, so time on 17β-estradiol is shorter to avoid toxicity, and E) one 'cycle' of 17β-estradiol/anti-estrogen therapy in this study is 6 months, which is a benchmark for determination of clinical benefit. In a prior study of 34 patients, this planned dose of 17β-estradiol was associated with Grade 3-4 serious adverse events (SAEs) in 12% of patients (1); Grade 3 adverse events included Low Na+ levels (1 case), Pain (6 cases), CNS ischemia (1 case), Infection (2 cases), and 6 other instances of event(s); the only observed Grade 4 adverse event was 1 case of Thrombosis/Embolism. Grade 1-2 SAEs were observed in 30 of 34 (88%) patients.

Patients with metastatic, or locally recurrent and not amenable to surgery with curative intent, ER+/HER2- breast cancer are eligible. We anticipate that the majority of patients participating in this study will have received anti-estrogen therapy in the advanced/metastatic setting, and thus treatment with 17β-estradiol will be 2nd line or later therapy for advanced disease. Patients with newly diagnosed advanced breast cancer that recurred on anti-estrogen therapy in the adjvant setting, if on adjuvant hormonal therapy for >2 years, are also eligible, and such breast cancer is also predicted to be sensitive to 17β-estradiol therapy.

Participation in this study is of greater-than-minimal risk, has the potential for direct clinical benefit by offering the patient an under-utilized therapeutic (*i.e.*, 17β-estradiol) in an innovative strategy (*i.e.*, alternating antagonistic estrogen/anti-estrogen therapies), and has the potential to generate important generalizable knowledge for identifying biomarkers to direct treatment decisions in future patients with breast cancer. Physical risks of study participation include risks associated with 17β-estradiol and aromatase inhibitor therapies, which are FDA-approved drugs with extensive histories of clinical use, and risk that switching therapies (17β-estradiol and AI) prior to disease progression may be less effective than continuing one therapy until disease progression. Loss of patient confidentiality is a risk of study participation, and this risk will be minimized by the following: pathology specimens from the patient (tumor, blood, plasma) will be labeled only with the patient case number; the key matching patient identification with the patient case number will be kept in a protected environment; only the PI and his designated representatives will have access to this key; no one will have access to the study records except the Investigators and CRCs, the DH IRB, the FDA, the OHRP, government regulatory bodies, and Dartmouth compliance and quality assurance groups permitted to conduct study-related audits and inspections.

Study Objectives

Primary Objective:
Determine the rate of clinical benefit from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer.

Secondary Objectives:
1) Determine the objective response rate of patients treated with 17β-estradiol therapy for 8 weeks.

2) Determine the progression-free survival from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer.

3) Determine the adverse event profiles of 17β-estradiol and aromatase inhibitors in this patient population.

4) Determine whether specific tumor genetic lesions in *ESR1* are predictive of response to 17β-estradiol and/or subsequent aromatase inhibitor therapies.

Exploratory Objectives:
1) Determine the rate of clinical benefit and interval of progression-free survival from continuous aromatase inhibitor therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy.

2) Determine the rate of clinical benefit and interval of progression-free survival from continuous 17β-estradiol therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy.

3) Identify molecular biomarkers predictive of response to 17β-estradiol and/or alternating 17β-estradiol/aromatase inhibitor therapies.

4) Determine whether genetic lesions in *ESR1* become detectable in circulating DNA in plasma at the time of disease progression on alternating 17β-estradiol/aromatase inhibitor therapies.

5) Identify molecular changes that occur in response to 17β-estradiol therapy in ER+/HER2- breast tumors.

Study Design

3.1 General Design

This is a single-arm phase II study of post-menopausal patients with locally recurrent or metastatic ER+/HER2- breast cancer. Eligible patients will have received an anti-estrogen [common medications include aromatase inhibitors (AIs) such as Letrozole, Anastrozole, Exemestane, Formestane, and Aminoglutethimide; Tamoxifen; Toremifene; Endoxifen; Raloxifene; Fulvestrant] at some time in their disease history. The most recent line of therapy must not have included Fulvestrant within the prior 16 weeks. Patients with disease that has been treated in the advanced/metastatic setting must have had a PFS interval of ≥3 months with the most recent line of therapy (except in the case of investigational therapies, or investigational therapy combinations that include an endocrine agent); multiple lines of prior anti-estrogen therapy are allowed; one line of chemotherapy in the advanced setting is allowed. Patients with newly diagnosed advanced disease (untreated in the advanced/metastatic setting) must have been treated with adjuvant anti-estrogen therapy with a RFS interval of ≥2 years. Prior (neo)adjuvant chemotherapy is allowed.

In cases of non-bone metastatic disease (*i.e.*, any tumor site besides bone) in which a biopsy is clinically indicated, excess tumor tissue must be acquired for research purposes during the biopsy procedure.

Alternatively, archived tumor tissue obtained within the past 4 months prior to study enrollment may be used if the tissue specimen provides adequate tissue for research purposes.

This study is a single-arm treatment design with a primary objective of measuring the rate of clinical benefit from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer. Although PFS is a common endpoint in therapeutic studies of advanced breast cancer, this patient population has widely varying PFS intervals. Median TTP or PFS on first-line anti-estrogen therapy in the setting of advanced disease ranges in studies from 6 to 24 months (3-7), and median PFS or TTP on second-line or later anti-estrogens is 3.7 to 5 months (8-10). It is well-established that PFS intervals shorten substantially with subsequent lines of anti-estrogen therapy in ER+ breast cancer. Since this study will enroll patients with any number of prior lines of anti-estrogen therapy, and ≤1 prior line of chemotherapy for advanced disease, we anticipate a wide range of PFS intervals. Hence, clinical benefit will provide a more accurate reflection of the clinical efficacy of alternating 17β-estradiol/AI therapy.

Patients will have had PET/CT, or conventional CT [chest, abdomen, and pelvis (CAP)] and bone scan for staging. In cases where a tumor was biopsied within the past 4 months or just prior to study entry, the tumor must be ER+/HER2- as determined by immunohistochemistry for ER, and IHC or FISH for HER2. Patients with bone-only metastatic disease with a history of ER+/HER2- breast cancer are eligible, and bone biopsy is not required. Patients with non-bone metastatic disease are eligible without a tissue biopsy if they do not have a site of metastatic disease that is amenable to being safely and accurately biopsied.

Blood samples (20 mL) for research use will be obtained at baseline (prior to 17β-estradiol treatment), after 12-14 days of 17β-estradiol (optional), and after the first instance of disease progression. This blood may be obtained at the same time as clinically indicated blood draws.

Patients will be treated with 17β-estradiol (2 mg PO t.i.d.) for 8 weeks. After 12-14 days of 17β-estradiol therapy, an optional research tumor biopsy is requested from patients with non-bone disease for whom this biopsy procedure is considered safe; the biopsied tumor would ideally be the same locally recurrent or metastatic site that was biopsied at baseline, but may also be a different site if the former site is not amenable to repeat biopsy. Patients will then be treated with an AI for 16 weeks, alternating between 17β-estradiol and AI therapies every 8 and 16 weeks, respectively, until disease progression.

Patients will be monitored for tumor response per standard of care using either PET/CT or conventional CT (CAP) every 8 weeks, and a bone scan as clinically indicated (see Table 6.2 for detailed study requirements). Disease progression, objective response, and clinical benefit will be determined using RECIST 1.1 criteria (46):

CR (complete response) = disappearance of all target lesions
PR (partial response) = ≥30% decrease in the sum of the longest diameters of target lesions
PD (progressive disease) = ≥20% increase in the sum of the longest diameters of target lesions
SD (stable disease) = changes that do not meet above criteria.

Objective Response (OR) = CR + PR
Clinical Benefit (CB) = SD at 24 weeks + CR + PR

Deaths that occur while on study treatment will be counted as progression events for calculation of progression-free survival. If a patient experiences adverse events due to AI therapy (or has experienced such events previously that led to AI discontinuation), the treating physician has the option of switching to another AI during this study. It is preferable to use only one AI during this study.

Tumor biopsies (obtained at baseline prior to 17β-estradiol treatment and, optionally, after 12-14 days of 17β-estradiol) will be analyzed to identify molecular biomarkers predictive of, and correlated with, response to 17β-estradiol and alternating 17β-estradiol/AI therapy. Blood samples (20 mL) to monitor markers of disease progression will be obtained at baseline, after 12-14 days of 17β-estradiol (optional), and at the time of disease progression, typically at the same times as clinically indicated blood draws.

