Phase II trial of exemestane with immunomodulatory cyclophosphamide in ER and/or PR-positive and HER2/neu negative metastatic breast cancer

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Study Summary

Title	Phase II trial of exemestane with immunomodulatory *cyclophosphamide* in ER and/or PR-positive and HER2/neu-negative metastatic breast cancer.
Short Title	Combination of endocrine and immune therapy in advanced breast cancer.
Phase	Phase 2
Methodology	Single-arm open label
Hypothesis	The treatment regimen of low dose immunomodulatory cyclophosphamide in combination with exemestane will result in a target 3-month PFS of $\geq$ 75% (extrapolated from median PFS of 6.9 months seen in the combination arm of Bolero 2, current standard of care exemestane + everolimus but with significant toxicity).
Study Duration	Three years
Study Center(s)	NYU Cancer Center, Bellevue Hospital
Objectives	To estimate progression-free survival (PFS) and PFS rate at 3 months, response rate (RR), clinical benefit rate (CBR) and evaluate the safety of immunomodulatory doses of cyclophosphamide with exemestane after progression on at least one line of endocrine therapy.
Number of Subjects	23
Diagnosis and Main Inclusion Criteria	Metastatic ER or PR positive, Her2 negative breast cancer Progression on aromatase inhibitor (AI), tamoxifen and/or fulvestrant Postmenopausal
Study Product, Dose, Route, Regimen	Cyclophosphamide at 50 mg tablet po daily and exemestane at 25 mg tablet po daily.
Duration of administration	Treatment until progression or removal from study
Statistical Methodology	This is an open label single arm Phase II study to estimate the median PFS and the PFS on this regimen at 3 months. The primary outcome, PFS rate at 3 months, will be estimated with an exact 95% confidence interval at the conclusion of the study.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

1.1.1 Metastatic Breast Cancer

Breast cancer is the most common cancer among women and is after lung cancer a leading cause of mortality in women. The majority of breast cancer related deaths are a result of complications from recurrent or metastatic disease. As an initial presentation, metastatic breast cancer (MBC) is uncommon, occurring in less than 10% of newly diagnosed cases [1]. However, up to 30% of the women who are initially diagnosed with early stage breast cancer eventually develop recurrent or metastatic disease. In the metastatic setting, treatment goals are generally to prolong life, control symptoms, and maintain quality of life. Many of these patients are likely to have experienced recurrences after receiving prior adjuvant chemotherapy and/or endocrine therapy. A major limitation of treatments in the recurrent and metastatic settings is the high incidence of tumor resistance to endocrine agents. Over the last decade therapeutic innovation has only resulted in modest improvements in survival rates [2]. It is therefore clear that novel therapeutic approaches and/or combinations are needed.

1.1.2. ER/PR positive, HER2 negative metastatic breast cancer and treatment options for 2nd line endocrine therapy

The majority of breast cancers are hormone receptor (HR) positive breast cancers. The prognostic and predictive value of hormone receptor status has been well established. Sensitivity to endocrine therapies is typically suggested by a long disease-free interval, a high level of estrogen/progesterone receptor (ER/PR) expression in tumors, metastatic disease confined to non-visceral sites and the absence of HER2 overexpression/amplification. Although there are limited data from RCTs (randomized controlled trials), results from non-randomized trials regarding the effects of endocrine sequence suggest that response to first-line therapy predicts response to subsequent endocrine therapy.

While there is no optimized treatment sequence for hormonal therapies, therapeutic options for second-line treatment of HR positive metastatic disease have been evaluated in separate RCTs and include anti-estrogens and aromatase inhibitors (**Table 1**). Third generation AIs are distinguished into non-steroidal (anastrazole and letrozole) and steroidal (exemestane) AIs. With non-steroidal AIs now being widely used in the adjuvant setting for postmenopausal women with HR positive breast cancer, the endocrine options for first-line treatment after relapse during or shortly after completion of adjuvant therapy are fulvestrant 500 mg, steroidal AI, steroidal AI with everolimus or tamoxifen (with or without everolimus).

Fulvestrant is an ER antagonist without known agonistic properties that downregulates cellular levels of ER in a dose-dependent manner. In a phase III RCT, the Comparison of Faslodex in Recurrent or Metastatic Breast Cancer trial (**CONFIRM)** [3] two different doses of fulvestrant (given by intramuscular injection) were evaluated—the initially approved dose (250 mg every 28 days) and a higher dose regimen including a day 14 loading dose (500 mg on days 0, 14, and 28, and every 28 days thereafter). Approximately 50% of enrolled patients had experienced relapse on adjuvant endocrine therapy or were within 1 year from completion of adjuvant endocrine therapy. For patients who experienced relapse after more than 1 year from completion of adjuvant endocrine therapy or for patients presenting with de novo advanced disease, eligibility required a previous treatment with either an antiestrogen (57.5% of patients) or an aromatase inhibitor (42.5%) as a first-line therapy. High-dose fulvestrant (500 mg monthly), as compared with standard dose fulvestrant, provided only a modest improvement in median progression-free survival, from 5.5 to 6.5 months (hazard ratio, 0.80; P = 0.006). This improvement was less pronounced in patients whose most

recent therapy was an aromatase inhibitor (hazard ratio, 0.85; P = 0.20) and in those who were considered to have had a response to the most recent endocrine therapy (hazard ratio, 0.85; P = 0.12).

Exemestane is an oral, steroidal irreversible inhibitor of aromatase. It did not show a difference in PFS in the international **EFECT** trial of fulvestrant 250 mg versus exemestane [4]. Enrolled patients were refractory to letrozole or anastrozole, defined as recurrence during or within 12 months after the end of adjuvant treatment or progression during or within 1 month after the end of treatment for advanced disease. Prior additional endocrine or chemotherapy was allowed and letrozole or anastrozole did not have to be the most recent treatment before randomization, approximately 15% of patients had received an anti-estrogen (TAM or fulvestrant) and approximately 10% of patients had received chemotherapy (max 1 line) in the metastatic setting.

In the fulvestrant versus anastrozole Phase III trial **North American trial** [5] the primary analysis of time to progression (TTP) showed fulvestrant 250 mg to be at least as effective as anastrozole after failing one line of endocrine therapy (96% of patients received tamoxifen).
It is therefore evident that standard approaches (switch to steroidal AI or fulvestrant) perform poorly once acquired resistance is observed and novel therapies for metastatic disease are needed. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway has been implicated in HR positive cancers and endocrine resistance [6]. This has led to studies combining various signaling inhibitors with aromatase inhibitors to enhance endocrine responsiveness and delay, or even reverse resistance.

BOLERO 2 [7]: This trial evaluated the addition of everolimus, which targets mTOR, to a steroidal AI after failure of at least one prior endocrine therapy (letrozole or anastrozole (100%), tamoxifen (48%), fulvestrant (16%) although 26% of patients had also received prior chemotherapy in the metastatic setting. The study showed longer PFS when everolimus is added to exemestane (published as **6.9 months for the combination versus 2.8 months for single agent exemestane**). Final PFS data updated for San Antonio's breast conference 2012 showed 7.8 vs 3.2 months PFS.

This observed benefit of the combination exemestane and everolimus compares favorably with that of all alternative options, including fulvestrant at a dose of 250 mg with a median PFS of 3.7 months and fulvestrant at 500 mg with a PFS of 6.5 months (see table below). The BOLERO 2 results also compare favorably to those achieved by (first-line) chemotherapy with capecitabine and taxanes or anthracyclines, with a median PFS of 6.2 months and 8.2 months, respectively, in patients with HR-positive disease (RIBBON-1, arms without bevacizumab [8]. Everolimus (Afinitor) therefore received FDA approval for metastatic breast cancer in July 2012 based on the prolongation of PFS in combination with exemestane.

