A phase IIB study of neoadjuvant ZT regimen (Enzalutamide therapy in combination with weekly paclitaxel) for androgen receptor (AR)-positive triple-negative breast cancer

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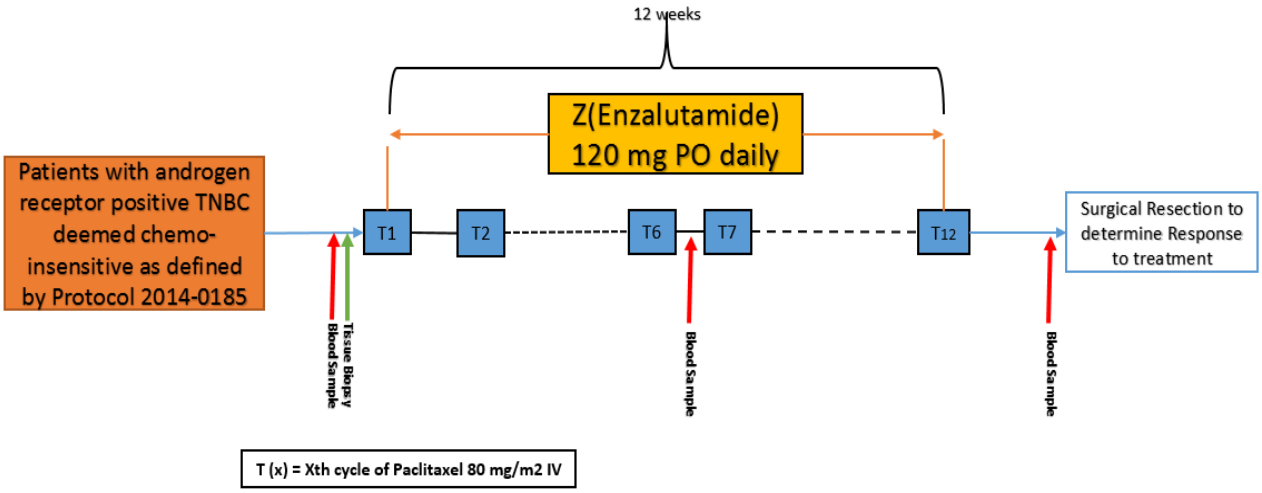
Table of Contents:

Study Schema



1. Introduction

Patients with localized TNBC are preferably treated with systemic chemotherapy in the neoadjuvant setting (NACT) as this allows for close monitoring of response in the intact primary tumor and often results in 'down staging' of tumors, which increases the feasibility of breast conserving surgery. The response to NACT at the time of surgical resection can be determined by measuring the amount of residual cancer remaining in the breast and draining lymph nodes during routine pathologic evaluation and is such a powerful indicator of prognosis that the Food and Drug Administration (FDA) has recognized significant improvement in complete response to neoadjuvant therapy as a pathway to drug approval. Investigators at MDACC have developed and validated a scoring system known as the Residual Cancer Burden (RCB) to quantify the extent of residual disease remaining after NACT and surgical resection.[2] Approximately 50% of patients with localized TNBC treated with standard taxane/anthracycline-based NACT will have either pathologic compete response (pCR/RCB-0) or minimal residual disease (RCB-I) at the time of surgical resection and those patients have identical and exceptionally good long-term prognosis with less than 10% risk of developing distant metastatic disease within 5 years. Unfortunately, those with more extensive residual disease (RCB-II or RCB-III) have a much worse prognosis, with 50%-80% of patients developing distant metastatic disease within 3 years of initial diagnosis.[3] Additionally, clinical trials of NACT in breast cancer have demonstrated that patients without response to their first chemotherapeutic regimen have very low chance (5%) of achieving pCR after their second neoadjuvant chemotherapy regimen.[4] However, this has not been the case with targeted regimens such as trastuzumab in HER2+ tumors, suggesting that intrinsic resistance to chemotherapy can be overcome with appropriate targeted therapy.[5] Though several targeted agents have been tested for treatment in TNBC, so far none have been successful. The underlying causes of this failure are commonly attributed to the molecular heterogeneity of tumors classified within the 'catch all' category of TNBC as well as the dilution effects of chemotherapy sensitive disease which requires clinical trials to enroll larger number of patients to show benefit of combined targeted therapy/chemotherapy regimens over standard chemotherapy. Additionally, clonal selection of resistant cells with escape mechanisms also likely develops. Recent advances in gene expression profiling have identified subgroups of triple- negative breast cancer (TNBC) with distinct molecular features that, if appropriately selected, may be more responsive to targeted therapy with existing FDA-approved drugs, leading to rapid improvement of outcomes in this high-risk breast cancer population[1, 6-8].

Prediction of chemosensitivity and molecular aberrations associated with TNBC subtypes: Our collaborator, Dr. Symmans, and his team have developed microarray-based predictive signatures of chemotherapy response using fresh primary tumor tissue obtained from a routine biopsy of breast cancer at the time of diagnosis or surgery. This approach has been retrospectively validated and tested for feasibility using a prospective registry study (MDACC clinical trial 2011-0007).[9] The proposed predictor was tested on an independent cohort of breast cancers irrespective of receptor status (N=198, 99% with clinical Stage II-III) who received neoadjuvant (N=180) or adjuvant (N=18) taxane-anthracycline chemotherapy. Predictions were accurate if ER+/HER2- (30% predicted sensitive, Distant relapse-free survival (DRFS) 97%, CI 91-100; Absolute risk reduction (ARR) 11%, CI 0.1-21) or ER-/HER2- (26% predicted sensitive, DRFS 83%, CI 68-100; ARR 26%, CI 4-48) (Fig 1).[9] Predicted treatment sensitivity was significantly associated with lower relapse risk (HR 0.19, CI 0.07-0.55) in a multivariate model including ER status, tumor and nodal stage, grade, age, and type of taxane treatment (paclitaxel or docetaxel).

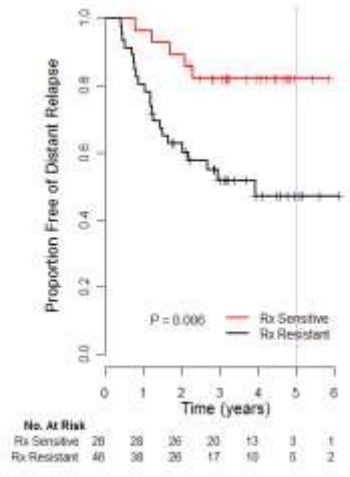


Fig 1: Distant RFS by genomic prediction of chemosensitivity in ER-/HER2- breast cancer