At the time of the first instance of disease progression, the patient will either A) be managed off-study as per standard of care (no further data is required to be collected after this point), or B) optionally be treated as follows. Optional: After disease progression on alternating 17β-estradiol/AI therapy, at the discretion of the treating physician, patients may be switched to the alternate therapy and treated (without alternation) until disease progression. For example, if disease progresses while a patient is taking AI therapy, and her physician thinks that the cancer is sensitive to 17β-estradiol therapy, she could be switched back to 17β-estradiol and remain on 17β-estradiol until another instance of disease progression. If the physician administers this continuous treatment, data (PFS, response, clinical benefit, adverse events) will be collected for this study during this time frame.

Expected duration of subject participation: 12 months

3.2 Primary Study Endpoints

- Clinical benefit and objective response (in patients with measureable disease) from alternating 17β-estradiol/aromatase inhibitor therapy.

3.3 Secondary Study Endpoints

- Toxicities and adverse events.
- Disease progression during alternating 17β-estradiol/AI therapy, and during subsequent continuous therapy with either 17β-estradiol or an AI.
- Objective response (in patients with measureable disease) from the first 8 weeks of 17β-estradiol therapy.
- Tumor and plasma mutations in *ESR1*.

Subject Selection and Withdrawal

4.1 Inclusion Criteria

1) Women ≥18 years of age with clinical stage IV ER+/HER2- breast cancer, or with locally recurrent ER+/HER2- disease not amenable to therapy for curative intent.

2) Patient must have been treated with an anti-estrogen at any time in their disease history. Combination regimens that include an anti-estrogen and any biologic, or targeted therapy, are permitted (*e.g.*, any CDK inhibitor, everolimus, or any other novel biologics), and are considered to be a single hormonal therapy based regimen.
- Any number of prior lines of anti-estrogen (*i.e.*, hormonal) therapy is permissible.
- One line of prior chemotherapy for advanced/metastatic disease is permissible.

3) Histologic documentation of ER strongly+/HER2- breast cancer by core needle biopsy, fine needle aspiration, incisional biopsy, or surgical biopsy of ≥1 site(s) of metastatic or locally advanced disease performed as standard of care within the past 4 months for assessment of eligibility for study participation (except as noted below in c/d/e).

a) ER strongly+ status defined as ER staining by immunohistochemistry in ≥50% of malignant cell nuclei with an intensity ≥2+ on a scale of 0-3+. These criteria are equivalent to an Allred score ≥6.

b) HER2-negative status is defined as immunohistochemistry score of 0-1+, or with a FISH ratio of <2 if IHC is 2+ or if IHC has not been done (as per ASCO/CAP definitions). In cases of borderline or equivocal HER2 status, eligibility will be determined by the PI.

c) Archived tumor specimens: Excess tumor tissue must be available for research purposes. This will include tumor tissue sufficient to make ≥10 five-micron sections; more tumor tissue is preferred.

Freshly acquired tumor specimens: As part of a clinically indicated biopsy procedure, an additional 1-3 cores or tissue fragments will be obtained by core needle or surgical biopsy for research purposes and FFPE.

d) Patients with bone-only metastatic disease with a history of ER+/HER2- breast cancer are eligible, and bone biopsy is not required, providing their primary cancer is consistent with the above-described ER and HER2 criteria.

e) Patients with non-bone metastatic disease in whom a safe and accurate biopsy of recurrent/metastatic disease cannot be readily obtained are also eligible, providing their primary cancer is consistent with the above-described ER and HER2 criteria.

4) Patient must be a candidate for treatment with 17β-estradiol and an aromatase inhibitor.

5) If the most recent therapy was in the adjuvant setting, the recurrence-free interval (time from initiation of adjuvant anti-estrogen therapy to clinical evidence of disease recurrence) must have been ≥2 years.

If the most recent therapy was in the advanced/metastatic setting, the progression-free interval must have been ≥3 months (except in the case of investigational hormonal therapies, or investigational combinations that include an endocrine agent).

6) Patient must be post-menopausal based on either a history of an oophorectomy, or ≥1 year of amenorrhea. An elevated serum gonadotropin level and estradiol level in the postmenopausal range (as locally defined) can be used to confirm menopausal status in a subject with <1 year of amenorrhea.

7) Baseline radiographic staging performed as standard of care, including specifically either PET/CT, or CT (CAP) and bone scan.

8) Patient must be capable and willing to provide informed written consent for study participation.

9) The following laboratory values must be confirmed for eligibility within 28 days prior to initiation of study therapy:

Hematology panel
- hemoglobin > 9 g/dL
- white blood cell (WBC) count (≥ 2,000/μL)
- platelet count ≥ 75,000/μL

Serum biochemistry/metabolic panel
- creatinine ≤ 1.5 x upper limits of normal (ULN)
- total bilirubin ≤ 1.5 x upper limits of normal (ULN)
- ALT and AST ≤ 3.0 x upper limits of normal (ULN)
  For patients with liver metastasis: < 5 x upper limits of normal (ULN)

4.2 Exclusion Criteria

1) Treatment with fulvestrant within 16 weeks prior to study enrollment.

2) Any other concurrent systemic anti-cancer treatments, including conventional chemotherapeutic agents and biological agents, during the study period.

Anti-resorptive bone therapies (*e.g.*, bisphosphonates, denosumab) are permitted.

3) Any investigational cancer therapy in the last 3 weeks.

4) Known CNS disease, unless clinically stable for ≥ 3 months.

5) History of any of the following:
deep venous thrombosis
pulmonary embolism
stroke
acute myocardial infarction
congestive heart failure
previous malignancy not treated with curative intent, or with an estimated recurrence risk ≥30%

Study Drugs

5.1 Description

This study will use 17β-estradiol and aromatase inhibitors (AIs). All of these drugs are dispensed in tablet form, specifically 2 mg 17β-estradiol, 2.5 mg letrozole, 1 mg anastrozole, 25 mg exemestane.

5.2 Treatment Regimen

This study will use an 8-week/16-week alternating regimen of 17β-estradiol therapy and aromatase inhibitor (typically either letrozole, anastrazole, or exemestane) therapy until the first instance of disease progression. Use of only one AI throughout the study is preferred, although switching AIs is permitted if clinically indicated.

17β-estradiol: One tablet containing 2 mg 17β-estradiol will be taken orally three times daily, for a total dose of 6 mg/day.

Letrozole: One tablet containing 2.5 mg letrozole will be taken orally once per day.

Anastrozole: One tablet containing 1 mg anastrozole will be taken orally once per day.

Exemestane: One tablet containing 25 mg exemestane will be taken orally once per day.

5.3 Method for Assigning Subjects to Treatment Groups

Study is single-arm, non-randomized.

5.4 Preparation and Administration of Study Drug

All medications used in this study are in routine clinical use, non-investigational.

5.5 Packaging

All medications used in this study are in routine clinical use and available through hospital and commercial pharmacies.

5.6 Blinding of Study Drug

Study is not blinded.

. Study Procedures

6.1 Registration

Each participant must sign and date an informed consent form before undergoing any study-specific procedures. The Project Manager at the Dartmouth Cancer Center Clinical Research Office is to be contacted once consent is signed. The Project Manager will assign a participant identification number. Once a participant has completed all pre-treatment evaluations and eligibility criteria have been met, participants will be registered to the study.

The registration form (Including local PI-signed or designee-signed eligibility checklist), primary source documentation including pathologic documentation of ER+/HER2- advanced/metastatic breast cancer, and staging studies should be faxed or emailed to the Project Manager. The Project Manager will send a confirmatory email that these materials have been received.

Eligibility criteria will then be verified by the Dartmouth PI or designee at the time of registration. At that point, a decision will be made regarding enrollment. Once the participant is determined to be eligible, the subject will be registered. A completed registration form will be sent confirming registration. Following completion of registration, the patient may begin study treatment. Each participant will then be entered into Velos by the Project Manager.

To register a patient, the following documents need to be completed and faxed (603-650-7799) or e-mailed to the Project Manager:

- Completed registration form, with eligibility checklist signed by PI or designee
- Pathology report for advanced/metastatic breast tumor specimen and staging studies
- Copy of required laboratory reports, clinical records and notes as needed

Within 2 business days, the Project Manager will:

- Assign a participant study ID number
- Fax or e-mail the participant study ID number to the participating site
- Verify eligibility with the Dartmouth PI or designee
- Register the participant to the study
- Contact the participating site and confirm registration

6.2 Schedule (also see Table 6.2)

6.2.1. Physical examination

Physical examination at screening, baseline, and during the treatment period consists of ECOG performance status, body weight, and vital signs (temperature, blood pressure, heart rate). Major body systems are reviewed at screening and baseline visits only. The symptom-directed examination is a physical examination relevant to the patient's symptoms and is performed during the treatment period.