However, the combination of exemestane and everolimus was associated with a higher incidence of adverse events than exemestane alone, serious adverse events were reported in 23% of participants. The adverse events observed with everolimus plus exemestane include stomatitis, fatigue and asthenia, anemia, diarrhea, cough, dyspnea, pneumonitis, fever, and hyperglycemia. Despite permitted dose interruptions and/or reductions, a high percentage of patients discontinued everolimus because of intolerability (19% of patients) or withdrawal of consent (5%). 1% of patients in the everolimus arm died due to an adverse event.

Table 1: Outcomes of Phase III RCT of endocrine therapy in patients with metastatic hormone-receptor positive breast cancer who failed a non-steroidal aromatase inhibitor or an anti-estrogen

Trial	Agents	RR^	CBR^	PFS^	OS
		percent		months	
BOLERO 2 2nd line after AI, n=724	Exemestane Exemestane + Everolimus	0.4 7*	n.a.	2.8 **6.9***	n.a.
EFECT 2nd line after AI, n=693	Fulvestrant 250 Exemestane	7.4 6.7	32.2 31.5	3.7 3.7	n.a.
CONFIRM 2nd line after TAM or AI, n=736	Fulvestrant 500 Fulvestrant 250	9.1 10.2	36.5 29.4	6.5* 5.5	25.1* 22.8

North American trial 2nd line after TAM, n=400	Fulvestrant 250 Anastrozole	17.5 17.5	42.2 36.1	5.4 3.4	27 27

*Statistically significant difference, n.a. not available, RR response rate CBR clinical benefit rate PFS progression-free survival OS overall survival
^Frequency of RECIST tumor assessment: Bolero 2 q 6 weeks, North American trial q 12 weeks, CONFIRM q 12 weeks, EFECT q 8 weeks (after 6 months q 12 weeks)

1.2 Investigational Agents

1.2.1 Exemestane

Exemestane (trade name aromasin) is an oral steroidal aromatase inhibitor indicated for the treatment of breast cancer in post-menopausal women. It is a synthetic androgen analogue (6-methylenandrosta-1,4-diene-3,17-dione), which binds irreversibly to and inhibits the enzyme aromatase, thereby blocking the conversion of cholesterol to pregnenolone and the peripheral aromatization of androgenic precursors into estrogens, the main pathway providing estrogen after menopause. Exemestane significantly lowers circulating estrogen concentrations in postmenopausal women, but has no detectable effect on adrenal biosynthesis of corticosteroids or aldosterone.
Aromasin was originally approved in the US in 1999 for the treatment of advanced breast cancer in postmenopausal women whose tumors have stopped responding to tamoxifen. On October 5, 2005, the U.S. Food and Drug Administration approved exemestane for adjuvant treatment of postmenopausal women with estrogen receptor -positive early breast cancer following two-to-three years of tamoxifen based on the results of the Intergroup Exemestane Study [9], showing superior DFS in a double-blind, multicenter, international clinical trial in 4724 patients randomly assigned to either continue tamoxifen (20-30 mg/day) or switch to exemestane (25 mg/day) to complete a total of five years of adjuvant hormonal therapy. The most common adverse events on the IES that occurred more frequently on the exemestane arm included hot flashes, fatigue, arthralgia, headache, insomnia, increased sweating, hypertension, and dizziness. Cardiac ischemic events occurred in 1.6 percent of patients in the exemestane arm of the IES compared to 0.6 percent of patients in the tamoxifen arm. Changes in bone mineral density (BMD) were evaluated in a sub-study of the IES and in a supporting safety study (027), which compared the effects of two years of exemestane to placebo. Mean decreases in BMD of the lumbar spine and femoral neck were more pronounced with exemestane than with either tamoxifen or placebo. On the IES, osteoporosis was reported in 4.6 percent of patients treated with exemestane compared to 2.8 percent of patients receiving tamoxifen.

1.2.2 Cyclophosphamide

Cyclophosphamide (former trade name cytoxan, CTX) is a synthetic alkylating agent chemically related to the nitrogen mustards with antineoplastic and immunosuppressive activities. In the liver, CTX is converted to the active metabolites aldophosphamide and phosphoramide mustard, which bind to DNA, thereby inhibiting DNA replication and initiating cell death. While conventional (high) dose CTX is cytotoxic and a mainstay of treatment in breast cancer, it causes cytopenias and is generally considered as an immunosuppressive agent.

Metronomic cyclophosphamide ("frequent and homogeneously spaced low-dose" administrations)

1.3 Preclinical Data

In contrast, low dose metronomic CTX can enhance immune responses. Initially, preclinical models provided evidence of the ability of CTX to selectively deplete regulatory T cells (Tregs), thereby improving anti-tumor immune responses (summarized in **Table 2**).

Table 2: Pre-clinical studies of cyclophosphamide alone or in combination with immunotherapies

Reference	Murine Model	CTX dose and schedule	Combination agent	Immune responses	Outcome
Hermans Ca Res 2003 [10]	Melanoma	175 mg/kg every 6 days or 150 mg/kg every other day over 6 days followed by 15 days of rest	Vaccine d1, boosted 14-60 days later	Higher in combination with 175 mg/kg	Improved survival with 175 mg/kg
Lutsiak Blood 2005 [11]	Healthy	100 mg/kg day 0	none	Decrease in CD4+25+ Treg number (halved on d4), function and enhanced apoptosis	
Motoyoshi Oncol Rep 2006 [12]	Hepato-cellular cancer	200 mg/kg or 20 mg/kg after tumor inoculation	none	high dose decreased all T cell subsets while low dose decreased Tregs selectively	Anti-tumor effect with low dose, only in immuno-competent mice and if given after tumor inoculation
Malvicini Oncoimmunol 2012 [13] and Mol Oncol 2011 [14]	Colorectal and pancreas cancer	50mg/kg once day 8 versus 25mg/kg 3x/week	Adenovirus expressing IL-12	Both initially decreased Treg and MDSC but 25 mg/kg did not sustain, better IR with 50 mg/kg	Seen with 50 mg/kg (RR and OS)
Mkrtichyan Eur J Immunol 2011 [15]	TC-1 tumor cells	50mg/kg the day before vaccine	Anti-PD-1 HPV16 E7 peptide /GM-CSF/anti-CD40		Tumor regression and increased survival
Sevko JID 2012 [16]	Melanoma	50 and 125 mg/kg once	none	Treg decrease but MDSC expansion	No benefit
Son J Immunother 2012 [17]	Colon cancer	30 mg/kg 3 days before every iDC injection 3x/week	RT and intratumoral iDC	Treg decrease, increased anti-tumor immune response	Improved survival
Dewan CCR 2012 [18]	Breast cancer	100 mg/kg once	RT and imiquimod	reduces Tregs and IL-10 production, enhances tumor-specific IFN-g production by TDLNs	Prevented tumor recurrence and rejected tumor challenge

1.4 Clinical Data to Date

More recently low dose CTX has been studied in patients based on the preclinical evidence discussed above. Extensive reviews of low-dose CTX in combination with immunotherapies demonstrate an excellent safety profile [19-21]. CTX (50 or 100 mg/d) is well tolerated even in elderly and/or heavily pretreated patients. Toxicities are usually grade I/II and manageable, only when combined with bevacizumab, have severe adverse events (hemorrhagic complications) been reported.

Several of the clinical trials confirmed immune modulating properties of low dose CTX; relevant studies are summarized in **Table 3**. Numerous single agent and combination studies have demonstrated some signs of activity, although most clinical studies reported are non-randomized. In small but randomized studies CTX was confirmed to be effective and safe as both, single agent in refractory solid tumors [22] and in combination with letrozole in the neoadjuvant treatment of ER positive breast cancers [23].