As important as predicting chemosensitivity may be, outcomes for TNBC patients will not be improved unless targeted therapy is developed for patients with chemoresistant disease. Recent advances in molecular profiling have identified subsets of TNBC (Fig. 2) with distinct molecular features targetable with existing FDA approved drugs.[1, 7, 10, 11] Basal-like TNBCs (BL1 and BL2) are heavily enriched in cell cycle and cell division pathways. Gene expression profiling has revealed that these tumors have high expression of proliferation genes such as aurora kinases, *MYC*, *NRAS* and *PLK1* as well as elevated DNA damage response (BL1 subtype). These tumors may have higher rates of pCR to standard chemotherapy compared to other TNBC subtypes. The BL2 subset displays gene ontologies involving growth factor signaling such as EGFR and IGF1R pathways. Cell lines classified as basal-like had high rates of sensitivity to cisplatin and relative resistance to PI3K directed therapy in both cell culture and xenograft models. Though most triple-negative breast cancers are classified as basal-like (BL1 and BL2) by microarray analysis, approximately 30% of triple negative tumors are molecularly distinct from basal-like breast cancers as they display mesenchymal features, including enrichment in epithelial-to-mesenchymal transition (EMT) and stem-cell like characteristics. Subgroups with these features have been identified by independent investigators and have been termed mesenchymal (M), mesenchymal-stem cell like (MSL) and claudin-low. Mesenchymal-like TNBCs carry a high rate of molecular aberrations that activate the PI3K/Akt/mTOR axis suggesting that this subgroup may be responsive to therapeutic regimens targeting this pathway. Approximately 10-15% of TNBC are associated with expression of androgen receptor (AR+ or LAR) and approximately 20-25% are immune modulatory (IM), expressing genes associated with immune activation. Approximately 10-20% of patients with TNBC are carriers of germline BRCA1/2 mutations. When treated with NACT, pCR rates similar to slightly higher than non-carriers.[12] BRCA1 and BRCA2 are required for homologous recombination repair of DNA strand breaks leading to a defect in DNA repair in cancers harboring such mutations. As such, these tumors may be more sensitive to chemotherapy inducing DNA breaks or poly ADP-ribose polymerase (PARP) inhibitors.

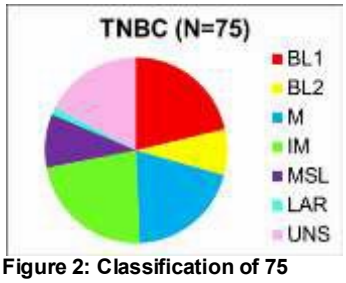


Figure 2: Classification of 75 TNBC samples at MDACC using signatures published by Pietenpol et al.[1]

Notably, investigators at MDACC also determined that rates of pCR/RCB-I to standard NACT were not statistically different amongst the subtypes, though the lowest rates of pCR were seen in the BL-2 subtype and the highest rates of RCB-III disease was within the mesenchymal subtypes, further validating the need for both predictors of chemotherapy sensitivity as well as molecular characterization of predicted chemotherapy-insensitive disease to determine appropriate therapy for patients with localized TNBC.[13]

Given the disparity of treatment outcomes from NACT for TNBC, a molecular triaging protocol (MDACC 2014-0185) has been developed and IRB approved as a diagnostic platform to identify patients whose tumors are likely or unlikely to achieve pCR or RCB-I, in order to direct predicted responders toward standard chemotherapy and to direct predicted chemotherapy insensitive patients toward potentially more effective experimental therapies within clinical trials (Figure 3). Previously untreated patients with localized TNBC who are candidates for standard NACT (anthracycline →taxane based therapy) will be enrolled into 2014-0185, a non-therapeutic, randomized trial where they will undergo biopsy of the primary

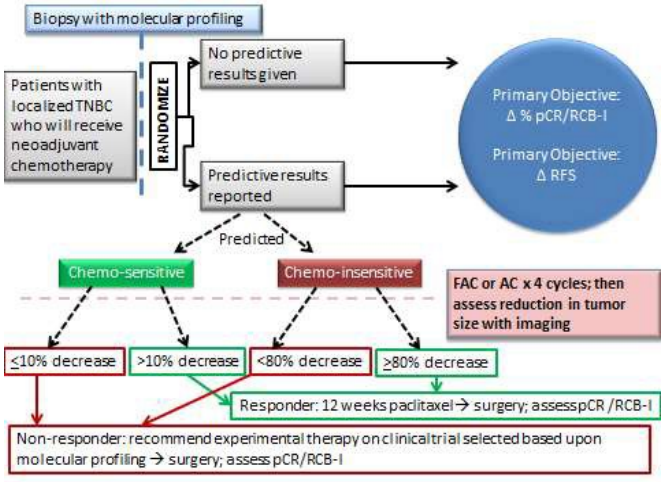


Fig. 3 Clinical Trial Schema for 2014-0185

tumor for molecular characterization within the MDACC CLIA certified diagnostic lab, including predicted sensitivity to chemotherapy using gene signatures described above. Patients will be randomized 2:1 to know the results of the molecular characterization (given within 4-6 weeks after biopsy) or not know the results (treating physician blinded as well). *All patients will be treated the same except for this randomization (Figure 3).*

All patients will then initiate a planned 4 cycles of standard neoadjuvant anthracycline based chemotherapy based upon physician's choice (AC, dose-dense AC, FAC, EC or FEC are all allowed) with assessment of response by diagnostic imaging after 4 cycles of therapy (or at the time of progression in patients who develop clinical evidence of disease progression on chemotherapy). In our retrospective analysis of molecularly characterized patients with available diagnostic imaging, approximately 10-15% of patients will have obvious, extreme discordance between volumetric measurement of tumor response by ultrasound after 4 cycles of NACT and response prediction by gene signature with the diagnostic imaging best predicting outcome in these extreme cases of response or lack thereof (parameters outlined in Fig. 3). As such, patients with discordant imaging/molecular predictor results will be advised of these results and counseled to proceed based upon imaging. After response assessment, all patients will be offered the option of continuing forward with standard taxane (paclitaxel or docetaxel) or participation in clinical trials (Figure 4). Molecular characterization of the tumor (if known) can also be used by the treating physician to select for clinical trials within the BMO neoadjuvant clinical trials portfolio (Figure 4).

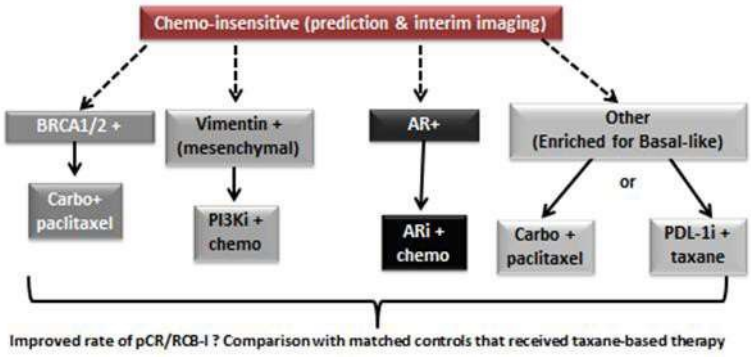


Figure 4: BMO Neoadjuvant Portfolio for Treatment of Chemotherapy Insensitive TNBC

Additionally, because of the 2:1 randomization in 2014-0185 (Figure 3), a subgroup of patients molecularly profiled will not know the results for decision making (control arm). Like the experimental arm, patients on the control arm will be allowed to choose between study participation or to receive standard chemotherapy with paclitaxel based regimen for 12 additional weeks. Essentially, those who do not know the results of the molecular profiling are offered the same options as those who do, they simply will not have the molecular analysis to guide their decision making. Since all patients enrolled in 2014-0185 have biopsies for molecular characterization and diagnostic imaging, for research purposes all patients can be characterized as chemosensitive or chemoresistant and further subtyped into the TNBC molecular subtypes mentioned above. As such, those patients characterized with chemoresistant, subtype specific disease who receive standard NACT with taxane-based regimens can be compared with matched patients treated on clinical trials with targeted agents, such as Enzalutamide. Though this comparison will not be a randomized comparison, it will provide evidence as to whether patients treated on a single arm phase II trial of targeted therapy derive additional clinical benefit and the comparison can be used to provide more robust data for confirmatory phase III clinical trial design. Likewise, patients whose tumors do not have luminal androgen receptor (LAR) features can be enrolled on this protocol and receive Enzalutamide, which will allow a control group of non-LAR TNBCs to serve as a control to determine the predictive value of potential biomarkers to define the mesenchymal population.