6.2.2. Laboratory assessments

Local institutional laboratories will perform all clinically indicated laboratory tests. Evaluations performed as part of routine care, before obtaining informed consent, can be utilized as screening evaluations if done within 28 days before the first dose of 17β-estradiol study treatment. Blood samples for hematology and biochemical/metabolic profiles will be prepared using standard procedures. Laboratory panels in order to monitor patient safety will be done according to local standard of care.

6.2.3. Research Assessments

Post-menopausal patients with a history of ER+/HER2- breast cancer who received prior anti-estrogen therapy and currently exhibit symptoms of disease recurrence or progression will undergo routine clinical staging [PET/CT, or CT (CAP) and bone scan] and have a diagnostic biopsy confirming metastatic breast cancer, or locally advanced breast cancer not amenable to treatment with curative intent; an extra 1-3

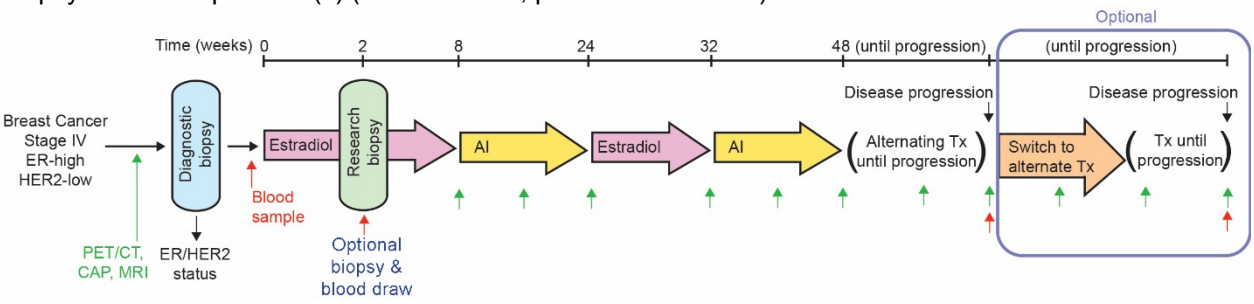
cores/fragments of tumor tissue for research use is required to be obtained during this diagnostic biopsy procedure; an exception is patients who underwent a biopsy procedure within the past 4 months, where excess tissue from the biopsy specimen is adequate and available for research purposes. The metastatic/recurrent disease must be determined to be ER+ and HER2-. In cases of bone-only metastatic disease and in other patients as described in eligibility criteria (Section 4.1), a biopsy is not required, and ER+/HER2- status may have been determined from the primary tumor. Before initiation of 17β-estradiol therapy, a 20-mL "baseline" blood sample drawn into two 10-mL purple-top (EDTA) tubes will be obtained for research purposes.

- Blood will be separated into plasma and buffy coat fractions by centrifugation at 1,000 x *g* for 30 min at 4°C.
- Plasma will be aliquotted in 2-mL cryotubes and frozen at -80°C.
- Buffy coat will be split between 2 cryotubes and frozen at -80°C.
- Frozen plasma and buffy coat samples will be sent to the Miller Lab, 6th floor of Rubin Research Bldg. at Dartmouth-Hitchcock Medical Center.

6.2.4. Treatment Schedule

A Cycle is defined as 28 days (4 weeks). Study medication schedules/changes and clinical assessments will occur on a 4-week Cycle (+/- 4 days).

Patients will be treated with 17β-estradiol (6 mg/d total, taken as 2 mg PO t.i.d.), and re-evaluated after 8 weeks (or less) as per standard of care by imaging and clinical evaluation. After 12-14 days on 17β-estradiol therapy (during Cycle 1), a research tumor biopsy (optional) and a 20-mL blood sample (optional) are requested from patients with soft-tissue disease; the same tumor site biopsied at baseline is preferred for this repeat biopsy, but if unavailable, research biopsy of another tumor site is acceptable. Research biopsy tumor specimens (1-3 cores) should be processed in the same manner as the baseline biopsy research specimen(s) (formalin-fixed, paraffin-embedded).



Following 8 weeks of treatment with 17β-estradiol, patients will be switched to treatment with an AI. If a patient experiences adverse events due to treatment with one AI, the treating physician has the option of switching to another AI for use in this study. It is preferable to use only one AI during this study. Patients will continue to be evaluated as per standard of care once every 4 weeks (+/- 4 days) by clinical evaluation, and by imaging as per standard of care. After 16 weeks (4 Cycles) of AI therapy, patients will be switched back to 17β-estradiol therapy. This alternating 17β-estradiol (Cycles 1, 2, 7, 8, etc.) / AI (Cycles 3, 4, 5, 6, 9, 10, 11, 12, etc.) treatment regimen will continue until the time of disease progression (determined by imaging or clinical evidence of progression), or until withdrawal from study participation. Adverse events (AEs) that occur during study treatment will be managed as per standard of care. At the time of disease progression (on alternating therapy), or upon study withdrawal, patients will be managed off-study as per standard of care (except as noted below). When a clinically indicated blood sample is obtained for routine diagnostic testing within 4 weeks following disease progression, an extra 20-mL blood sample will be obtained for research purposes and processed as above. If the treating physician chooses, she/he may then switch treatment to the alternate therapy; for example, if a patient progresses on AI therapy, she could be switched to 17β-estradiol therapy (which may be beneficial) and continuously treated until another instance of disease progression. If the treating physician administers such treatment,

we request that follow-up data (PFS, response, clinical benefit, adverse events) and an end-of-study blood sample be collected for this study.

Treatment interruption is permissible. A drug washout period between 17β-estradiol and an AI is not required. Dose reductions are not permitted.

Table 6.2- Study Calendar

	Screening[9]	Cycle 1* Day 1	Cycle 2 Day 1 +/- 2 days	Cycle 3 Day 1 +/- 2 days	Cycle 4 Day 1 +/- 2 days	Cycle 5 Day 1 +/- 2 days	Cycle 6 Day 1 +/- 2 days	After First Instance of Disease Progression (EOS)
Assessment								
Consent	X							
Physical Exam and Medical History [1]	X	X	X	X	X	X	X	X
Con Meds	X							
AE Review		X	X	X	X	X	X	X
PET/CT or CT [2]	X		X		X		X	X
Bone Scan [2,3]	X		X		X		X	X
CBC [4]	X	X	X	X	X	X	X	X
CMP [4]	X	X	X	X	X	X	X	X
CEA, CA 27-29 and/or CA 15-3 [5]	X							X
Study Blood Tests [6]	X	Optional (day 12-14)						X
Research tumor biopsy [7]	X	Optional (day 12-14)						
Treatment								
17β-Estradiol [8]		X	X					Optional
AI (Letrozole, Exemestane or Anastrozole) [8]				X	X	X	X	Optional

*A Cycle is defined as 28 days.

Table 6.2 Footnotes

[1] Physical Exam includes height and weight, vital signs and ECOG status. Screening assessment within 4 weeks prior to initiation of study therapy. Height is measured only at Screening.

[2] Staging by PET/CT, or conventional CT (CAP) and bone scan. Baseline staging must occur within 4 weeks prior to initiation of 17β-estradiol therapy. Subsequent staging must occur Q 8 weeks +/- 1 week, or Q 2 cycles. The first staging will occur 8 weeks (+/- 1 week) after start of 17β-estradiol therapy. Restaging should generally use the same method as performed at baseline. Refer to Section 6.3 of the protocol.

[3] Baseline staging by bone scan (if baseline PET/CT not performed). Baseline staging must occur within 4 weeks prior to initiation of 17β-estradiol therapy. Subsequent re-staging by bone scan only as clinically

indicated, except for patients with bone-only disease, in whom re-staging will be performed Q 8 weeks (+/- 1 week).

[4] CBC and CMP to be done on Day 1 of each Cycle (+/- 2 days).

[5] CEA, CA 27-29 and/or CA 15-3 will be performed as per local standard of care as clinically indicated.

[6] 20 mL of blood will be drawn in two 10-mL purple-top (EDTA) tubes. Blood is to be drawn concurrently with routine, clinically indicated blood draws when possible, including at baseline and at the time of the first instance of disease progression (within 4 weeks after detecting disease progression). Cycle 1 blood draw to be drawn after 12-14 days of estradiol therapy only if optional tumor biopsy is performed. Refer to section 6.2 of the protocol.