Table 3: Clinical trials of low dose cyclophosphamide alone or in combination therapy

Reference	Setting	CTX regimen	Immune changes	Safety and efficacy
Single agent cyclophosphamide				

Ghiringhelli, CII 2007 [24]	End-stage cancer (n=9)	1.4 mg/kg CTX per day (i.e. 50 mg BID) for weeks 1 and 3 of 4 week cycle	Selective depletion of Tregs, restored NK and T effector function, (non-selective depletion of all leukocytes and decreased NK and T function at 100 mg BID)	
Greten, J Immunother 2010 [25]	HCC (n=13)	150, 250, or 350 mg/m2 (3.4, 5.7, and 6.8 mg/kg) on d 1 and d29	Selectively depletes Tregs in low groups only (reduction of 34.1% by d 8, 80% by d 29, and 65% by d 71, endogenous Ag-spec T cell responses unmasked in 6/13	No DLT
Ge, CII 2012 [26]	Breast cancer (n=12)	50 mg CTX daily x 3 mos (0.7 mg/kg)	Transiently reduced Tregs, rebounded after d 42	Anti-tumor response and SD, no grade III/IV toxicity, grade I leucopenia in all patients
Penel, Br J Cancer 2010 [22]	End-stage cancer (n=88; no breast cancer)	50 mg BID CTX (1.4 mg/kg/d) continuously versus megestrol acetate	Not evaluated	Safe, PFS 20%, 11% and 4% at 2, 4 and 6 months, TTP 2 months both arms
Viaud, Ca Res 2011 [27]	Advanced cancers (n=21)	50 mg/d CTX daily (0.7 mg/kg) x 3 weeks	Expansion of Th17 cells in periphery and tumor	
Cyclophosphamide in combination with immune-, biological or chemotherapy				
Hoon, Cancer Res 1990 [28]	Melanoma	75, 150 and 300 mg/m2 (1.7, 3.4 and 6.8 mg/kg) CTX or no CTX prior to vaccine	Selective depletion of CD8+ suppressor T cells, greater with first 2 cycles	Possibly benefit
Laheru, CCR 2008 [29]	Pancreatic cancer	250 mg/m2 (5.7 mg/kg) CTX versus none on day -1 prior to each vaccination	Greater CD8-T cell response with CTX	Safe, improved OS with CTX
Emens, JCO 2009 [30]	Breast cancer	200, 250, and 350 mg/m2 (4.5, 5.7 or 6.8 mg/kg) CTX prior to each vaccine versus no CTX	HER2-specific immunity only in lowest dose (and no) CTX groups	Safe
Greten, BMC Cancer 2010 [25]	Liver cancer	300 mg/m2 CTX (6.8 mg/kg) d-3 prior to peptide vaccine and GM-CSF	Decreased Tregs, but no vaccine response	Safe
Slingluff, JCO 2011 [31]	Melanoma	300 mg/m2 (6.8 mg/kg) CTX given with each vaccination versus no CTX	No impact on Ag-specific immunity	Safe (g 4 hypoglycemia?)
Chu, CII 2011 [32]	Ovarian cancer	300 mg/m2 CTX (6.8 mg/kg) intravenous CTX two days before vaccine	No change in Tregs	Safe
Cerullo, Mol Ther 2011 [33]	Advanced cancers	50 mg/day oral (x 7 days before vaccine) (=0.7 mg/kg/d), 1000 mg (15 mg/kg) IV x1, or a combination of oral and IV CTX prior to vaccine	Decreased Tregs without compromised anti-tumor / anti-viral IR in both po arms	Safe, best OS in third group
Berd, Cancer Res 1987/88 [34, 35]	Melanoma	300 mg/m2 (6.8 mg/kg) CTX 3 d prior to vaccine	Increased Ag-specific immunity in CTX arm	Safe, tumor regression and 2 CRs
Colleoni, Ann Oncol 2002 [36]	Breast cancer	50 mg/d CTX (0.7 mg/kg) continuously, with low dose MTX	Not evaluated	Safe, 19% RR, TTP 2.8 months
Orlando, Antica Drugs 2006 [37]	Breast cancer	50 mg/d CTX (0.7 mg/kg) continuously, with low dose MTX	Not evaluated	Safe, 15% RR, one durable CR>4 years
Dellapasqua, JCO 2008 [38]	Breast cancer	50 mg/d CTX (0.7 mg/kg) continuously, with capecitabine and bevacizumab	Not evaluated	Safe, 48% RR
Wong, JCO 2010 [39]	Breast cancer	50mg/d CTX (0.7 mg/kg) continuously with dalteparin, MTX and prednisone	Not evaluated	Safe, 17% RR
Bottini, JCO 2006 [23]	Breast cancer	Preoperative letrozole +/- CTX 50 mg/d (0.7 mg/kg) continuously for 6 months	Not evaluated	Increased RR with CTX, greater decrease in Ki67
Colleoni, Ann Oncol 2006 [40]	Breast cancer	50 mg/d CTX (0.7 mg/kg) continuously with low dose MTX +/- thalidomide	Not evaluated	Without thalidomide: safe, 21% RR, TTP 3.8 months
Ongoing trials utilizing immunomodulatory cyclophosphamide				
Emens (NCT00971737):	Breast cancer	CTX 200 mg/m2 IV (4.5 mg/kg) over 30 minutes on day before each vaccine (q 4-6 weeks)		
Knutson (NCT01606241)	Breast cancer	CTX 100 mg CTX orally daily (1.4 mg/kg) for 1 week followed by 1 week rest, then another 1 week of CTX, followed by vaccine 7-10 days after		

Bhardwaj (NCT01740401)	Melanoma	CTX 300 mg/m2 orally (6.8 mg/kg), 2 days prior to each ipilimumab dose

Conversions based on assumptions of a patient with BSA 1.6 m2 and 70kg: for example CTX 300 mg/m2 = 480 mg = 6.8 mg/kg or CTX 50 mg total = 0.7 mg/kg

1.5 Dose and Schedule Rationale

The beneficial anti-tumor effects of low dose CTX are mediated by selectively depleting circulating Tregs, thereby restoring levels to those seen in healthy volunteers [24, 26]. Circulating Tregs are increased in patients with cancer (approximately 5% of circulating CD4 T cells) and have a higher proliferative capacity compared with healthy individuals [26]. Tregs can exert functional inhibition on tumor-specific T cells and blunt the innate arm of immunity by inhibiting NK cell proliferation and effector functions. Therefore, Tregs can limit the induction of anti-tumor immune responses, immune rejection via innate and adaptive mechanisms and promote disease progression, all of which likely contribute to the positive correlation of Treg –especially T cells infiltrating the tumor- with worse outcomes in a variety of solid tumors, including breast cancer [41].

Treatment of cancer patients with low dose CTX has been shown to:

1. Selectively deplete Tregs, thereby restoring levels to those seen in healthy volunteers ,
2. limit the suppressive function of remaining Tregs
3. restore NK cell-dependent cytotoxicity (innate killing)
4. restore peripheral T cell proliferation
5. unmask pre-existing (endogenous) tumor antigen-specific T cell responses
6. induce T cell responses to endogenous tumor antigens
7. normalize proliferative ability of effector T cells, especially CD8 T cells
8. increase Th17 cells
9. enhance vaccine responses

These effects of CTX were only seen at certain doses (for instance with CTX 100 mg/d but not with 200 mg/d as higher doses depleted lymphocytes in a non-selective manner and decreased NK cell-dependent cytotoxicity and T cell proliferation).

Treg depletion is usually transient. With the CTX 50 mg daily for 3 months regimen used by Ge et al [26], Treg numbers were reduced for 4-6 weeks, then rebounded to pre-treatment levels at week 8, remaining at that level until the end of treatment; the proliferative capacity of Treg recovered as well. Interestingly, induced and unmasked anti-tumor T cell responses continued to increase throughout the 3 months treatment period (despite an only transient decrease of Tregs) and correlated with clinical benefit. In a study by Ghiringhelli et al utilizing CTX 50 mg BID 1 week on and 1 week off [24], Treg were significantly reduced at 4 weeks, residual Treg had lost their suppressive function and T and NK effector function were restored. As with continued treatment in the study by Ge, Treg numbers also returned to baseline when measured 2 months after stopping treatment in the study by Ghiringhelli.