Rationale for Enzalutamide in luminal androgen receptor TNBC: Androgen receptor (AR) is a steroid hormone and is positive in 10-50% of TNBC patients by IHC. [14-16] Based on the molecular features, 20 out of 130 TNBC patients (15%) are classified to LAR. Among the seven

molecular subtypes, based on their gene profiles, LAR is the most distinct in the sense that its gene ontologies are heavily enriched in hormonally regulated pathways including androgen/estrogen metabolism regardless of a negative IHC for estrogen receptor.[1, 13] LAR has other distinguishing clinical characteristics as well. Our previous data shows that the pCR rate differed according to molecular subtype within TNBC and that the LAR subtype is associated with a low pCR rate (0.1%; 95% CI: 0.003-0.23) but with a high RCB-I rate, however, the survival outcome is relatively better compared to the other subtypes such as IM, MSL, or BL2.[13] This characteristic of low pCR rate and yet better survival outcome is similar to that of hormone receptor positive breast cancer. As a supporting data, genome-wide transcript analysis using DNA microarrays shows that a subset of ER/PR-negative cancer resembles ER-positive breast cancer, and this subtype contains AR-responsive genes. Thus, therapies targeting AR could be utilized to treat ER/PR-negative breast cancer with such characteristics.[17] In vitro experiment using MDA-MB-453 cell line, which molecularly resembles to ER positive breast cancer based on the clustering results, it is demonstrated that cell line incubation with only antiestrogens had no effect on overall cell viability. In the experiment using the same cell line, incubation with a synthetic androgen (R-1881) stimulated growth and this proliferative effect was abrogated by the addition of the AR antagonist flutamide, confirming that the response was AR dependent and ER independent. [17] These data suggest that therapeutic AR blockade warranted consideration as a potential endocrine therapy for the subset of patients with ER negative breast cancer.[18]

In the previous Phase II study investigating the efficacy of Bicalutamide in patients' estrogen receptor and progesterone receptor negative, androgen positive (IHC >10% nuclear staining) metastatic breast cancer, the 6-month clinical benefit rate was 19% (95% CI, 7-39%), and the median PFS was 12 weeks (95% CI, 11-22 weeks).[19] Enzalutamide is an androgen-receptor–signaling inhibitor and is distinct from the currently available antiandrogen agents in that it inhibits nuclear translocation of the androgen receptor, DNA binding, and coactivator recruitment. It also has a greater affinity for the receptor, induces tumor shrinkage in xenograft models (in which conventional agents only retard growth), and has no known agonistic effects. [20, 21]

Of note, preclinical data in prostate cancer cell lines have shown that enzalutamide has a synergic effect with taxane via the AR pathway, and the prostate cancer cell lines may have a cross-resistance between taxanes and enzalutamide when these drugs were used sequentially.[22, 23] A phase I study of prostate cancer patients showed that enzalutamide does not have a clinically significant effect on docetaxel PK when these drugs were used concurrently.[24]

Study Rationale:
Based on the above mentioned data, it is hypothesized that among patients with AR-positive TNBC who were non-responders to initial anthracycline and cyclophosphamide chemotherapy, ZT regimen (the combination of enzalutamide and weekly paclitaxel) administered in the neoadjuvant setting increases pCR and RCB-I rates compared with conventional taxane-based chemotherapy.

Rationale for Correlative Studies:
Optional tumor tissue will be collected prior to beginning enzalutamide therapy and at the time of surgery. Peripheral blood will be collected prior to beginning enzalutamide, after six cycles of paclitaxel, and at the time of surgery (see above "Study Schema" on page3). These samples will be used to perform correlative studies aimed at identifying predictive markers and to enhance our understanding of the biology of AR positive TNBC. All studies will be performed at MD Anderson.

Gene profiles and reverse phase protein assay (RPPA)/RNA sequences: The experiments using breast cancer cells are limited since the mechanism and cross talk of androgen receptor signaling is still unclear in breast cancer. However, both in vitro and in vivo experiments using prostate cancer cells have been performed to investigate the roles of androgen receptor signaling pathway. In prostate cancer, it is thought that there are two signaling pathways; androgen-dependent and androgen-independent signaling [25, 26] PI3K/Akt pathway has been shown to promote prostate cancer cell survival and

growth via enhancing AR-mediated transcription. Both PTEN and the PI3K inhibitor negatively regulate this process. It is shown that the cross talk between the androgen and PI3K/Akt pathways is mediated through the modulation of the PI3K/Akt downstream effector GSK3 β. Its inactivation by phosphorylation results in increased nuclear levels of β-catenin, which augment AR activity. These findings delineate a novel mechanism by which PI3K/Akt and PTEN regulate the androgen pathway during prostate cell growth and survival. [27] AR, the progesterone receptor (PR), and the estrogen receptor (ER) are able to activate the MAPK/ERK through a mechanism independent of their transcriptional activity. AR has been found to interact with the intracellular tyrosine kinase Src. One of the targets of Src is the adapter protein Shc, an upstream regulator of the MAPK pathway. [26]

Given there are some related proteins in AR signaling in prostate cancer, reverse phase protein assay (RPPA) and RNA sequences of relevant signaling pathways including ERK, PI3K, SRC, PTEN, Akt, p53, EGFR,GSK3, β-catenin , IGF-IR, Raf, and MAPK will be performed on all biopsies to better understand the molecular mechanism related to AR signaling and their changes by enzalutamide. The expression level of those AR related genes will be also evaluated using an Affymetrix GeneChip.

Circulating tumor cells (CTCs): CTCs are found in human blood when cancers undergo metastatic dissemination and spread to distant organs.[28, 29] CTCs have been reported as a surrogate marker for tumor response and linked to shorter survival in metastatic breast, prostate and colorectal lung cancer patients.[28, 30-34] CTCs also carry highly specific oncogenic mutations.[35] In prostate cancer cell lines, it has been known that androgen receptor signaling is activated by translocation of AR from the cytoplasm to the nucleus where AR activates AR-target genes. In vivo experiment using castrate resistant prostate cancer patients, taxane inhibits AR nuclear localization and AR cytoplasmic localizations correlated with clinical response to taxane. This may suggest that monitoring AR subcellular localization in the CTCs might predict clinical response to treatment blocking AR translocation such as enzalutamide. [36]

2.0 Objectives

2.1 Primary Objective:

2.1.1 To evaluate the pCR and RCB-I rates of patients with TNBC who were non-responders to initial anthracycline and cyclophosphamide chemotherapy and who were treated with Enzalutamide in combination with weekly paclitaxel in the neoadjuvant setting.