[7] A baseline diagnostic tumor biopsy specimen(s) is required; this specimen may have been obtained within 4 months prior to study enrollment and excess tumor tissue is available for research use; or a new clinically indicated biopsy specimen is being obtained and extra cores/fragments will be collected for research use.
An optional tumor biopsy for research purposes after 12-14 days of 17β-estradiol therapy is requested from patients with non-bone disease, in cases in which this biopsy is considered safe. When possible, the repeat research biopsy should be obtained from the same site/metastasis that was biopsied at Baseline; if unavailable, research biopsy of a different site is acceptable. If this biopsy is performed, 20 mL of blood should also be collected as per footnote 6.

[8] Patients will be treated with 17β-estradiol for 8 weeks (Cycles 1-2), then treated with an Aromatase Inhibitor (AI) for 16 weeks (Cycles 3-6), and continue to alternate between 17β-estradiol and AI therapies every 8 and 16 weeks, respectively, until disease progression. At the time of disease progression, the treating physician may optionally choose to treat continuously with 17β-estradiol or an AI until another instance of disease progression. Response and PFS will be collected for patients who are given this optional segment of therapy. Refer to section 6.2 of the protocol.

[9] Screening assessments within 28 days prior to initiation of study therapy.

6.3 Assessment of response

Patients will be seen by their treating physician every 4 weeks (+/- 4 days). Patients will have had PET/CT, or conventional CT (CAP) and bone scan for staging prior to study enrollment. The same imaging method will be used to monitor tumor response and disease progression per standard of care [at 8-week intervals (+/- 1 week) for PET/CT; bone scans as clinically indicated], or earlier if patients exhibit symptoms suggestive of disease progression. Tumor response and disease progression will be determined using RECIST criteria (46) [CR (complete response) = disappearance of all target lesions; PR (partial response) ≥ 30% decrease in the sum of the longest diameter of target lesions; PD (progressive disease) ≥ 20% increase in the sum of the longest diameter of target lesions; SD (stable disease) = small changes that do not meet above criteria]. In patients with measurable disease, at least 1 measurable lesion [defined by (RECIST) criteria (46)] will be followed. Deaths that occur while on study treatment will be counted as progression events.

6.4 Clinical data collection

We will record the following patient information for analyses in this study:

1) Previous anti-cancer agents administered, dates of administration, and reasons for discontinuation.
2) Disease and health history.
3) Pre-existing co-morbidities and adverse events experienced through the study.
4) Progression-free survival on alternating 17β-estradiol/AI therapy.
5) Clinical response (RECIST criteria) to 17β-estradiol and alternating 17β-estradiol/AI therapies.
6) Optional: Time to the second instance of disease progression (or death) on continuous 17β-estradiol or AI therapy, and clinical response (RECIST criteria).

6.5 Retrospective analysis of ESR1 mutation status through circulating tumor DNA

These analyses will be performed after all patients have completed study treatment.
Mutations in exons 4-8 of *ESR1,* which encode the ligand-binding domain of ER, have been shown to confer estrogen-independent activity in cultured cells. Such mutations have recently been detected in tumors from ~18% of patients with anti-estrogen-resistant breast cancer, and these mutations were present in the recurrent but not the primary tumors (30, 47-49). However, analyses of larger numbers of patients are required to determine whether there is a significant causal association between such *ESR1* mutations and anti-estrogen resistance. We will determine whether *ESR1* mutations A) are associated with resistance/sensitivity to 17β-estradiol/AI therapy, B) arise during the course of 17β-estradiol/AI therapy, and C) are detectable in plasma at the time of clinical evidence of disease progression.

Plasma samples obtained from patients at routine clinical visits at DHMC and non-DHMC sites will be stored frozen at -80°C until delivery to Dartmouth (Todd Miller laboratory) for analysis. We will extract DNA from plasma samples from each patient; this analysis will focus on patients who did not have an *ESR1* mutation detected in their baseline advanced/metastatic tumor. We will use PCR primers to amplify exons 4-8 of *ESR1*, ligate adapters with barcodes to multiplex samples, and sequence using the Illumina platform in the DCC Genomics Shared Resource.

6.6 Retrospective genetic analysis of tumors

These analyses will be performed after all patients have completed study treatment.
Tumor samples from the archived primary tumor (if available), any other archived tumor biopsy specimens obtained throughout a patient's disease course (if available), and the diagnostic biopsy specimen (required; obtained within 4 months prior to the start of 17β-estradiol therapy) will be used for genetic analyses. FFPE tumor sections will be sent to Dartmouth (Todd Miller laboratory) for analysis. DNA will be extracted from 4-8 five-micron sections of tumor tissue, and from leukocytes (buffy coat) of blood, and used to generate libraries for whole-exome sequencing. Tumor genetic lesions will be identified by comparison to the normal (blood) genome. Tumor genetic lesions will be compared between specimens from the same patient to determine whether new lesions arose during the course of disease,

which may have been driven/selected by prior therapy. Tumor genetic lesions will also be compared to patient outcome upon treatment with 17β-estradiol, and with alternating 17β-estradiol/AI therapies, to identify predictive genetic biomarkers.

6.7 Retrospective transcriptional analysis of tumors

Tumor samples from the archived primary tumor (if available), any other archived tumor biopsy specimens obtained throughout a patient's disease course (if available), the diagnostic biopsy specimen (required; obtained within 4 months prior to the start of 17β-estradiol therapy), and the research biopsy specimen obtained after 12-14 days of 17β-estradiol therapy (optional) will be used for genome-wide gene expression analyses. FFPE tumor sections will be sent to Dartmouth (Todd Miller laboratory) for analysis. RNA will be extracted from 4-8 five-micron sections of tumor tissue and used to generate libraries for RNA sequencing. Gene expression profiles will be compared to identify changes induced by 17β-estradiol therapy, and changes that occurred during the evolution of anti-estrogen resistance in tumors.

Statistical Plan

7.1 Sample Size Determination

There are no historical data on Clinical Benefit Rate (CBR) in "control" patients with advanced/metastatic ER+ breast cancer with disease that recently progressed on an endocrine therapy who continue on the same therapy; we conservatively predict that such a CBR would be 10%. This study was designed using Simon's two-stage design (50). The null hypothesis that the true Clinical Benefit Rate (CBR) is 10% will be tested against a one-sided alternative. In the first stage, 10 patients will be accrued. If ≤1 patient experiences CB among these first 10 patients, the study will be stopped for futility. Otherwise, 19 additional patients will be accrued for a total of 29 patients. The null hypothesis will be rejected if ≥6 patients experience CB among 29 total patients. This design yields a type I error rate of 5% and power of 80% when the true CBR is 30%.

7.2 Statistical Methods

Objective: Determine the rate of clinical benefit from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer.
Method: CB is defined as CR, PR, or SD at 24 weeks per RECIST criteria. The CBR and the 95% exact binomial confidence interval will be calculated.

Objective: Determine the objective response rate of patients treated with 17β-estradiol therapy for 8 weeks.
Methods: Objective Response is defined as CR + PR as per RECIST criteria; the proportion of patients experiencing Objective Response on 17β-estradiol will be calculated to determine ORR. Mean ORR will be calculated along with the 95% exact confidence interval.

Objective: Determine the interval of progression-free survival (PFS) from alternating 17β-estradiol/aromatase inhibitor therapy in patients with advanced ER+ breast cancer.
Methods: PFS is defined as the time period from the start of a treatment until the time of cancer progression or death from any cause. Cancer progression will be determined as per RECIST criteria. The time of last observation for patients remaining in the study, the time at which alternating 17β-estradiol/AI treatment is terminated, and the time of withdrawal of consent will be treated as censored data. PFS will be estimated using the Kaplan-Meier estimator. Hazard rate of progression as a function of time will be estimated as well.

Objective: Determine the rate of clinical benefit and interval of progression-free survival from continuous 17β-estradiol therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy.
Methods: PFS is defined as the time period from start of a treatment until the time of cancer progression or death from any cause. Cancer progression will be determined as per RECIST criteria. The time of last observation for patients remaining in the study, the time at which continuous 17β-estradiol treatment is terminated, and the time of withdrawal of consent will be treated as censored data. PFS will be estimated using the Kaplan-Meier estimator and hazard rate will be estimated. Clinical benefit is defined as CR + PR + SD ≥6 months (as per RECIST criteria); the proportion of patients experiencing clinical benefit on continuous 17β-estradiol therapy will be calculated to determine CBR. Mean CBR will be calculated along with the 95% exact confidence interval.