While there is no documented superiority of a specific immune modulating dose/regimen in patients, in the proposed clinical trial we will use CTX at 50 mg by mouth daily on a continuous basis, per schedule studied by Ge et al, as this regimen has shown a decrease in Tregs for the first 2 months, the induction of breast cancer antigen-specific immunity and was exclusively tested in breast cancer patients. Furthermore this dose and schedule has been safely and effectively combined with an aromatase inhibitor in the neoadjuvant setting for breast cancer [23].

Rationale for the current trial and the combination of exemestane with immunomodulatory cyclophosphamide

There is an **unmet need** for therapeutics in hormone-receptor positive metastatic breast cancer after failure to respond to non-steroidal aromatase inhibitors or an antiestrogen. The phase III RCTs in this setting (Table 1) demonstrated that single agent exemestane as second line treatment has low response rates in the 0.4-6.7% range and a short PFS of 2.8-3.7 months. It is therefore evident that standard approaches perform poorly once acquired resistance is observed and novel therapies for metastatic disease are needed. Notably, the addition of the targeted agent and mTor inhibitor everolimus to exemestane in the Bolero 2 trial led to a significantly better outcome for patients with a four month improvement of PFS over single agent exemestane alone (RR remained low at 7%). The benefit of the combination led to its regulatory approval in the US in 2012 and offers a new benchmark for clinical benefit in sequential hormonal manipulation for metastatic breast cancer. However, the significant toxicity of the combination regimen limits its generalized adaptation and patient compliance, with a dropout rate of 24% of patients receiving everolimus [42].

In the current trial we will investigate if exemestane combined with low dose CTX, a known immunomodulator, which is also the most commonly used metronomic chemotherapeutic and is exceptionally well tolerated [21], can provide a significant clinical benefit similar to that seen with exemestane + everolimus in the Bolero 2 trial. Both regimens are available orally, but if successful, the exemestane + CTX combination would be a favorable alternative due to its cost-effectiveness and tolerability, and a larger randomized trial would be pursued.

We hypothesize that low dose CTX in combination with exemestane will result in a target 3-month PFS rate of ≥ 75% (extrapolated from median PFS of 6.9 months seen in the combination arm of Bolero 2). This expected improvement of PFS over exemestane alone is based on the following reasons:

1. Both modalities have a distinct major mechanism of action (with non-overlapping toxicities)

Exemestane, as discussed above, is a steroidal aromatase inhibitor, which reduces levels of circulating estradiol via inhibition of its biosynthesis from androgen precursors. CTX at low immunomodulating doses selectively depletes Tregs as discussed above, thereby restoring innate and adaptive anti-tumor immunity.

2. Both drugs have the potential to diminish the pool and suppressor function of Tregs

The Treg modulating potential of low dose CTX has been discussed extensively in Section 2.4; and may lead to the induction and/or unmasking of anti-tumor immune responses which could provide a beneficial and long-lasting effects on tumor control.
Anti-estrogen therapies may also modulate Tregs. Endogenous estrogens at physiological (late follicular phase of menstrual cycle and pregnancy) and pharmacological ranges have been shown to drive expansion of $CD4^+CD25^+$ Tregs after their activation, increase the expression of Foxp3 and IL-10 genes as well as stimulate the conversion of $CD4^+CD25^-$ T cells (which express ER) into $CD4^+CD25^+$ T cells with similar regulatory function as naturally occurring Tregs [43-46]. As the conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ T cells stimulated by E2 could be inhibited by a specific inhibitor of ER [47] and numbers of Tregs generally decrease with lower estradiol levels, it is possible that lowering circulating estradiol levels via exemestane may contribute to a diminished Treg pool in patients. In a rat model of human arthritis, administration of anastrozole for instance suppressed the differentiation of naive T cells to Tregs as well as induced IFN-γ and IL-12 (Th1) and decreased levels of IL-4 and IL-10 (Th2 cytokines) [48]. In elderly breast cancer patients, neoadjuvant treatment with the non-steroidal AI letrozole decreased intratumoral Tregs, especially in responding tumors, which was potentiated by the addition of daily low dose CTX at 50 mg/d, albeit not statistically significant [49].

3. Potential other mechanisms of action to overcome endocrine resistance

It is not known if immunomodulatory CTX in combination with exemestane may overcome endocrine resistance, however some older studies suggest that non-specific immune stimulation, for instance with IFN-γ can restore antiestrogen sensitivity and that the combination of IFN-*α* and tamoxifen had a greater anti-proliferative effect on ZR-75-1 breast cancer cells than either drug alone [50]. Pilot studies suggest that IFN-*β* can improve clinical benefit in metastatic breast cancer, and a planned study will test IL-2 and an antiestrogen [51].

Importantly, based on two randomized trials performed in the neoadjuvant setting, the efficacy of the combination of letrozole with low dose CTX is similar to the one observed with letrozole and everolimus, albeit with fewer side effects necessitating treatment interruption or dose reduction (Table 4).

Table 4: Randomized neoadjuvant Phase II trial comparing a single agent aromatase inhibitor with the combination of aromatase inhibitor and everolimus (or CTX)

	Baselga et al, JCO 2009, n= 270		**Bottini et al, JCO 2006, n=114**	
	Letrozole	**Letrozole + everolimus**	**Letrozole + low dose CTX**	**Letrozole**
Clinical response	59%	**68%**	**88%**	72%
Ki67 reduction	75%	**90%**	**88%**	73%
Pathologic response	0.8%	**1.4%**	**3.5%**	3.5%
Dose delay or reduction for AE due to combination agent (everolimus or CTX)		**53%**	**2%**	

1.6 Research Risks & Benefits

1.6.1 Risk of Study Drug

Expected adverse events (AEs) for low dose CTX and exemestane are listed in section 8. While this is a greater than minimal risk study, exemestane (alone or in combination with everolimus) is a standard of care regimen in the proposed setting, and the addition of low dose CTX to an AI has not demonstrated a significantly increased rate of AEs in a randomized trial of preoperative letrozole and CTX in breast cancer [23].

1.6.2 Other Risks of Study Participation

Additional risks to study participation are listed in the consent form and include breach of confidentiality, not deriving benefit from the study treatments, procedures performed for research only (immune blood draws and tumor FNAs). Privacy protection procedures are in place and good clinical practice guidelines are followed for the study to minimized risks associated with research procedures and participation.

1.6.3 Potential benefits

It is not known if participation in the study will be beneficial for the patient, although it is anticipated that patients will derive clinical benefit from the combination with prolongation of PFS based on the data discussed above. The major theoretical benefits lie in a potentially as effective but less toxic alternative for exemestane + CTX (instead of everolimus) and the induction of a long-lasting protective anti-tumor immune response.

1.7 Correlative Studies Background

If funding is available, we plan to do the following correlative studies in all or a subset of trial participants to assess a baseline status and changes with treatment.

Phenotypic and functional profile of peripheral lymphocytes and myeloid lineages

Low dose CTX treatment has beneficial anti-tumor effects which have been attributed to relatively selective depletion of circulating Tregs, thereby restoring levels to those seen in healthy volunteers [24, 26]. Tregs can potentially exert functional inhibition of tumor-specific T cell activation and may also blunt the NK and innate

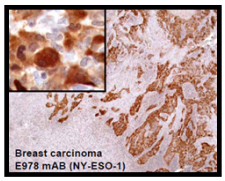
arm of immunity through suppression of their effector functions. Therefore, Tregs can limit the induction of anti-tumor immune responses, immune rejection via innate and adaptive mechanisms and promote disease progression. It is therefore reasonable to hypothesize that low dose CTX treatment enhances anti-tumor immunity through selective depletion of Tregs, as has been previously suggested. However, these studies were limited in their analysis of Treg subsets and corollary effector T cell subsets. Further, the perturbations in other regulatory compartments, such as myeloid suppressor cells or impact of CTX on the innate immunity could contribute to its beneficial effects. As such, we propose to assess the immune compartments in high resolution before and after CTX treatment to identify a profile that can provide both biomarker signatures and mechanistic insights. These immune profiling studies of Tregs and other lymphoid/myeloid subsets at baseline and selected time points during treatment to confirm an effect of CTX by established flow cytometry based methodology is described in section 6.3.