2.2 Secondary Objectives:

2.2.1 To estimate progression free survival (PFS) distribution of AR-positive TNBC patients who were non-responders to initial anthracycline and cyclophosphamide chemotherapy, treated with Enzalutamide in combination with weekly paclitaxel in the neoadjuvant setting.

2.2.2 To determine the safety of administering Enzalutamide in combination with weekly paclitaxel in the neoadjuvant setting.

2.3 Exploratory Objectives:

2.3.1 To investigate the association between biomarkers in the peripheral blood and tumor tissue with safety and efficacy for TNBC patients who were treated with Enzalutamide and treatment in combination with weekly paclitaxel in the neoadjuvant setting.

2.3.2 To investigate the correlation between CTC characteristics and/or gene profiles and treatment response of enzalutamide and taxane.

3.0 Selection of Patients

To be included in the study, the subject must have:

3.1 Inclusion Criteria:

1) Signed written informed consent

2) Patients with histologically confirmed intact primary cancer that is confirmed invasive carcinoma of the breast, with at least 1.0 cm residual disease as measured by mammography, ultrasound, or breast MRI after neoadjuvant anthracycline based chemotherapy.

3) Triple-negative breast cancer defined as ER<10%; PR<10% by immunohistochemistry (IHC) and HER2 0-1+ by IHC, or 2+ FISH non-amplified.

4) Androgen Receptor will be quantified using CLIA-compliant assays for AR on a biopsy specimen obtained prior to initiation of treatment.. AR-positivity is defined as $\geq$ 10% of nuclear staining.

5) AJCC 7th edition stage I-III Breast Cancer

6) Men or women 18 years of age or older.

7) Patients must have a performance status of (0-1) on the ECOG performance scale

8) Negative serum or urine pregnancy test must be done within 72 hours before the first dose of the study medication for women of childbearing potential as per institutional guidelines. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not required to undergo pregnancy test.

9) Men on study must use a condom if having sex with a pregnant woman.

10) Male patient and his female partner who is of childbearing potential must use 2 acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at screening and continuing throughout the study period and for 3 months after final study drug administration

11) Patient must have adequate organ function as determined by the following laboratory values:
    - Absolute neutrophil count ≥ 1,500 /µL
    - Platelets ≥ 100,000 / µL
    - Hemoglobin ≥ 9 g/dL
    - Creatinine Clearance $\geq$ 50 ml/min
    - Total Bilirubin $\leq$ 1.5 x ULN
    - ALT/AST $\leq$ 2.5 x ULN

3.2 Exclusion Criteria:

1) Patients who have received any previous antitumor therapies (other than anthracycline-based neoadjuvant chemotherapy for the current cancer event).

2) Female patients must not be breast-feeding at screening or planning to become pregnant during the course of therapy.

3) Patients having major surgery within 21 days before Cycle 1, Day 1.

4) Patients with known history of hypersensitivity to paclitaxel that did not resolve with pre- medication.

5) Patients with left ventricular ejection fraction <50% or 10% decrease from baseline on echocardiogram after anthracycline based chemotherapy.

6) Patients with gastrointestinal impairment that would affect the absorption of Enzalutamide; or previous history of colitis.

7) Subjects requiring daily corticosteroids, other than those given as premedication for the anthracycline-based chemotherapy.

8) Patients with known or suspected brain metastasis or active leptomeningeal disease.

9) History of seizure or any condition that may predispose to seizure (e.g., prior cortical stroke, significant brain trauma) at any time in the past. Also, history of loss of consciousness or transient ischemic attack within 12 months of Day 1 visit.

10) Myocardial infarction within 6 months before starting therapy, symptomatic congestive heart failure (New York Heart Association > class II), unstable angina, or unstable cardiac arrhythmia requiring medication.

4.0 Registration Procedures

- Patients must not start protocol treatment prior to registration.
- The following information will be requested:
  - Protocol Number
  - Investigator Identification
    - Investigator's name
  - Patient Demographics
    - Patient's initials
    - Sex
    - Birth date (mm/yyyy)
    - Race
    - Ethnicity

Eligibility Verification

Patients must meet all of the eligibility requirements listed in section 4. Baseline measurements and evaluations must be obtained within 4 weeks of registration to the study. All areas of disease should be recorded in order to assess response and uniformity of response to therapy.

Additional Requirements:

All patients must be provided with a signed and dated copy of the informed consent.

Instructions for Patients who do Not Start Assigned Protocol Treatment

If a patient is found ineligible, baseline and follow-up data will still be collected and submitted into the electronic database. Documentation of the reason for not starting protocol treatment will also be recorded.

5.0 Treatment Plan

5.1 Treatment

Patients will initiate therapy with enzalutamide and paclitaxel at the doses and schedules listed in study schema (page 3) and as defined in Table 3. Each cycle will be defined as 7 days of therapy with oral enzalutamide taken daily. Patients will undergo a planned 12 cycles of therapy prior to surgical resection.
Patients who are unable to receive combination therapy due to toxicity will be considered non-responders. Patients withdrawing prior to the completion of 4 cycles for any reasons other than (1) toxicity or (2) for progression of disease will be replaced. For drug administration guidelines and dose reductions, see Therapy Administration and Dose Reductions.

All patients will undergo surgical resection of their primary tumor unless they are deemed not fit for surgery after 12 weeks of therapy. Patients may undergo either lymph node sampling or complete axillary dissection as considered standard of care by their surgeon.

Review of adverse events will be done within 28 days before start of study drug, every week during treatment, at the time of surgery or progression, and 30 days after the discontinuation of study drug.

5.2 Study Calendar

Trial Period	Screening Phase		Treatment Cycles			End of Treatment	Post Treatment
	Screening		1-6	7	8-12	Discontinuation	Safety Follow-up
Scheduling Window (Days)	-28 to -1	-10 to -1	+/- 3	+/-3	+/-3	At Time of Surgery or Progression	30 Post Discontinuation (+/-3 Days)
Administrative Procedures							
Informed Consent	x						
Eligibility	x						
Medical History	x						
Clinical Procedures / Assessments							
Review Adverse Events	x		x		x	x	x
Physical Exam / Weight / Vitals[G]	x		x		x	x	x
ECOG / Performance Status	x		x		x	x	x
Enzalutamide, Oral Daily			x	x	x		
Paclitaxel, IV weekly			x	x	x		
Laboratory Procedures							
Serum or Urine Pregnancy Test [F]		x					
CBC with Differentials [B]		x	x	x	x		x
Chemistry Panel [A,B]		x	x	x	x		x
CTCs, Plasma, PBMCs [E]		x		x		x	
Tumor Tissue Biopsy / Analysis							
Optional Tissue Biopsy	x					x [C]	
RPPA / RNA seq	x					x	
Response Measurements							
Tumor Imaging [D]		x				x	

A. Total Bilirubin, ALT, AST, Alkaline Phosphatase, Albumin, BUN, Creatinine, Sodium, Potassium, Bicarbonate, Chloride, Calcium, Magnesium, Glucose
B. Blood Tests must be performed within 48 hours prior to starting each cycle of paclitaxel.
C. For patients having surgery, biopsy will not be performed, instead a surgical specimen will be collected.
D. Tumor Imaging ideally should be as close to start of treatment as possible, however baseline imaging within 28 days of start is acceptable. Imaging can also be performed at the time of suspected Clinical Progression. Acceptable Imaging includes: Mammogram, Ultrasound and/or MRI.
E. CTCs**,** Plasma, PBMCs will be collected at Baseline, Post Cycle 6 just Prior to Cycle 7, End of Treatment or Discontinuation.
F. Women of Child-Bearing Potential only. (WCBP)
G. Physical Exams will be done prior to start of cycles 1, 5, & 9; or if clinically indicated.