Objective: Determine the rate of clinical benefit and interval of progression-free survival (PFS) from continuous aromatase inhibitor (AI) therapy in patients with advanced ER+ breast cancer who had received alternating 17β-estradiol/aromatase inhibitor as the prior line of therapy.
Methods: PFS is defined as the time period from start of a treatment until the time of cancer progression or death from any cause. Cancer progression will be determined as per RECIST criteria. The time of last observation for patients remaining in the study, the time at which continuous AI treatment is terminated, and the time of withdrawal of consent will be treated as censored data. PFS will be estimated using the Kaplan-Meier estimator and hazard rate will be estimated. Clinical benefit is defined as CR + PR + SD ≥6 months (as per RECIST criteria); the proportion of patients experiencing clinical benefit on continuous AI therapy will be calculated to determine CBR. Mean CBR will be calculated along with the 95% exact confidence interval.

Objective: Determine the adverse event profiles of 17β-estradiol and aromatase inhibitors in this patient population.
Method: Adverse events will be recorded as Grade 1, 2, 3, or 4. Incidences will be compared within patients over time. Paired *t*-test and non-parametric Wilcoxon test will be used to compare 17β-estradiol vs. aromatase inhibitors.

Objective: Determine whether specific tumor genetic lesions in *ESR1* are predictive of response to 17β-estradiol and/or subsequent aromatase inhibitor therapies.
Method: The rates of clinical benefit from 17β-estradiol will be compared between patients with tumors with vs. without genetic lesions in *ESR1* by Fisher's exact test. The rates of clinical benefit from subsequent AI therapy will be compared between patients with tumors with vs. without genetic lesions in *ESR1* by Fisher's exact test.

Objective: Identify molecular biomarkers predictive of response to 17β-estradiol and/or alternating 17β-estradiol/aromatase inhibitor therapies.
Method: Retrospective genetic analysis of tumor tissues will be performed. Genetic profiles of tumors from patients who did vs. did not exhibit clinical benefit will be compared (in single-gene and pathway-specific-gene manners) by Fisher's exact test to identify genetic alterations predictive of clinical benefit.

Objective: Determine whether genetic lesions in *ESR1* become detectable in circulating DNA in plasma at the time of disease progression on alternating 17β-estradiol/aromatase inhibitor therapies.
Method: In patients with *ESR1*-wild-type tumors at baseline, we will quantify levels of *ESR1*-mutant DNA per mL of plasma in blood samples in triplicate reactions, and compare levels within each patient over time. The goal is to determine whether *ESR1* mutations are detectable in plasma DNA at the time of clinical evidence of disease progression. Paired *t*-test and non-parametric Wilcoxon test will be used to compare genetic lesions at baseline vs. upon disease progression. Mixed effect models or generalized estimating equations (GEE) approach will be used to estimate the trajectory of levels of *ESR1*-mutant DNA over time.

Objective: Identify molecular changes that occur in response to 17β-estradiol therapy in ER+ breast tumors.

Method: Tumor specimens obtained at baseline and after 12-14 days of estradiol therapy will be used for RNA-seq. Preprocessed reads will be mapped to the human genome using Tophat, and used to quantify mRNA expression levels with the 'fragments per kilobase of exon per million mapped fragments' (FPKM) method using Cufflinks. Base substitutions (SNVs, mutations, indels) will be detected with VarScan 2. mRNAs differentially expressed between pre- vs. post-E2 tumor specimens will be identified by paired t-tests of $\log_2$-transformed FPKM values of each transcript and using Benjamini-Hochberg multiple testing correction-based FDR-adjusted p≤0.05. Patients will be stratified based on molecular data (e.g., high vs. low gene expression), and groups will be compared by Fisher's exact test (OR, CB) and log-rank test (PFS) to determine whether molecular events predict therapeutic benefit. Conversely, patients will be stratified based on OR, CB, or PFS, and groups will be compared by t-test of molecular data. Molecular data will also be analyzed by Cox proportional hazards regression (univariate and multivariate) to identify association with PFS.

7.3 Subject Population(s) for Analysis

Patients who proceed to treatment with 17β-estradiol will be eligible for analysis of the primary endpoints of Clinical Benefit and Objective Response if one of the following are observed: an objective response (at any time point), disease progression (at any time point), or disease stabilization for 6 months per RECIST criteria.
Patients who proceed to treatment with 17β-estradiol for ≥4 weeks will be eligible for analysis of the secondary endpoint of toxicity.

Safety and Adverse Events

8.1 Adverse events

Adverse event (AE) recording and reporting is a routine part of every clinical trial. All AEs and serious AEs (SAEs), whether reported by the participant or noted by authorized study personnel, will be recorded in the participant's medical record, on the AE or SAE log, and the case report forms. AE recording will begin on the first day of study treatment (with 17β-estradiol) and end on the last day of treatment with alternating 17β-estradiol/AI therapy. Grade 1 or Grade 2 noncardiac AEs ongoing at the end of study treatment must be followed for an additional 30 days. In cases where the treating physician, after the first instance of disease progression during the study, switches treatment to the alternate therapy for continuous treatment, ongoing Grade 1 or Grade 2 noncardiac AEs and SAEs will also be recorded/reported until the second instance of disease progression, and for an additional 30 days after the end of study treatment. Abnormal lab values, even if they are expected, must be recorded appropriately as AEs. Each site must record the onset and duration of each AE.

All Grade 3-4 AEs considered related to study treatment must be followed until recovery to Grade ≤1. Cardiac AEs of Grade 2 or higher will be followed until improvement to Grade ≤1. These AEs ongoing at the end of study treatment must also be followed until improvement to Grade ≤1, or until, in the opinion of the Dartmouth Lead PI (in discussion with the treating physician), resolution can no longer be expected.

AEs and SAEs that are anticipated to be potentially caused by 17β-estradiol treatment in the study described herein are noted in Table 8-1 [from ref. (1)], as these events were previously observed in this patient population with the dosing regimen of 17β-estradiol that will be used in the study described herein (6 mg/day, administered as 2 mg PO t.i.d.). Notably, among 34 such patients, none withdrew from the study due to AEs.

Aromatase inhibitors (AIs) that will be used in the study described herein are in routine clinical use (i.e., letrozole, anastrozole, exemestane). AEs and SAEs are noted in package inserts for AIs.

	6 mg (n = 34)	
Toxicity Class	**Grade 3**	**Grade 4-5**
Nausea/vomiting	0	0
Low sodium	1 (3)	0
Pleural effusion	0	0
Pain	6 (18)	0
Thombosis/embolism	0	1 (3)
Central nervous system ischemia	1 (3)	0
Infection	2 (6)	0
Hypercalcemia	0	0
Other	6 (18)	0
	Grade 1-2 /Grade 3	**Grade 4 /Grade 5**
Serious adverse event incidence	30 (88)/3 (9)	1 (3)/0

Table 8.1- Grade 3 and Higher Toxicities and the Incidence of Serious Adverse Events in patients treated with 6 mg/d 17β-estradiol. Data were extracted from ref. (1). Quoting from ref. (1): "Adverse effects were generally characteristic of estradiol therapy. Most notably, there were fewer patients with high-grade nausea and vomiting, electrolyte disturbance, and problems with pleural effusion in the 6-mg group [compared with the 30 mg group].… Grade 1 or 2 vaginal bleeding was observed in 17 patients… and was well-controlled with progestin therapy either orally or as an intrauterine device. The rate of thrombosis was low with 1 event in each study group."
Note- in this randomized study, patients received either 6 or 30 mg/d 17β-estradiol. The 30 mg/d group showed considerably more toxicity. The association between dose and vaginal bleeding was not reported.

8.1.1. Methods of assessment

Assessment of AEs and SAEs will be performed during routine clinical visits as per standard-of-care. If abnormal findings emerge or worsen from baseline assessment, then the AE page of the CRF should be completed for these findings. If a finding meets the criteria for an SAE, then the appropriate procedures for reporting such events will be followed as described below.

8.2 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form, etc.)
- Related or possibly related to participation in the research (i.e., 'possibly related' means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness, or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality meets one of the following criteria:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that meets one of the following criteria:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event (defined below)

Important medical events are those that may not be immediately life-threatening, but are clearly of major clinical significance. They may jeopardize the welfare of the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (e.g., change of dose, discontinuation of study medication, more frequent follow-up assessments, further diagnostic investigation, etc.).

Hospitalization, Prolonged Hospitalization, or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances: hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic, and the outcome was uneventful.