Examination of tumor antigen-specific CD4 and CD8 T cell responses

Induction of tumor antigen-specific immunity would imply that the treatment facilitates cross-priming and/or is unmasked by the inhibition/depletion of Tregs. Antigen-specific immune monitoring is more feasible than autologous whole tumor cell monitoring and has been demonstrated to correlate with clinical benefit in metastatic breast cancer, when Her2-specific CD4 T cell response was examined during therapy with trastuzumab and chemotherapy [52]. Paraffin-embedded tumor tissue (archived or study entry) will be tested for the expression of five antigens: 1. PRAME (PReferentially expressed Antigen of Melanoma), 2. WT-1 (Wilms' Tumor gene), 3. Her2, 4. NY-ESO-1 and 5. MAGE A3. These tumor antigens (TAA) are selected due to their expression in breast cancers (frequencies listed in table below, based on which >90% of patients will express at least 2 of the 5 antigens), inclusion of CTL epitopes and the availability of overlapping peptide pools. In addition, pre-existing immune responses have been observed for some antigens in breast cancer patients [53] and anti-tumor activity has been observed for some antigens when used for vaccination [54-57].

Antigen	Expression in breast cancer	IHC mAb (Source)	References
PRAME	27-53%	AB32185 (Abcam)	[58, 59]
WT-1	33-87%	6F-H2 (Dako)	[60, 61]
Her2	24%	Commercial, FISH for 2+	[62]
NY-ESO-1	5-42%	E978 (LICR)	[63, 64] and unpublished data
MAGE A3	10-19%	M3H67 (LICR)	[65] and unpublished data

With the exception of Her2 antigen, for which results will be used from routine clinical testing, IHC procedures follow standard techniques as previously published by our group with collaborator Dr Jungbluth [66], an example IHC stain is shown for NY-ESO-1+ breast cancer), antigens expressed in at least 10% of tumor cells will be selected for T cell monitoring for a given patient.



2 Study Objectives

2.1 **Primary Objective**

To estimate the median PFS and PFS rate at 3 months with the addition of immunomodulatory doses of cyclophosphamide to exemestane in patients with ER and/or PR-positive, Her2-negative metastatic breast cancer who progressed on endocrine therapy with an aromatase inhibitor, fulvestrant and/or tamoxifen.

Patients treated with exemestane alone in a second line setting have a median PFS of 2.8 months; whereas patients treated with a combination of exemestane and everolimus have a median PFS of 6.9 months with added toxicity based on the randomized Bolero 2 trial data. For the current trial a target 3-month PFS of $\geq$ 75% (based on the assumption of an exponential survival distribution and median PFS of 6.9 months) is considered promising. Based on the data from Bolero 2 summarized above, the estimated PFS at 3 months on the combination regimen is 75% assuming that the distribution of progression free survival is exponential. [Calculations from PASS, NCSS, 2008, J. Hintze, Kaysville, Ut.].

2.2. **Secondary Objectives**

a. To estimate the response rate of the combination of exemestane and cyclophosphamide.

   Published data suggest that patients treated with exemestane alone or in combination with everolimus in a second line setting have a RR of less than 10 %.

b. To estimate the clinical benefit rate (defined as objective response plus stable disease for at least 24 weeks) of the combination of exemestane and cyclophosphamide.

c. To determine the adverse event profile in patients treated with exemestane and cyclophosphamide (CTCAE v 4.0).

2.3. **Translational Objectives (if additional funding available)**

i. To assess changes in circulating T regulatory effector T cells and innate subsets during combination treatment with exemestane and immunomodulatory cyclophosphamide.

ii. To measure changes in tumor antigen-specific T cells from baseline and during combination treatment with exemestane and immunomodulatory cyclophosphamide.

iii. To assess if tumor-infiltrating T regulatory lymphocytes in archival paraffin embedded tumor specimens from either the primary tumor or metastatic disease is predictive of PFS.

3 Study Design

3.1 General Design

This is a Phase II open label non-randomized single arm trial of exemestane and cyclophosphamide to estimate the median PFS and the PFS rate at 3 months in patients with metastatic ER/PR positive, HER2 negative breast cancer who progress on at least one line of endocrine therapy. The median progression-free survival estimated from the Bolero 2 trial for a single agent exemestane is 2.8 months, whereas the combination exemestane + everolimus achieved a median PFS of 6.9 months but added significant toxicity. For the current trial a target 3-month PFS of $\geq$ 75% (based on the assumption of an exponential survival distribution and median PFS of 6.9 months) is considered promising. With a single stage design, we can reject the null hypothesis that the progression-free survival rate at 3 months is 50% or less versus that alternative that the progression free survival rate at 3 months is 75 % or greater with a alpha of 0.05 and power of 80% with 23 patients enrolled over 2-2.5 years. If the number of patients surviving progression-free at 3 months is 15 or fewer, then the regimen will be rejected.

3.2 Primary Study Endpoints

The primary endpoint is Progression-free survival (PFS). PFS is defined as the time from first treatment day until objective disease progression or death from any cause. Assessment of disease progression (eg, tumor measurements as per RECIST 1.1) will be performed every 12 weeks on study.

3.3 Secondary Study Endpoints

Response rates and clinical benefit rates will be estimated using exact 95% confidence intervals. Changes from baseline in circulating T regulatory cells during combination treatment and in tumor antigen-specific T cells during treatment will be summarized using descriptive statistics at each time point and mixed effects regression models to incorporate repeated observations within a patient and missing data.

3.4 Safety Endpoints

Adverse events will be recorded along with grade (CTCAE v 4.0), attribution, onset, duration, outcome and treatment if indicated. The proportions of patients with various type/grade of adverse events will be summarized and reported.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

4.1.1 Patients must have histologically confirmed breast cancer that is ER positive and/or PR positive, and HER2/neu negative and have disease that is metastatic (stage IV [$T_{any}N_{any}M1$])

- HER2/neu negative disease determined using commercially available/approved assay in local institutional or reference laboratory, according to ASCO/CAP guidelines (IHC 0-1+ or 2+ with HER2/17 ratio on FISH $\leq$ 1.8).
- ER/PR expression performed by standard immunohistochemical assay and classified as ER and/or PR-positive according to ASCO/CAP guidelines (1-100% expression)
- Histologic and/or cytologic confirmation of metastatic disease is encouraged whenever feasible, furthermore, if feasible, the biopsy should confirm that the metastatic tumor is ER and/or PR positive and HER2/neu negative. For patients in whom histologic biopsy confirmation and/or assessment of ER/PR/HER2 of metastatic disease is not feasible, it is required that the primary tumor be ER and/or PR-positive and HER2/neu negative.

4.1.2 Measurable disease (RECIST 1.1) or non-measurable (assessable) disease

4.1.3 Patients must have had progressive disease during at least one line of endocrine therapy for metastatic disease or have recurrent disease while or within 12 months of receiving adjuvant endocrine therapy. Prior treatments accepted include a non-steroidal aromatase inhibitor, tamoxifen, fulvestrant or combinations.

4.1.4 Patients taking bisphosphonates for bone disease are permitted to enter the trial, but their bone lesions are not considered to be assessable for response, although they are assessable for progression.