5.3 Scheduled Evaluations:

5.3.1 Pretreatment Evaluation

- History and physical examination.
- Laboratory studies: CBC with differential, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, albumin, alkaline phosphatase, total bilirubin, AST, ALT, serum pregnancy test (women of child bearing potential only).
- Radiologic evaluation of measurable disease within 4 weeks of starting treatment.
- Patient must sign IRB-approved informed consent prior to any study-specific procedures unless such procedures are part of the standard of care.

5.3.2 Evaluation During Study

- Physical examination (including vital signs, weight, performance status): on day 1 (or up to 3 days prior) of cycles 1, 5 and 9. Clinic visits are also to be scheduled if indicated, i.e. toxicities, progression of disease, or if treatment has been held for greater than 7 days.
- CBC with differential, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, albumin, alkaline phosphatase, total bilirubin, AST, ALT within 48 hours prior to starting each cycle of weekly paclitaxel.
- Patients will undergo repeat imaging (mammogram, ultrasound and/or MRI, as clinically indicated) of the involved breast and axillary nodal basin as standard of care prior to surgical resection (after 12 weeks of therapy) or at the time of clinically suspected disease progression.

5.4 Correlative Studies :

All molecular correlative studies except for CTC analysis, RPPA, and RNA sequencing will involve molecular profiling data generated through biopsy of the primary breast tumor and profiled within the existing BMO molecular triaging protocol that is currently IRB approved (MDACC 2014- 0185). Potential biomarkers of response will be correlated with pathologic response to enzalutamide and taxane treatment using appropriate statistical analyses for the biomarker of interest.

Tissue biopsy

RNA sequences and reverse phase protein assay (RPPA)
Up to three core biopsies may be obtained prior to initiation of enzalutamide and paclitaxel therapy. Tissue collected from participating subjects will be de-linked from all personal identifiers per institutional tissue banking procedures.
The following tissue analyses will be completed if sufficient tissue can be obtained:

- Immunohistochemistry of AR and reverse phase protein assay (RPPA) of relevant signaling pathways including ERK, PI3K, SRC, PTEN, Akt, p53, EGFR, and MAPK will be performed.
- The expression level of genes related to the response to enzalutamide will be evaluated using a molecular method such as Affymetrix.

Peripheral Blood

These samples will be used to perform correlative studies aimed at identifying predictive markers and to enhance the understanding of androgen receptor inhibition in the neoadjuvant setting.

CTC
7.5 mL of peripheral blood will be collected at three time points: prior to first dose of paclitaxel (baseline), between end of cycle 6 and prior to 7 cycle (mid-treatment), and prior to surgery.

Plasma and PBMCs
20 mL of peripheral blood will be collected at three time-points: baseline, mid-treatment and before surgery. Baseline plasma and PBMC samples will be used to isolate circulating tumor DNA (cfDNA) and RNAseq for future analysis if needed. These samples will be bio-banked for future analysis.

6.0 Drug Information:

6.1 Enzalutamide (Xtandi®) [38]

Enzalutamide is an androgen receptor inhibitor that acts on different steps in the androgen receptor-signaling pathway. Enzalutamide has been shown to competitively inhibit androgen binding to androgen receptors and inhibit androgen receptor nuclear translocation and interaction with DNA.

The most common adverse reactions (≥ 5%) observed with Enzalutamide are asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension. The most clinically important adverse effects are discussed below:

Seizure

In the randomized phase 3 study of patients with CRPC previously treated with docetaxel (CRPC2), 0.9% of patients receiving enzalutamide (160 mg/daily) experienced a seizure. Seizure events occurred from 31 to 603 days after initiation of enzalutamide. No seizures occurred in patients treated with placebo. In the randomized phase 3 study of chemotherapy naive patients treated with enzalutamide (MDV3100-03), 1 enzalutamide-treated patient experienced a seizure and 1 placebo-treated patient experienced a seizure, leading to an incidence of 0.1% in both treatment groups. Patients experiencing seizure were permanently discontinued from therapy and all seizure events resolved. There is no clinical trial experience re-administering enzalutamide to patients who experienced seizure. Limited safety data are available in patients with predisposing factors for seizure because these patients were generally excluded from the trials. These exclusion criteria included a history of seizure, underlying brain injury with loss of consciousness, transient ischemic attack within the past 12 months, cerebrovascular accident, brain metastases, and brain arteriovenous malformation. CRPC2 excluded the use of concomitant medications that may lower the seizure threshold, whereas MDV3100-03 permitted the use of these medications. Because of the risk of seizure associated with enzalutamide use, patients should be advised of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.

Laboratory Abnormalities

In the randomized clinical trial, Grade 1-4 neutropenia occurred in 15% of patients on XTANDI (1% Grade3-4) and in 6% of patients on placebo (no Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was similar in both arms; 0.5% of patients on XTANDI and 1% on placebo experienced Grade 3-4 thrombocytopenia. Grade 1-4 elevations in ALT occurred in 10% of patients on XTANDI (0.3% Grade 3-4) and 18% of patients on placebo (0.5% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of patients on XTANDI and 2% of patients on placebo.

Infections

In the randomized clinical trial, 1.0% of patients treated with XTANDI compared to 0.3% of patients on placebo died from infections or sepsis. Infection-related serious adverse events were reported in approximately 6% of the patients on both treatment arms.

Falls and Fall-related Injuries

In the randomized clinical trial, falls or injuries related to falls occurred in 4.6% of patients treated with XTANDI compared to 1.3% of patients on placebo. Falls were not associated with loss of consciousness or seizure. Fall-related injuries were more severe in patients treated with XTANDI and included non-pathologic fractures, joint injuries, and hematomas.

Hallucinations

In the randomized clinical trial, 1.6% of patients treated with XTANDI were reported to have Grade 1 or 2 hallucinations compared to 0.3% of patients on placebo. Of the patients with hallucinations, the majority was on opioid-containing medications at the time of the event. Hallucinations were visual, tactile, or undefined.