8.3 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document, and also in the appropriate AE module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedure results should be recorded in the source document, though should be grouped under one diagnosis.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. AEs that are still ongoing at the end of the study period must be followed up to determine the final outcome for 30 days.

8.4 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators must conform to the AE reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,

- unexpected, and
- serious or involve risks to subjects or others

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.4.1 Investigator reporting: notifying the Dartmouth IRB

This section describes the requirements for safety reporting by investigators who are Dartmouth faculty, affiliated with a Dartmouth research site, or otherwise responsible for safety reporting to the Dartmouth IRB. The Dartmouth IRB requires reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Dartmouth IRB requires researchers to submit reports of *any incident, experience, or outcome that meets each of the following criteria:*

- ***Unanticipated*** *in terms of nature, severity, or frequency given: (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and consent document; and (b) the characteristics of the subject population being studied; and*
- ***Possibly related*** *to participation in the research means there is a reasonable possibility that the incident, experience, or outcome may have been associated with research participation; and*
- *The problem suggests that the research places subjects or others at a* ***greater risk of harm*** *(including physical, psychological, emotional, economic, legal, or social harms) than was previously known or recognized.*

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Dartmouth IRB using the form: “CPHS - UPIRSO, SAE, UADE reporting form.”

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

Other Reportable events:

For clinical trials, the following events are also reportable to the Dartmouth IRB:

- Report to Dartmouth IRB using the “CPHS –UPIRSO, SAE, UADE reporting form: Any adverse experience, defined as an untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s participation in research, whether or not considered related to the subject’s participation in the research), that is considered:
  - Serious: Death; a life-threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability or incapacity; or a congenital anomaly or birth defect; and
  - Unexpected: Any adverse experience, the specificity or severity of which is not consistent with the current investigator brochure or consent form; and
  - Possibly related: There is a reasonable possibility that the incident, experience, or outcome may have been associated with the procedures involved in the research; and
  - Is experienced by a participant in a trial open at a site subject to Dartmouth IRB review

Report to Dartmouth IRB via online submission of “Reportable New Information” in RAPPORT system:

- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol deviation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.4.2 Investigator reporting: Notifying a non-Dartmouth IRB

Investigators who are not Dartmouth faculty or not affiliated with a Dartmouth research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file and sent to the Dartmouth Lead Investigator. The Dartmouth Lead Investigator will report information on participants to the Data Safety Monitoring and Accrual Committee (DSMAC) of the Dartmouth Cancer Center

8.4.3 Expedited Adverse Event Reporting

Any SAEs which occur once the first dose of study treatment has been administered, or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol-specific diagnostic procedures or interventions must also be reported.

All SAEs as defined in 21 CFR 314.80 (whether initial or follow-up) must be reported to the local IRB per IRB requirements and to the Project Manager at Dartmouth-Hitchcock Medical Center by facsimile to Dartmouth-Hitchcock Medical Center (603-650-7799), or email the project manager within 1 business day of becoming aware of the SAE. The SAE report must comprise a full written summary detailing relevant aspects of the AEs in question. Where applicable, information from relevant hospital case records and autopsy reports should be included. Follow-up information should be forwarded to Dartmouth-Hitchcock Medical Center as soon as available. As the sponsor, Dartmouth-Hitchcock Medical Center will report SAEs to the Dartmouth IRB per their requirements

The Principal Investigator shall use his/her judgment to determine the relationship between the Serious Adverse Drug Experience and the Study Drug.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the investigator will contact Dartmouth-Hitchcock, or a designee, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be documented. If the deviation meets guidelines for reporting, it must be reported to the institutional IRB, as well as the Committee for the Protection of Human Subjects at Dartmouth. The deviation form used for each institution can be submitted to the Project Manager at Dartmouth-Hitchcock Medical Center.

9. Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

Following closure of the study, the investigator will maintain all site study records in a safe and secure location. The records are maintained to allow easy and timely retrieval when needed and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Upon completion of study analysis, research information is stored in Dartmouth College Records Management off-site storage located at 6218 Etna Road, Hanover, NH 03755. Documents are shredded on-site after 50 years of storage.

Electronic case report forms, participant, and study information will be kept in the Velos eResearch password-protected database (or equivalent) indefinitely.

10. Ethical Considerations

This study is to be conducted according to applicable government regulations, DHMC and non-DHMC site research policies and procedures.

The study will be initiated only after the protocol and a copy of the informed consent document have been reviewed and approved by the DH IRB. Upon approval by the DH IRB, the protocol will be submitted to non-DHMC site IRBs for approval. All protocol amendments by non-DHMC site IRBs will be reviewed by the Lead PI and submitted to the DH IRB for approval.

If the protocol or the informed consent document that the DH IRB has approved is amended during the study, the investigator is responsible for ensuring the DH IRB reviews and approves these amended documents. The investigator must follow all applicable regulatory requirements pertaining to the use of an amended informed consent document, including obtaining DH IRB approval of the amended form before new subjects consent to take part in the study using this version of the form.

11. Study Finances

11.1 *Funding Source*

This study is financed through the DCC Early-Phase Trials Program.

11.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Dartmouth-Hitchcock investigators will follow the Dartmouth-Hitchcock conflict of interest policy.

11.3 Subject Stipends or Payments

Subjects will not receive payment for participation in this study.

12. Study monitoring, auditing, and inspecting

12.1 Safety and Data Monitoring

This study will be monitored by the Data Safety Monitoring and Accrual Committee (DSMAC) of the Dartmouth Cancer Center. DSMAC is a multidisciplinary committee charged with overseeing monitoring of safety of participants, conduct, progress, and validity and integrity of the data of all clinical trials at DCC at Dartmouth. It meets quarterly to review accrual rates and information from studies that have accrued participants. The Committee will also review accrual and adverse events observed at non-DHMC sites. The Clinical Cancer Review Committee (CCRC) determines the frequency of DSMAC data and safety review. The DSMAC has the authority to suspend or to recommend termination to the CCRC of all research activities that fall within its jurisdiction. In the event that a study is suspended or terminated, that information will be forwarded to the Dartmouth IRB.

12.2 Monitoring

The expectation is that representatives of Dartmouth-Hitchcock Medical Center will be allowed to visit all study site locations as needed to assess the regulatory compliance, data quality, and study integrity. On site, they will review study records and directly compare them with source documents, discuss the conduct of the study with the local investigators, and verify that the facilities remain acceptable. They may request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents must be guaranteed by the local investigators, who must provide support at all times for these activities.

12.2.1. Monitoring at DHMC

Clinical research monitoring is conducted by appropriately trained staff of DCC–OCR and/or the Dartmouth Clinical Trials Office according to a study-specific internal monitoring plan. This monitoring will include periodic assessment of the regulatory compliance, data quality, pharmacy records, and study

integrity. Study records will be reviewed and directly compared to source documents, and the conduct of the study will be discussed with the Lead Dartmouth PI. Monitors may request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents is guaranteed by the investigator, who must provide support at all times for these activities.

12.2.2. Monitoring at non-DHMC Sites

Involvement in this study implies acceptance of potential monitoring, auditing or inspections, including source data verification, by representatives designated by the local PI or institution. A monitoring plan for non-DHMC sites will be determined as sites are added with consideration of the monitoring resources available at that site. The purpose of this monitoring is to determine whether study-related activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

Data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

12.3 Auditing and Inspecting

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Dartmouth and institutional compliance and quality assurance offices. Investigators will permit study-related audits and inspections by the Dartmouth and local IRB, CTO, government regulatory bodies, and Dartmouth or institutional compliance and quality assurance groups of all study-related documents (e.g., source documents, regulatory documents, data collection instruments, study data). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., diagnostic laboratory).

12.4 Investigator Compliance

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by, Dartmouth-Hitchcock. The investigator must not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard(s) to study participants. Any significant deviation must be reported to the coordinating site, reported to the IRB if it meets their guidelines for reporting, and documented in the CRF.