4.1.5 Female or male subjects age $\geq$ 18 years.

4.1.6 ECOG performance status 0, 1, or 2.

4.1.7 Patients must have normal organ and marrow function as defined below:

– absolute neutrophil count	$\geq$1,200/mcL
– platelets	$\geq$100,000/mcL
– hemoglobin	$\geq$ 9g/dl
– total bilirubin	$\leq$2 X ULN [unless due to Gilbert's disease]
– AST(SGOT)	$\leq$2.5 X ULN
– ALT(SGPT)	$\leq$2.5 X ULN
– creatinine	$\leq$ 1.5 X ULN

4.1.8 Patients must be able to swallow and tolerate oral medications.

4.1.9 Postmenopausal status, defined as 60 years and older, being 45 years and older and having amenorrhea x 12 months or follicle stimulating hormone levels within postmenopausal range, OR having undergone a bilateral oophorectomy.

4.1.10 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Patients may not be receiving any other investigational agents.

4.2.2 Prior treatment for breast cancer with a steroidal aromatase inhibitor; with the exception of patients who were started on the combination of exemestane with everolimus less than 4 weeks prior to study entry and discontinued everolimus due to poor tolerability.

4.2.3 Presence of life threatening metastatic visceral disease (defined as extensive hepatic involvement or symptomatic pulmonary lymphangitic spread) or uncontrolled brain metastases.

4.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.3 Subject Recruitment and Screening

Patients will be recruited from physicians at the NYU Medical Center and Bellevue as well as through outside referrals. Consenting, screening and treatment takes place at the NYU Clinical Cancer Center and Bellevue Cancer Center under the supervision of the PI. Prospective subjects receive detailed information about this study and its investigational nature, required study procedures, alternative treatments, risks and potential benefits of the study. They also receive the informed consent document to read. All questions are answered by the PI and qualified research personnel.

Recruitment and consenting will take place in a private area such as an exam room to protect the patient's privacy. The informed consent process and documentation follows the established procedures of the NYUCI Clinical Trials Office.

4.4 Early Withdrawal of Subjects

Subjects may be withdrawn from the study prior the expected completion of that subject for the following reasons:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations
- Intercurrent illness that prevents further administration of treatment
- Disease progression
- Unacceptable adverse events regardless of grade
- Termination of study
- Subject consent withdrawal
- Decision by the Investigator that termination is in the patient's best medical interest, for instance for significant disease progression
- Unrelated medical illness or complication

5 Study Drug

5.1 Description

Cyclophosphamide (former trade name cytoxan, CTX) is a synthetic alkylating agent chemically related to the nitrogen mustards, which at the low dose utilized in this study is an immunomodulatory agent.

Cyclophosphamide: Bioavailability >75% (oral)
Protein binding >60%,
Metabolism: Hepatic, CTX is metabolized by cytochrome P450 (CYP2B6, 2C9 and 3A4 (with 2A6, 2C8 and 2C19 making more minor contributions)), glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH) enzymes
Half-life: 3-12 hours
Excretion: Renal

Exemestane (trade name aromasin) is an oral steroidal aromatase inhibitor indicated for the treatment of breast cancer in post-menopausal women. It is a synthetic androgen analogue (6-methylenandrosta-1,4-diene-3,17-dione), which binds irreversibly to and inhibits the enzyme aromatase

Exemestane: Bioavailability 42% (oral)
Protein binding 90%.
Metabolism: Hepatic, exemestane is metabolized by cytochrome P 450 3A4 (CYP 3A4) and aldoketoreductases.
Half-life: 24 hours
Excretion: Renal and GI

5.2 Treatment Regimen

Treatment will be administered on an outpatient basis. A treatment cycle is defined as 4 weeks (28 days).

Exemestane: Exemestane will be given as one tablet (25mg) daily by mouth (PO). The first dose of exemestane should be given the same day as CTX. Dosing for patients on concurrent CYP3A4 inducers is detailed in section 5.6

Cyclophosphamide: Cyclophosphamide will be given as one tablet (50mg) daily by mouth (PO). The first dose of cyclophosphamide should be given the same day as exemestane.

Patients who enroll into the study who have received exemestane with everolimus and have stopped everolimus due to intolerance, may resume exemestane throughout and start CTX once toxicities have resolved to Grade 1 or less. The day CTX is started is considered day 1 of the study.

Agent	Dose	Route	Schedule	Cycle Length
Exemestane	One 25mg tablet	PO	Daily continuously	28 days
Cyclophosphamide	One 50mg tablet	PO	Daily continuously	28 days

5.3 Dose modifications

- Exemestane: no dose modifications planned
- Cyclophosphamide: discontinue cyclophosphamide for grade 3 or greater cystitis, neutropenia, anemia or thrombocytopenia. Patients are allowed to continue exemestane alone if they had to stop CTX due to toxicity

5.4 Preparation, Storage and Administration of Study Drug

Both, cyclophosphamide and exemestane are commercially available and will be prescribed by the treating physician and can be filled at a pharmacy. Patients will follow the study doctor's instruction, take exemestane 25 mg po and CTX 50 mg po daily. Both, CTX and exemestane tablets should be stored out of reach of children, at room temperature (25°C (77°F)); the temperature is not to exceed 30°C (90 F°).

5.5 Subject Compliance Monitoring

The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be reviewed by study personnel at all study visits and returned to research staff at the end of study.

5.6 Concomitant Therapy

Cyclophosphamide is minimally emetogenic when administered as a low daily oral dose. No prophylactic antiemetics are needed, however, patients may be pre-medicated with prochloperazine or an alternative antiemetic if clinically indicated.

No investigational or commercial agents or therapies other than CTX, exemestane and bisphosphonates may be administered with the intent to treat the patient's malignancy.

As both, CTX and exemestane are metabolized by cytochrome P450, physicians should be aware of possible interactions with other medicines with metabolism by CYP450 and patients should report all medications (prescription and nonprescription), herbal remedies, and vitamins to the study team.

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6 Per the January 2013 exemestane package insert the recommended dose of exemestane for patients receiving a potent CYP3A4 inducer such as rifampicin or phenytoin is 50mg po daily. No significant effect on pharmacokinetics of exemestane is expected for inhibitors of CYP3A4 such as ketoconazole. **Study Procedures**

6.1 STUDY CALENDAR

The following guidelines will be followed:

- Screening/baseline evaluations are to be conducted within 6 weeks prior to start of therapy.
- Tumor evaluation (eg, tumor measurements as per RECIST 1.1 by CT scan) will be performed every 12 weeks on treatment, or earlier if medically indicated. It is preferred but not mandated to use the identical imaging technique throughout the study period.
- Each treatment cycle is 4 weeks (28 days). The study calendar shows only 3 cycles, study procedures of subsequent cycles are identical (except research bloods are optional). Patients should continue treatment until disease progression, prohibitive toxicity, or other reasons listed in section 4.4.

	Pre-Study	Wk 1	Wk 5	Wk 9	Wk 12
		Cycle 1	Cycle 2	Cycle 3	
Informed consent	X				
Concurrent meds	X	X	X	X	
History/physical exam, ECOG PS	X	X	X	X	
CBC/Diff, Liver/renal function[a]	X	X[c]	X	X	
Exemestane		Continuous daily			
Cyclophosphamide		Continuous daily			
Review of treatment diary		X	X	X	
Adverse event evaluation		Throughout			
Tumor measurement[d],	X				X
CA 27-29		X			X
Baseline formalin preserved paraffin embedded biopsies (archived)	X				
Research bloods (if funding available), max 40cc per time point [b]		X	X		X

[a] Mandatory: total bilirubin, AST, ALT, creatinine, BUN

[b] Immune correlates: see section 6.3, for patients on trial > 3months, only required at the 6, 9, 12, 15 mos time points

[c] Not required if screening labs done within 2 weeks

[d] Imaging modality depending upon metastatic sites (see section 6.2.; CT scan, bone scan, PET/CT, MRI, clinical measurement)

6.2 . MEASUREMENT OF ANTITUMOR EFFECT (by RECIST 1.1)

For the purposes of this study, patients should be re-evaluated for response every 12 weeks. Confirmatory scans should be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. **Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used** in the RECIST criteria.