Posterior Reversible encephalopathy Syndrome (PRES)

Two post marketing cases of confirmed PRES were identified from the global safety database (estimated cumulative post marketing exposure of 17,704 patient treatment years as of 30August2014). In the first case, reported symptoms (confusional syndrome and aphasia; symptom onset on treatment day 27). The patient received acute treatment with intravenous corticosteroids. After discontinuing enzalutamide, reported symptoms resolved and a follow-up cerebral MRI revealed regression of abnormalities, indicative of positive de-challenge. In the second case, reported symptoms (altered mental status, confusion, and word-finding difficulties; with a blood pressure of 188/97 mm Hg; symptom onset on treatment day 98) and MRI findings were consistent with a diagnosis of PRES. The patient was treated with dexamethasone and an antihypertensive regimen of hydralazine and carvedilol. After discontinuing enzalutamide, reported symptoms resolved and follow-up cerebral MRI finding showed significant improvement, indicative of positive de-challenge. Discontinuation of enzalutamide in patients who develop PRES is recommended. Patients should be informed to contact the study physician as soon as possible if they experience rapidly worsening symptoms possibly indicative of PRES such as seizure, headache, and confusion, reduced eyesight, or blurred vision.

Table 1. Adverse Reactions in the Phase III Trials [39, 40]

	PREVAIL Trial				AFFIRM Trial			
	Enzalutamide (n=871)		Placebo (n=844)		Enzalutamide (n=800)		Placebo (n=399)	
	All Grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3	All grades	Grade ≥ 3
	Number of patients (percent)				Number of patients (percent)			
Fatigue	310 (36)	16 (2)	218 (26)	16 (2)	269 (34)	50 (6)	116 (29)	29 (7)
Constipation	193 (22)	4 (<1)	145 (17)	3 (<1)				
Musculoskeletal pain					109 (14)	8 (1)	40 (10)	1 (<1)
Back pain	235 (27)	22 (3)	187 (2)					
Arthralgia	177 (20)	12 (1)	135 (16)	9 (1)				
Decreased appetite	158 (18)	2 (<1)	136 (16)	6 (1)				
Hot flash	157 (18)	1 (<1)	65 (8)	0	162 (20)	0	41 (10)	0
Diarrhea	142 (16)	2 (<1)	119 (14)	3 (<1)	171 (21)	9 (1)	70 (18)	1 (<1)
Hypertension	117 (13)	59 (7)	35 (4)	19 (2)	(6.6)		(3.3)	
Asthenia	113 (13)	11 (1)	67 (8)	8 (1)				
Fall	101 (12)	12 (1)	45 (5)	6 (1)				
Weight loss	100 (11)	5 (1)	71 (8)	2 (<1)				
Peripheral edema	92 (11)	2 (<1)	69 (8)	3 (<1)				
Headache	91 (10)	2 (<1)	35 (4)	3 (<1)	93 (12)	6 (<1)	22 (6)	0
Any cardiac event	88 (10)	24 (3)	66 (8)	18 (2)	49 (6)	7 (1)	30 (8)	8 (2)
Afib[1]	16 (2)	3 (<1)	12 (1)	5 (1)				
ACS[2]	7 (1)	7 (1)	4 (<1)	2 (<1)	2 (<1)*	2 (<1)*	2 (<1)*	2 (<1)*
Acute renal failure	32 (4)	12 (1)	38 (5)	12 (1)				
Cerebrovascular event	12 (1)	6 (1)	9 (1)	3 (<1)				
Abnormality on liver function test	8(1)	2 (<1)	5 (1)	1 (<1)	8 (1)	3 (<1)	6 (2)	3 (<1)
Seizure	1 (<1)	1 (<1)	1 (<1)	0	5 (<1)	5 (<1)	0	0

[1]Atrial fibrillation
[2]Acute Coronary Syndrome
*Myocardial Infarction
In phase I trial of combination of enzalutamide and docetaxel, 9% experienced febrile neutropenia and 36% did neutropenia in any grades. (Data not shown)

Drug Interactions

Given previously reported CYP hepatic enzyme mediated interaction with other drugs – drugs that are known to have strong interaction through either CYP3A4 or CYP 2C9 should be avoided.

Drugs that are confirmed to have strong interactions are: midazolam, gemfibrozil, omeprazole, warfarin (attachment: IB page 41 for list of drugs). Other drugs will be evaluated by PI upon individual cases.

6.2 Paclitaxel (Taxol®)

Contraindications

Taxol is contraindicated in patients who have a history of hypersensitivity reactions to Taxol or other drugs formulated in Cremophor® EL (polyoxyethylated castor oil).
Taxol should not be used in patients with solid tumors who have baseline neutrophil counts of <1500 cells/mm3 or in patients with AIDS-related Kaposi's sarcoma with baseline neutrophil counts of <1000 cells/mm3.
However for the purposes of this trial, once patients have begun active treatment, patients must maintain an Absolute Neutrophil Count $\geq$ 1,000 cells/mm^3.

Warnings

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2 to 4% of patients receiving Taxol in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H2 antagonists
Patients who experience severe hypersensitivity reactions to Taxol should not be rechallenged with the drug.

Bone marrow suppression (primarily neutropenia)

Dose-dependent and is the dose-limiting toxicity. Neutrophil nadirs occurred at a median of 11 days. Taxol should not be administered to patients with baseline neutrophil counts of less than 1,500 cells/mm3 (<1000 cells/mm3 for patients with KS). Frequent monitoring of blood counts should be instituted during Taxol treatment. Patients should not be re-treated with subsequent cycles of Taxol until neutrophils recover to a level >1,000 cells/mm3 (>1000 cells/mm3 for patients with KS) and platelets recover to a level >100,000 cells/mm3.
Severe conduction abnormalities have been documented in <1% of patients during Taxol therapy and in some cases requiring pacemaker placement. If patients develop significant conduction abnormalities during Taxol infusion, appropriate therapy should be administered and continuous cardiac monitoring should be performed during subsequent therapy with Taxol.

Pregnancy

Taxol can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel during the period of organogenesis to rabbits at doses of 3.0 mg/kg/day (about 0.2 the daily maximum recommended human dose on an mg/m2 basis) caused embryo- and fetotoxicity, as indicated by intrauterine mortality, increased resorptions, and increased fetal deaths. Maternal

toxicity was also observed at this dose. No teratogenic effects were observed at 1.0 mg/kg/day (about 1/15 the daily maximum recommended human dose on an mg/m2 basis); teratogenic potential could not be assessed at higher doses due to extensive fetal mortality.
There are no adequate and well-controlled studies in pregnant women. If Taxol is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

Precautions

Contact of the undiluted concentrate with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted Taxol solutions should preferably be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene- lined administration sets. Taxol should be administered through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® filters which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP. IVEX-2® is the registered trademark of the Millipore Corporation.

Drug Interactions

In a Phase 1 trial using escalating doses of Taxol (110–200 mg/m2) and cisplatin (50 or 75 mg/m2) given as sequential infusions, myelosuppression was more profound when Taxol was given after cisplatin than with the alternate sequence (i.e. Taxol before cisplatin). Pharmacokinetic data from these patients demonstrated a decrease in paclitaxel clearance of approximately 33% when Taxol was administered following cisplatin.

The metabolism of Taxol is catalyzed by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when Taxol is concomitantly administered with known substrates (e.g., midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (e.g., atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), and inducers (e.g., rifampin and carbamazepine) of CYP3A4. Caution should also be exercised when Taxol is concomitantly administered with known substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), and inducers (e.g., rifampin) of CYP2C8. Potential interactions between Taxol, a substrate of CYP3A4, and protease inhibitors (ritonavir, saquinavir, indinavir, and nelfinavir), which are substrates and/or inhibitors of CYP3A4, have not been evaluated in clinical trials. Reports in the literature suggest that plasma levels of doxorubicin (and its active metabolite doxorubicinol) may be increased when paclitaxel and doxorubicin are used in combination.