13. References

1. Ellis, MJ, Gao, F, Dehdashti, F, Jeffe, DB, Marcom, PK, Carey, LA, Dickler, MN, Silverman, P, Fleming, GF, Kommareddy, A, Jamalabadi-Majidi, S, Crowder, R and Siegel, BA. Lower-dose vs high-dose oral estradiol therapy of hormone receptor-positive, aromatase inhibitor-resistant advanced breast cancer: a phase 2 randomized study. JAMA. 302: 774-80 (2009).
2. Hossain, T, Islam, MA, Pal, R and Saha, A. Exploring structural requirement and binding interactions of beta-amyloid cleavage enzyme inhibitors using molecular modeling techniques. Medicinal Chemistry Research. 22: 4766-4774 (2013).
3. Mouridsen, H, Gershanovick, M, Sun, Y, Perez-Carrion, R, Boni, C, Monnier, A, Apffelstaedt, J, Smith, R, Sleeboom, HP, Jaenicke, F, Pluzanska, A, Dank, M, Becquart, D, Bapsy, PP, Salminen, E, Snyder, R, Chaudri-Ross, H, Lang, R, Wyld, P and Bhatnagar, A. 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 (2003).
4. Robertson, JFR, Lindemann, JPO, Llombart-Cussac, A, Rolski, J, Feltl, D, Dewar, J, Emerson, L, Dean, A and Ellis, MJ. Fulvestrant 500 mg versus anastrozole 1 mg for the first-line treatment of advanced breast cancer: follow-up analysis from the randomized 'FIRST' study. Breast Cancer Research and Treatment. 136: 503-511 (2012).

5. Bergh, J, Jonsson, PE, Lidbrink, EK, Trudeau, M, Eiermann, W, Brattstrom, D, Lindemann, JPO, Wiklund, F and Henriksson, R. FACT: An Open-Label Randomized Phase III Study of Fulvestrant and Anastrozole in Combination Compared With Anastrozole Alone As First-Line Therapy for Patients With Receptor-Positive Postmenopausal Breast Cancer. J Clin Oncol. 30: 1919-1925 (2012).
6. Wolff, AC, Lazar, AA, Bondarenko, I, Garin, AM, Brincat, S, Chow, L, Sun, Y, Neskovic-Konstantinovic, Z, Guimaraes, RC, Fumoleau, P, Chan, A, Hachemi, S, Strahs, A, Cincotta, M, Berkenblit, A, Krygowski, M, Kang, LL, Moore, L and Hayes, DF. Randomized phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer. J Clin Oncol. 31: 195-202 (2013).
7. Paridaens, RJ, Dirix, LY, Beex, LV, Nooij, M, Cameron, DA, Cufer, T, Piccart, MJ, Bogaerts, J and Therasse, P. Phase III Study Comparing Exemestane With Tamoxifen As First-Line Hormonal Treatment of Metastatic Breast Cancer in Postmenopausal Women: The European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. J Clin Oncol. 26: 4883-4890 (2008).
8. Baselga, J, Campone, M, Piccart, M, Burris, HA, 3rd, Rugo, HS, Sahmoud, T, Noguchi, S, Gnant, M, Pritchard, KI, Lebrun, F, Beck, JT, Ito, Y, Yardley, D, Deleu, I, Perez, A, Bachelot, T, Vittori, L, Xu, Z, Mukhopadhyay, P, Lebwohl, D, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 366: 520-9 (2012).
9. Chia, S, Gradishar, W, Mauriac, L, Bines, J, Amant, F, Federico, M, Fein, L, Romieu, G, Buzdar, A, Robertson, JF, Brufsky, A, Possinger, K, Rennie, P, Sapunar, F, Lowe, E and Piccart, M. Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT. J Clin Oncol. 26: 1664-70 (2008).
10. Johnston, SRD, Kilburn, LS, Ellis, P, Dodwell, D, Cameron, D, Hayward, L, Im, YH, Braybrooke, JP, Brunt, AM, Cheung, KL, Jyothirmayi, R, Robinson, A, Wardley, AM, Wheatley, D, Howell, A, Coombes, G, Sergenson, N, Sin, HJ, Folkerd, E, Dowsett, M, et al. Fulvestrant plus anastrozole or placebo versus exemestane alone after progression on non-steroidal aromatase inhibitors in postmenopausal patients with hormone-receptor-positive locally advanced or metastatic breast cancer (SoFEA): a composite, multicentre, phase 3 randomised trial. Lancet Oncology. 14: 989-998 (2013).
11. Ingle, JN, Ahmann, DL, Green, SJ, Edmonson, JH, Bisel, HF, Kvols, LK, Nichols, WC, Creagan, ET, Hahn, RG, Rubin, J and Frytak, S. Randomized clinical trial of diethylstilbestrol versus tamoxifen in postmenopausal women with advanced breast cancer. N Engl J Med. 304: 16-21 (1981).
12. Gockerman, JP, Spremulli, EN, Raney, M and Logan, T. Randomized comparison of tamoxifen versus diethylstilbestrol in estrogen receptor-positive or -unknown metastatic breast cancer: a Southeastern Cancer Study Group trial. Cancer Treat Rep. 70: 1199-203 (1986).
13. Peethambaram, PP, Ingle, JN, Suman, VJ, Hartmann, LC and Loprinzi, CL. Randomized trial of diethylstilbestrol vs. tamoxifen in postmenopausal women with metastatic breast cancer. An updated analysis. Breast Cancer Res Treat. 54: 117-22 (1999).
14. Carter, AC, Sedransk, N, Kelley, RM, Ansfield, FJ, Ravdin, RG, Talley, RW and Potter, NR. Diethylstilbestrol: recommended dosages for different categories of breast cancer patients. Report of the Cooperative Breast Cancer Group. JAMA. 237: 2079-8 (1977).
15. Haddow, A, Watkinson, JM, Paterson, E and Koller, PC. Influence of Synthetic Oestrogens on Advanced Malignant Disease. Br Med J. 2: 393-8 (1944).
16. Belani, CP, Pearl, P, Whitley, NO and Aisner, J. Tamoxifen withdrawal response. Report of a case. Arch Intern Med. 149: 449-50 (1989).
17. Stein, W, 3rd, Hortobagyi, GN and Blumenschein, GR. Response of metastatic breast cancer to tamoxifen withdrawal: report of a case. J Surg Oncol. 22: 45-6 (1983).
18. Legault-Poisson, S, Jolivet, J, Poisson, R, Beretta-Piccoli, M and Band, PR. Tamoxifen-induced tumor stimulation and withdrawal response. Cancer Treat Rep. 63: 1839-41 (1979).
19. Canney, PA, Griffiths, T, Latief, TN and Priestman, TJ. Clinical significance of tamoxifen withdrawal response. Lancet. 1: 36 (1987).