6.2.1 **Definitions**

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.2.2 **Disease Parameters**

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Target lesions. **All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters

will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.2.3 Methods for Evaluation of Measurable Disease

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, for this study the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.2.4 **Response Criteria**

6.2.4.1 **Evaluation of Target Lesions**

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

6.2.4.2 **Evaluation of Non-Target Lesions**

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.2.4.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is

				Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.2.5 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.2.6 **Progression-Free Survival**

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.3. Correlative studies

Subjects and sample processing: Blood samples (20-40 ml) will be processed at the Unutmaz laboratory to isolate PBMC using Ficoll separation as previously described. The PBMC will be counted and viable cells will be frozen in 2-3 vials using freezing media and will be stored at -140 degrees along with serum for the following analysis. Samples will be stored for the intended immune analyses only, which may include improving methods for analyzing the patient's immune response, for instance by culturing immune cells. Samples will not be used nor stored for other research purposes.

Immune profiling panels:

One vial of cells will be thawed and stained with fluorescently conjugated antibodies for flow cytometry (FACS) analysis for immune profiling, using LSR-II FACS machine at Unutmaz laboratory. The data will be analyzed using Flowjo software.

1. T cell differentiation and activation panel: To identify following T cell subsets:
a) Naïve, central memory, effector and terminally differentiated and follicular helper subsets of CD4 or CD8+ T cell subsets
b) The proportion of T cells that express activation and differentiation markers CD38, PD1 and HLA-DR and CD57.

2. Chemokine receptor panel: To determine the proportion of T cells, including Tregs, with essential chemokine receptors, involved in tissue homing. Each chemokine receptor identifies tissue homing as well as T cells with different effector functions. For example, Th1 cells express higher CCR5 and CXCR3, whereas Th2 cells are higher in CCR4 expression. Cells expressing CLA home to skin, whereas CCR6+ cells home to mucosal tissues and contain most of the Th17/Th22 functional effector T cells.

3. Treg/Th17 cell subset panel: PBMC will be stained with T cell marker CD3, CD4 and memory marker CD45RO and following Treg markers. In addition cells will be stained with newly identified markers that define novel subsets of Tregs and Th17 cells. In this panel we perform stainings for intracellular transcription factors Foxp3 and Helios to identify Treg cells together with a viability dye to discriminate dead cells.

4. Treg differentiation and activation panel: In this panel we determine recently activated and cycling Treg and other T cell subsets, by using a cell cycle protein called Ki67 in conjunction with FoxP3, Helios and T cell activation markers.

5. Myeloid and dendritic cell panel: This panel identifies several myeloid lineages:
a) Monocytes
b) Two different dendritic cells subsets (CD1c+ and CD1c-)
c) Plasmacytoid DCs, which secrete IFN alpha
d) Myeloid suppressor cells (monocytic and granulocytic)

6. NK cells, gd-T cells and mucosa homing T cells panel: The goal of this panel is to determine the proportion of non-classical T cells and NK cells:
a) NK cells (both CD16+/- CD56+/- subsets)
b) Gammadelta T cells
c) Mucosal associated invariant T (MAIT) T cells
d) invariant Natural Killer T cells (iNKT), which recognize CD1d molecules

Potential pitfalls and Quality control: All of these panels are well-established in the Unutmaz laboratory and are routinely performed on hundreds of human samples. Every antibody in each of the panels is extensively tested to ensure optimal staining and reproducibility. We do not anticipate any problems. For quality control, in each staining we utilize one known donor for frozen in aliquots for each batch of staining to ensure quality of antibodies and FACS machines.

Analysis: Percent of each lymphoid and myeloid subset will be determined using FlowJo FACS software and entered into spreadsheets. The statistics and patient correlative data will be performed by analyzing the same donors before and after CTX treatment. We will first determine statistical changes in the proportion of Tregs and other subsets analyzed above before and after CTX-treatment. In the second layer analysis we will develop signatures that correlate with the effectiveness of the treatment or impact on specific regulatory or effector compartments, by stratifying patients at the end of the study.

Examination of tumor antigen-specific CD4 and CD8 T cell responses

T cell responses to the expressed tumor antigens will be monitored by intracellular cytokine staining (ICS) utilizing overlapping peptide (OLP) pools, as previously described by our group and others [67-69]). This method is suited for analysis of cryopreserved PBMCs, allows the detection of CD4 and CD8 T cell responses (optimized by use of 15-mer peptides with an overlap by 11 amino acid residues) and an assessment if multiple cytokines are being secreted by single cells. If antigen-specific T cells are detected, confirmatory analysis (ELISA in serum and/or IHC for protein expression in tumor) may be performed. If outside laboratories (for instance MSKCC, MSSM) are used, samples will be sent numbered, without personal identifiers and no PHI will be shared.

Antigen	Expression in breast cancer	IHC mAb (Source)	References
PRAME	27-53%	AB32185 (Abcam)	[58, 59]
WT-1	33-87%	6F-H2 (Dako)	[60, 61]
Her2	24%	Commercial, FISH for 2+	[62]
NY-ESO-1	5-42%	E978 (LICR)	[63, 64] and unpublished data
MAGE A3	10-19%	M3H67 (LICR)	[65] and unpublished data

Method: Peripheral blood mononuclear cells (PBMCs) are purified from blood and frozen (by the Unutmaz lab) for paired analysis of *ex vivo* and, if necessary, *in vitro* stimulated (IVS [67]) T cell responses. For the *ex vivo* assay, thawed pre- and post-treatment PBMCs are washed in complete R-10 medium supplemented with 20 IU/ml DNase I (Roche) and cultured overnight at 37°C. The following day, the number of viable cells are counted, 5 million viable cells/ml in complete R-10 medium are plated at 200 ml/well in 96-well V-bottom plates in the presence of 1 mcg/ml anti-CD28 and anti-CD49d antibodies (BD Biosciences), and either 1 mcg/ml TAA OLP (PRAME, WT-1, Her2, NY-ESO-1, MAGE A3; Proimmune) or control antigen (ProMix CEF and ProMix MOG; ProImmune) or no antigen. PMA/Ionomycin will serve as an additional positive control. A mixture of Brefeldin A and Monensin (GolgiPlug and GolgiStop, BD Biosciences) is added to each well after an 1 hour culture, before culturing for an additional 5 hours. Samples are then washed with PBS, stained for 20 min at room temperature with anti-CD8 PerCP-Cy5.5 and anti-CD4 FITC antibodies (BD Biosciences) and LIVE/DEAD violet (Invitrogen), washed again with PBS, then fixed and permeabilized for 20 min at room temperature using Cytofix/Cytoperm solution (BD Biosciences). Samples are then washed using Perm/Wash solution (BD Biosciences) and stained for intracellular antigens using anti-IFNg AlexaFluor 700 (BioLegend), anti-TNFa PE-Cy7, anti-IL-2 PE and anti-CD3 APC-H7 (BD Biosciences) antibodies for 20 min at room temperature. Samples are washed once with Perm/Wash solution, and acquired on a BD LSR II flow cytometer. 7-color compensation (parallel controls using cells singly stained for each color) and data analysis are done with FlowJo flow cytometry analysis software (TreeStar). Boolean analysis is used determine the percentage of single, double and triple cytokine-producing CD4 and CD8 T cells. The induction or boosting of TAA-specific T cell immunity for an individual patient is defined as a post-treatment value at week 9 at least 3-fold higher than baseline that is also 3-fold or greater than parallel negative controls (and at least 0.03) [67].

6.4 Follow Up

Following study completion, patients will enter routine follow-up with their primary oncologist, who may be contacted to provide follow-up information on the patient's clinical and disease status. Patients removed from study for adverse events will be followed until resolution or stabilization of the adverse event.