Hematology

Taxol therapy should not be administered to patients with baseline neutrophil counts of less than 1500 cells/mm3. In order to monitor the occurrence of myelotoxicity, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving Taxol. Patients should not be re-treated with subsequent cycles of Taxol until neutrophils recover to a level >1,000 cells/mm3 and platelets recover to a level >100,000 cells/mm3. In the case of severe neutropenia (<500 cells/mm3 for 7 days or more) during a course of Taxol therapy, a 20% reduction in dose for subsequent courses of therapy is recommended.
For patients with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma, Taxol, at the recommended dose for this disease, can be initiated and repeated if the neutrophil count is at least 1000 cells/mm3.

Hypersensitivity Reactions

Patients with a history of severe hypersensitivity reactions to products containing Cremophor® EL (e.g., cyclosporine for injection concentrate and teniposide for injection concentrate) should not be treated with Taxol. In order to avoid the occurrence of severe hypersensitivity reactions, all patients treated with Taxol should be premedicated with corticosteroids (such as dexamethasone), diphenhydramine and H2 antagonists (such as cimetidine or ranitidine). Minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia do not require interruption of therapy. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of Taxol and aggressive symptomatic therapy. Patients who have developed severe hypersensitivity reactions should not be rechallenged with Taxol.

Cardiovascular

Hypotension, bradycardia, and hypertension have been observed during administration of Taxol, but generally do not require treatment. Occasionally Taxol infusions must be interrupted or discontinued because of initial or recurrent hypertension. Frequent vital sign monitoring, particularly during the first hour of Taxol infusion, is recommended. Continuous cardiac monitoring is not required except for patients with serious conduction abnormalities. When Taxol is used in combination with doxorubicin for treatment of metastatic breast cancer, monitoring of cardiac function is recommended.

Nervous System

Although the occurrence of peripheral neuropathy is frequent, the development of severe symptomatology is unusual and requires a dose reduction of 20% for all subsequent courses of Taxol. Taxol contains dehydrated alcohol USP, 396 mg/mL; consideration should be given to possible CNS and other effects of alcohol.

Hepatic

There is limited evidence that the myelotoxicity of Taxol may be exacerbated in patients with serum total bilirubin >2 times ULN. Extreme caution should be exercised when administering Taxol to such patients.

Injection Site Reaction

Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of Taxol at a different site, i.e., “recall,” has been reported.
More severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been reported. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to 10 days.
A specific treatment for extravasation reactions is unknown at this time. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.

Carcinogenesis, Mutagenesis, Impairment of Fertility

The carcinogenic potential of Taxol (paclitaxel) has not been studied.
Paclitaxel has been shown to be clastogenic *in vitro* (chromosome aberrations in human

lymphocytes) and *in vivo* (micronucleus test in mice). Paclitaxel was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.
Administration of paclitaxel prior to and during mating produced impairment of fertility in male and female rats at doses equal to or greater than 1 mg/kg/day (about 0.04 the daily maximum recommended human dose on a mg/m2 basis). At this dose, paclitaxel caused reduced fertility and reproductive indices, and increased embryo- and fetotoxicity.

Pregnancy

Pregnancy (Section 9.2.2). Should a participant, or participant's partner, become pregnant while on study, refer to section 9.2.2 for IRB and sponsor notification.

7.0 Therapy Administration and Dose Reductions:

NOTE: All toxicities should be graded according to the NCI Common Toxicity Criteria (version 4.0)

Dose modification for paclitaxel due to drug interaction to enzalutamide is not necessary based on the phase I trial, combination therapy of enzalutamide and docetaxel in CRPCa patients. (Data not shown) AEs leading to dose reduction of enzalutamide in the two combined controlled population of CRPCa patients were infrequent (enzalutamide 2.1% vs placebo 1.5%). Dose adjustments of paclitaxel should be considered first with the exception of nervous system disorders, e.g., seizure, PRES, syncope. If the AEs persist after dose reduction of paclitaxel, dose reduction of enzalutamide should be considered. Treating physician should consult with PI prior to reduction of either, or both, drugs. Dose modification schedule was shown in Table 3.

In the case of nervous system toxicity, the trial treatment will be discontinued.

Enzalutamide and paclitaxel will be withheld for drug-related ≥ Grade 3 hematologic toxicities (excluding Grade 3 neutropenia, anemia, and thrombocytopenia), or Grade 3 or higher non-hematological toxicity, including laboratory abnormalities, and severe or life-threatening AEs as per Table 2 below. After those toxicities resolve to Grade 0-1 or baseline, treatment could be resumed with one level of dose reduction.

Should a delay of >14 days be required, then the patient should be discontinued from study treatment.

Table 2: Dose modification guidelines for drug-related adverse events.

Toxicity	Grade	Hold Treatment (Y/N)	Restart Treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Hematologic Toxicity	1 2	No	N/A	N/A	N/A
	3* *Excluding Grade 3 neutropenia, anemia, and thrombocyto-penia	Yes	Toxicity resolves to Grade 0-1 or baseline	One level of dose reduction	Toxicity not resolving within 14 days of dose. *Permanent discontinuation should be considered for any severe or life- threatening event*
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	One level of dose reduction	
Non-Hematologic Toxicity	1 2	No	N/A	N/A	N/A
	3 4	Yes	Toxicity resolves to Grade 0-1 or baseline	One level of dose reduction	Toxicity not resolving within 14 days of dose *Permanent discontinuation should be considered for any severe or life- threatening event*

Table3: Drug Dosing and Recommended Reductions for Toxicity [38]		
	Enzalutamide	Paclitaxel
Dose Level 1	120 mg PO daily	80 mg/m^2 weekly
Dose Level -1	120 mg PO daily	60 mg/^{2} weekly
Dose Level -2	80 mg PO daily	60 mg/^{2} weekly
Dose Level -3	Hold	Hold

7.1 Supportive Care

All supportive measures consistent with optimal patient care should be given throughout the study. Exceptions include the use of erythropoietin to treat therapy-induced anemia. Investigators who choose to use myeloid growth factors should administer either GCSF or pegylated GCSF beginning on either day 2 or 3 of each cycle. The use of growth factor support should follow ASCO guidelines.

7.2 Duration of Therapy

Patients will receive protocol therapy unless:

- Patient experiences unacceptable drug toxicity.
- Patient withdraws consent.
- Patient has progression of disease.
- The patient completes the required 12 weeks of therapy.

7.3 Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed until at least two years after surgical resection of the tumor.

7.4 Protection of human subjects

All current FDA, NCI, state federal and institutional regulations concerning informed consent will be followed.

In order to protect confidential patient information, all study participants will be assigned a study ID number. Specimens will be assigned a separate specimen ID number. All study information will be stored in locked file cabinets and in password-protected computer files. Only authorized study personnel will have access to these files.

8.0 Measurement of Effect

8.1 Pathologic response

The RCB is a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. RCB can be divided into four classes (RCB-0 to RCB-III) and will be collected as part of the study.