20. Bhide, SA and Rea, DW. Metastatic breast cancer response after Exemestane withdrawal: a case report. Breast. 13: 66-8 (2004).
21. Howell, A, Dodwell, DJ, Anderson, H and Redford, J. Response after withdrawal of tamoxifen and progestogens in advanced breast cancer. Ann Oncol. 3: 611-7 (1992).
22. Ariazi, EA, Cunliffe, HE, Lewis-Wambi, JS, Slifker, MJ, Willis, AL, Ramos, P, Tapia, C, Kim, HR, Yerrum, S, Sharma, CG, Nicolas, E, Balagurunathan, Y, Ross, EA and Jordan, VC. Estrogen induces apoptosis in estrogen deprivation-resistant breast cancer through stress responses as identified by global gene expression across time. Proc Natl Acad Sci U S A. 108: 18879-86 (2011).
23. Liu, H, Lee, ES, Gajdos, C, Pearce, ST, Chen, B, Osipo, C, Loweth, J, McKian, K, De Los Reyes, A, Wing, L and Jordan, VC. Apoptotic action of 17beta-estradiol in raloxifene-resistant MCF-7 cells in vitro and in vivo. J Natl Cancer Inst. 95: 1586-97 (2003).
24. Osipo, C, Gajdos, C, Liu, H, Chen, B and Jordan, VC. Paradoxical action of fulvestrant in estradiol-induced regression of tamoxifen-stimulated breast cancer. J Natl Cancer Inst. 95: 1597-608 (2003).
25. Song, RX, Mor, G, Naftolin, F, McPherson, RA, Song, J, Zhang, Z, Yue, W, Wang, J and Santen, RJ. Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol. J Natl Cancer Inst. 93: 1714-23 (2001).
26. Lewis, JS, Osipo, C, Meeke, K and Jordan, VC. Estrogen-induced apoptosis in a breast cancer model resistant to long-term estrogen withdrawal. J Steroid Biochem Mol Biol. 94: 131-41 (2005).
27. Lewis, JS, Meeke, K, Osipo, C, Ross, EA, Kidawi, N, Li, T, Bell, E, Chandel, NS and Jordan, VC. Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation. J Natl Cancer Inst. 97: 1746-59 (2005).
28. Miller, TW, Balko, JM, Fox, EM, Ghazoui, Z, Dunbier, A, Anderson, H, Dowsett, M, Jiang, A, Smith, RA, Maira, SM, Manning, HC, Gonzalez-Angulo, AM, Mills, GB, Higham, C, Chanthaphaychith, S, Kuba, MG, Miller, WR, Shyr, Y and Arteaga, CL. ERalpha-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. Cancer Discov. 1: 338-51 (2011).
29. Miller, TW, Hennessy, BT, Gonzalez-Angulo, AM, Fox, EM, Mills, GB, Chen, H, Higham, C, Garcia-Echeverria, C, Shyr, Y and Arteaga, CL. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. J Clin Invest. 120: 2406-13 (2010).
30. Li, S, Shen, D, Shao, J, Crowder, R, Liu, W, Prat, A, He, X, Liu, S, Hoog, J, Lu, C, Ding, L, Griffith, OL, Miller, C, Larson, D, Fulton, RS, Harrison, M, Mooney, T, McMichael, JF, Luo, J, Tao, Y, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 4: 1116-30 (2013).
31. Lonning, PE, Taylor, PD, Anker, G, Iddon, J, Wie, L, Jorgensen, LM, Mella, O and Howell, A. High-dose estrogen treatment in postmenopausal breast cancer patients heavily exposed to endocrine therapy. Breast Cancer Res Treat. 67: 111-6 (2001).
32. Zucchini, G, Armstrong, AC, Wardley, AM, Wilson, G, Misra, V, Seif, M, Ryder, WD, Cope, J, Blowers, E, Howell, A, Palmieri, C and Howell, SJ. A phase II trial of low-dose estradiol in postmenopausal women with advanced breast cancer and acquired resistance to aromatase inhibition. Eur J Cancer. 51: 2725-31 (2015).
33. Hata, AN, Yeo, A, Faber, AC, Lifshits, E, Chen, Z, Cheng, KA, Walton, Z, Sarosiek, KA, Letai, A, Heist, RS, Mino-Kenudson, M, Wong, KK and Engelman, JA. Failure to induce apoptosis via BCL-2 family proteins underlies lack of efficacy of combined MEK and PI3K inhibitors for KRAS-mutant lung cancers. Cancer Research. 74: 3146-56 (2014).
34. Stoll, BA. Hypothesis: breast cancer regression under oestrogen therapy. Br Med J. 3: 446-50 (1973).
35. Miller, TW and Paciello, RA. Discriminative dimensions of the MMPI as a function of age and psychopathology. J Clin Psychol. 36: 758-62 (1980).
36. Hata, AN, Niederst, MJ, Archibald, HL, Gomez-Caraballo, M, Siddiqui, FM, Mulvey, HE, Maruvka, YE, Ji, F, Bhang, HE, Krishnamurthy Radhakrishna, V, Siravegna, G, Hu, H, Raoof, S, Lockerman, E, Kalsy, A, Lee, D, Keating, CL, Ruddy, DA, Damon, LJ, Crystal, AS, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. Nat Med. 22: 262-9 (2016).

37. Hata, AN, Engelman, JA and Faber, AC. The BCL2 Family: Key Mediators of the Apoptotic Response to Targeted Anticancer Therapeutics. Cancer Discov. 5: 475-87 (2015).
38. Ham, J, Costa, C, Sano, R, Lochmann, TL, Sennott, EM, Patel, NU, Dastur, A, Gomez-Caraballo, M, Krytska, K, Hata, AN, Floros, KV, Hughes, MT, Jakubik, CT, Heisey, DA, Ferrell, JT, Bristol, ML, March, RJ, Yates, C, Hicks, MA, Nakajima, W, et al. Exploitation of the Apoptosis-Primed State of MYCN-Amplified Neuroblastoma to Develop a Potent and Specific Targeted Therapy Combination. Cancer Cell. 29: 159-72 (2016).
39. Castello-Cros, R, Bonuccelli, G, Molchansky, A, Capozza, F, Witkiewicz, AK, Birbe, RC, Howell, A, Pestell, RG, Whitaker-Menezes, D, Sotgia, F and Lisanti, MP. Matrix remodeling stimulates stromal autophagy, "fueling" cancer cell mitochondrial metabolism and metastasis. Cell Cycle. 10: 2021-2034 (2011).
40. Finn, RS, Martin, M, Rugo, HS, Jones, S, Im, SA, Gelmon, K, Harbeck, N, Lipatov, ON, Walshe, JM, Moulder, S, Gauthier, E, Lu, DR, Randolph, S, Dieras, V and Slamon, DJ. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 375: 1925-1936 (2016).
41. Finn, RS, Crown, JP, Ettl, J, Schmidt, M, Bondarenko, IM, Lang, I, Pinter, T, Boer, K, Patel, R, Randolph, S, Kim, ST, Huang, X, Schnell, P, Nadanaciva, S, Bartlett, CH and Slamon, DJ. Efficacy and safety of palbociclib in combination with letrozole as first-line treatment of ER-positive, HER2-negative, advanced breast cancer: expanded analyses of subgroups from the randomized pivotal trial PALOMA-1/TRIO-18. Breast Cancer Research. 18: 67 (2016).
42. Carlini, P, Michelotti, A, Ferretti, G, Ricci, S, Giannarelli, D, Pellegrini, M, Cresti, N, Di Cosimo, S, Bria, E, Papaldo, P, Fabi, A, Ruggeri, EM, Milella, M, Alimonti, A, Salesi, N and Cognetti, F. Clinical evaluation of the use of exemestane as further hormonal therapy after nonsteroidal aromatase inhibitors in postmenopausal metastatic breast cancer patients. Cancer Invest. 25: 102-5 (2007).
43. Carlini, P, Bria, E, Giannarelli, D, Ferretti, G, Felici, A, Papaldo, P, Fabi, A, Nistico, C, Di Cosimo, S, Ruggeri, EM, Milella, M, Mottolese, M, Terzoli, E and Cognetti, F. New aromatase inhibitors as second-line endocrine therapy in postmenopausal patients with metastatic breast carcinoma: a pooled analysis of the randomized trials. Cancer. 104: 1335-42 (2005).
44. Llombart-Cussac, A, Guerrero, A, Galan, A, Caranana, V, Buch, E, Rodriguez-Lescure, A, Ruiz, A, Fuster Diana, C and Guillem Porta, V. Phase II trial with letrozole to maximum response as primary systemic therapy in postmenopausal patients with ER/PgR[+] operable breast cancer. Clin Transl Oncol. 14: 125-31 (2012).
45. Krainick-Strobel, UE, Lichtenegger, W, Wallwiener, D, Tulusan, AH, Janicke, F, Bastert, G, Kiesel, L, Wackwitz, B and Paepke, S. Neoadjuvant letrozole in postmenopausal estrogen and/or progesterone receptor positive breast cancer: a phase IIb/III trial to investigate optimal duration of preoperative endocrine therapy. BMC Cancer. 8: 62 (2008).
46. Eisenhauer, EA, Therasse, P, Bogaerts, J, Schwartz, LH, Sargent, D, Ford, R, Dancey, J, Arbuck, S, Gwyther, S, Mooney, M, Rubinstein, L, Shankar, L, Dodd, L, Kaplan, R, Lacombe, D and Verweij, J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 45: 228-47 (2009).
47. Robinson, DR, Wu, YM, Vats, P, Su, F, Lonigro, RJ, Cao, X, Kalyana-Sundaram, S, Wang, R, Ning, Y, Hodges, L, Gursky, A, Siddiqui, J, Tomlins, SA, Roychowdhury, S, Pienta, KJ, Kim, SY, Roberts, JS, Rae, JM, Van Poznak, CH, Hayes, DF, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet. 45: 1446-51 (2013).
48. Toy, W, Shen, Y, Won, H, Green, B, Sakr, RA, Will, M, Li, Z, Gala, K, Fanning, S, King, TA, Hudis, C, Chen, D, Taran, T, Hortobagyi, G, Greene, G, Berger, M, Baselga, J and Chandarlapaty, S. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet. 45: 1439-45 (2013).
49. Jeselsohn, R, Yelensky, R, Buchwalter, G, Frampton, G, Meric-Bernstam, F, Gonzalez-Angulo, AM, Ferrer-Lozano, J, Perez-Fidalgo, JA, Cristofanilli, M, Gomez, H, Arteaga, CL, Giltnane, J, Balko, JM, Cronin, MT, Jarosz, M, Sun, J, Hawryluk, M, Lipson, D, Otto, G, Ross, JS, et al. Emergence of Constitutively Active Estrogen Receptor-alpha Mutations in Pretreated Advanced Estrogen Receptor-Positive Breast Cancer. Clin Cancer Res. 20: 1757-67 (2014).
50. Simon, R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 10: 1-10 (1989).