7 Statistical Plan

7.1 Sample Size Determination

This is a Phase II open label non randomized single arm trial of exemestane and cyclophosphamide to estimate the 3-month PFS in these patients. Based on the data from Bolero 2 summarized above, the estimated PFS at 3 months on the combination regimen is 75% assuming that the distribution of progression free survival is exponential. [Calculations from PASS, NCSS, 2008, J. Hintze, Kaysville, Ut.].

With a single stage design, we can reject the null hypothesis that the progression-free survival rate at 3 months is 50% or less versus that alternative that the progression free survival rate at 3 months is 75 % or greater with an alpha of 0.05 and power of 80% with 23 patients enrolled over 2-2.5 years. If the number of patients surviving progression-free at 3 months is 15 or fewer, then the regimen will be rejected.

7.2 Statistical Methods

Patient characteristics at baseline will be summarized using descriptive statistics (for quantitative variables, mean, median, quartiles, standard deviations, etc and graphical displays including boxplots; for qualitative variables, proportions and frequency distributions). The primary endpoint is Progression-free survival (PFS). PFS is defined as the time from first treatment day until objective disease progression or death from any cause. PFS will be estimated by the Kaplan-Meier method. Analyses will be based on the intent-to-treat population. Patients who drop out prior to disease progression (eg due to toxicity, non-compliance, or loss to follow-up) will be censored at the time of last study visit.

With 23 patients, the exact 95% confidence interval for the 3-month PFS will be estimated from the Kaplan Meier curve.

7.3 Subject Population(s) for Analysis

All patients who have received at least one dose of study drug will be included in the analyses of efficacy and safety.

8 Safety and Adverse Events

8.1 Expected Adverse Events

<u>Cyclophosphamide</u>

Side effects vary significantly based on the specific dose and duration of cyclophosphamide. The following events have been reported to occur in association with cytotoxic doses of cyclophosphamide:

A. Incidence More Frequent
1. Anemia, leucopenia, thrombocytopenia
2. Alopecia
3. Anorexia, nausea and vomiting
4. Gonadal suppression (azoospermia, missed menstrual periods) possibly resulting in infertility. Only applicable to men for this study as women are postmenopausal.
5. Hemorrhagic cystitis,
6. Infection

B. Incidence Less Frequent
1. Stomatitis

C. Incidence Rare
1. Anaphylaxis (tachycardia, shortness of breath, wheezing, tightness in throat)
2. Flushing or redness of face
3. Diarrhea
4. Skin rash, pigmentation changes in skin and nails
5. Pneumonitis or interstitial pulmonary fibrosis
6. Syndrome of inappropriate antidiuretic hormone (SIADH)
7. Secondary malignancies
8. Blurred vision
9. Cardiac toxicity (congestive heart failure, mycodarditis, pericarditis)
10. Ovarian fibrosis
11. Hemorrhagic colitis, oral mucosal irritation, jaundice
12. Malaise and asthenia

Exemestane

A. Incidence More Frequent
1. Fatigue, hot flashes, pain, malaise
2. Edema (peripheral edema, leg edema)
3. Increased sweating
4. Depression, insomnia, anxiety
5. Dizziness, headache
6. Nausea, vomiting, abdominal pain, anorexia, constipation, diarrhea, increased appetite
7. Dyspnea
8. Cough
9. Hypertension
10. Pain at tumor sites

B. Incidence Less Frequent
1. Fever
2. Generalized weakness, paresthesia, asthenia, hypoesthesia
3. Pathological fracture
4. Bronchitis, sinusitis, pharyngitis, rhinitis
5. Rash, itching
6. Urinary tract infection, upper respiratory tract infection
7. Lymphedema
8. Chest pain,
9. Confusion
10. Dyspepsia
11. Arthralgia, back pain, skeletal pain
12. Alopecia

C. Incidence Rare
1. Stroke
2. Heart failure

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, the investigators brochure, 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 adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality: 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 or 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 is: Fatal, life-threatening, requires or prolongs hospital stay, results in persistent or significant disability or incapacity, a congenital anomaly or birth defect or an important medical event.

Adverse Event Reporting Period
The study period during which adverse events must be reported is defined for this study as the period from the first day of study treatment until 30 days following the last administration of study treatment.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event 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 or the abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event 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 adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.3 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF).

All adverse events 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. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome.

8.4 Reporting of Serious Adverse Events and Unanticipated Problems

8.4.1 Investigator reporting: notifying the IRB

This section specifies the NYULMC IRB requirements for investigator reporting of unanticipated problems posing risk to subjects or other, including adverse events. The IRB requirements reflect the current guidance documents released by the Office of Human Research Protections (OHRP), and the Food and Drug Administration (FDA) and are respectively entitled "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" and "Guidance for Clinical Investigators, Sponsors, and IRBs: Adverse Event Reporting – Improving Human Subject Protection."

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events* that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation). Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

8.5 Unblinding Procedures

N/A

8.6 Stopping Rules

N/A

8.7 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10). Medical monitoring will include a regular assessment of the number and type of serious adverse events. The independent medical monitor assigned to the trial (Dr. A. Pavlick) is able to review all unexpected events and to evaluate/grant any waivers required for enrollment of study conduct in accordance to institutional guidelines.

8.7.1 Data Monitoring Committee

This investigator initiated study will be monitored by the Data Safety Monitoring Committee (DSMC) of the New York University Cancer Institute (NYUCI). The DSMC operates based on the 2011 National Cancer Institute approved Charter. It is a multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses and research administration staff knowledgeable of research methodology and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for clinical trials conducted in the NYUCI that are not monitored by another institution or agency. The DSMC reports to the Director of the NYUCI (William L. Carroll, M D). Per the NYUCI Institutional Data Safety and Monitoring Plan, this phase 2 trial will be monitored by DSMC annually (from the date the first patient is enrolled) and at the completion of the study prior to study closure. This review includes accrual data, subject demographics and adverse events. Principal Investigators are required to attend the review of their studies. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc.

9 Data Handling and Record Keeping

9.1 Confidentiality

The study team will maintain clinical and laboratory data in a designed manner to ensure patient confidentiality. All study personnel have passed human subject protection courses. If applicable, tissue samples sent to collaborators outside of NYU/Bellevue will only be labeled with an assigned protocol-patient identification number without patient identifiers. Systems used for electronic data capture are compliant with FDA regulations in 21 CFR Part 11 and applicable local regulatory agency guidelines. All documents are kept in strictly confidential files and are only made accessible for review of sponsors, monitors and authorized representatives of regulatory agencies as described in the informed consent document.

9.2 Confidentiality and HIPAA

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.

9.3 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, subjects' diaries or evaluation checklists, pharmacy dispensing records, 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, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.4 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.

9.5 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the formal closure of the study.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The proposed trial entails moderate risks to subjects.

At the NYU Cancer Institute, all investigator-initiated protocols are subject to a standardized data and safety monitoring, which includes scientific peer review, IRB review, Phase I/II committee review and DSMC review as well as internal auditing.

The review of AEs and trial conduct for this trial occurs at several levels:

(1) Principal Investigator: Adverse events are evaluated monthly by the principal investigator in conjunction with the research nurses, data manager and research team.

(2) Internal Phase I/II Committee: Study progress, enrollment and AEs are also reviewed monthly by the NYU Phase I/II Committee. This review includes nurses and data managers as well.

(3) DSMC: See 8.7.1.

(4) Institutional Review Board (IRB): An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with demographics, protocol violations, and current status of subjects as well as available research data.

(5) In addition, the internal audit committee will inspect the source documents, including consent forms for randomly selected enrolled participants at regular intervals throughout the trial to verify adherence to the protocol; the completeness, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to the NYU Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

The consenting process and documentation will follow Standard Operating Procedure no 30 (Obtaining Informed Consent for Clinical Trials) of the NYUCI CTO.

12 Study Finances

12.1 Funding Source

This investigator-initiated trial is supported by the NYU Cancer Institute .

12.2 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 NYULMC investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

N/A

13 Publication Plan

The study PI holds the primary responsibility for publication of the results of the study.

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