RCB-0 (pCR), Minimal RCB (RCB-I), Moderate RCB (RCB-II), and Extensive RCB (RCB-III).

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

The largest two dimensions (mm) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)

Submission of the entire largest cross-sectional area of the residual tumor bed for histologic mapping, with specific identification of those slides in the pathology report (e.g. "the largest cross-sectional area of primary tumor bed was submitted in cassettes A5 - A9")

If the residual tumor is large (i.e. largest diameter > 5 cm), then at least 5 representative cassettes from the largest cross-sectional area are sufficient, but should be identified in the original pathology report (e.g. "representative sections from the largest cross- sectional area of primary tumor bed were submitted in cassettes A5 - A9")

Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following:

0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%

To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.

When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.

Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed. e.g. if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.

Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following:

0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%

The number of positive (metastatic) lymph nodes

The largest diameter (mm) of the largest nodal metastasis

The RBC can be accessed online: www.mdanderson.org/breastcancer_RBC

8.2 Radiographic Imaging

Radiographic criteria of response will be based on the on regional ultrasound examination (decrease in size of the primary tumor and/or fatty replacement in regional lymph nodes)

A decrease in size of the product of the two largest dimensions should =/> 50% will be considered a partial response.

Complete disappearance of the primary tumor by physical exam and or ultrasound and normalization of the lymph nodes by ultrasound will be considered a complete clinical response.

Progression of disease will be defined as 30% increase in the size of the primary tumor and/or lymph nodes on physical exam and/or ultrasound.

9.0 Adverse Event Reporting

9.1 Definition of Adverse Events (AEs)

An adverse event (AE) is defined as any untoward medical occurrence in a subject administered a study drug and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets one of the following criteria:

- Induces clinical signs or symptoms.
- Requires active intervention.
- Requires interruption or discontinuation of study medication.
- The abnormality or investigational value is clinically significant in the opinion of the investigator.

All adverse events, whether or not related to the study drug, must be documented in the electronic database.

9.2 Definition of Serious Adverse Events (SAEs)

An AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in death,
- Is life threatening (an AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.),
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions,
- Results in congenital anomaly, or birth defect,
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious),
- Other medically important events.

9.2.1 Serious Adverse Event Reporting

Any serious adverse event will be reported to both sponsor, and institution as per guideline outlined below.

Report to Astellas:
Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the Investigator will complete and submit a MedWatch 3500A Form to FDA, containing all required information (reference 21 CFR 312.32). The Investigator will submit a copy of this MedWatch 3500A form to Astellas by either e-mail or fax, within the same timeframe. If submission of this

SAE to FDA or Astellas or is not possible within 24 hours, the Investigator's local drug safety contact (IRB, etc.) should be informed by phone.

The SAE documentation, including the MedWatch 3500A Form and available source records should be emailed or faxed to:

Astellas Pharma Global Development – United States
Email: Safety-us@us.astellas.com
Fax number: (847) 317-1241

The following minimum information is required:

- Study number/IIT regulatory identifier
- Subject number, sex and age
- The date of report
- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug

Follow-up information for the event should be sent within promptly (within 7 days) as necessary.

Report to MD Anderson IRB

All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy on Reporting Serious Adverse Events". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to ORERM, regardless of attribution (within 5 working days of knowledge of the event). All life-threatening or fatal events, expected or unexpected, and regardless of attribution to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in ORERM. The MDACC "Internal SAE Report Form for Prompt Reporting" will be used for reporting to ORERM. Serious adverse events will be captured from the time the patient signs consent until 30 days after the last dose of drug. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event. Serious adverse events will be forwarded to FDA by the Safety Project Manager ORERM according to 21 CFR 312.32

9.2.2 Procedure in Case of Pregnancy

The effect of enzalutamide in pregnant and lactating women is not known, and the exposure of a fetus or nursing infant is considered a potential risk. Enzalutamide can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Subjects receiving enzalutamide are advised to use 2 acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of screening for an enzalutamide study and continuing throughout the course of treatment and for at least three months after enzalutamide is discontinued.

If during the conduct of the clinical trial, a male subject impregnates his partner, the subject should report the pregnancy to the Investigator. The Investigator should report the pregnancy to the Sponsor as an SAE within 24 hours of awareness of the event. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

The Investigator should report the outcome of the pregnancy (independent of outcome, e.g. full term delivery, pre-term delivery, spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a miscarried fetus, etc.] in accordance with the same reporting procedure as for SAEs. The date of outcome of the pregnancy, gestational age, date of birth and neonatal data etc., should be included in this information.

10. Statistical Considerations

Overview

This is a non-randomized open label phase IIB study. Counting pCR (RCB-0) or RCB-I as response, using a Simon optimal two-stage design with alpha = beta = 10%, and then setting the threshold for an acceptable pCR or RCB-I rate at 20%. We will enroll 12 patients into the first stage. If we see 0 patients with pCR or RCB-I, we will stop the study after the first stage. If we see at least one patient with pCR or RCB-I, we will continue to enroll 25 more patients for a total of 37 patients. We would declare the treatment worthy of further study if we see at least 4 of the 37 patients with pCR or RCB-I. This design has a 54% probability of early termination after the first stage if the true pCR or RCB-I probability is 5%.

Patients who are unable to complete the combination therapy due to toxicity will be considered non- responders. Patients withdrawing early for any reasons other than (1) toxicity or (2) for progression of disease will be replaced. Additionally, a secondary efficacy analysis will be based on a modified intent-to-treat population receiving at least one dose of experimental therapy such that patients withdrawing early for any reason are considered non-responders. We will require 37 patients enrolled in order to ensure a sufficient number of evaluable patients at the final protocol analysis.

Secondary Objectives and Correlative Studies:

Potential biomarkers of response will be correlated with pathologic response to this treatment using appropriate statistical analyses for the biomarker of interest.

A secondary objective of this study is to also estimate the PFS distribution. PFS is defined as the time from enrollment to progression of disease (>30% increase in tumor size as defined in Section 8.2) or death whichever comes first.

We will estimate the proportion of patients with pCR (RCB-0) or RCB-I as the response rate along with an appropriate 95% confidence interval. We will estimate the proportion of patients in the remaining RCB categories with confidence intervals as well. We will estimate the PFS distribution using the Kaplan-Meier method from the date of enrollment onto this study until the date of progression or death without evidence of progression. Patients alive and disease-free at the latest clinical evaluation will be censored at the date of that evaluation.

Early drop outs (i.e., patients not getting to surgery) should be counted as treatment failures (i.e., non-responders with RCB > 1).

If no RCB-0 or -1 response has been observed prior to enrolling the 13th patient, enrollment will stop until all of the 12 patients in the first stage are evaluable and resume only if a response has been documented.

Inclusion of Minorities

The study will be available to all eligible patients regardless of race, or ethnic origin. There is no information currently available regarding differential effects of this regimen in subsets defined by race or ethnicity.

Protocol Monitoring

The study will be monitored by the PI. Bi-monthly safety review meetings/telephone conferences will be held to discuss toxicities, serious adverse events, and study progress. Meeting invitees will include the study PI, co-PI, study nurse, and data coordinator.

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