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A Phase II, Open label Study to evaluate Denosumab in patients with ER and/or PR-positive, HER2-negative Metastatic Breast Cancer (MBC) with bone metastases and detectable circulating tumor cells (CTCs)

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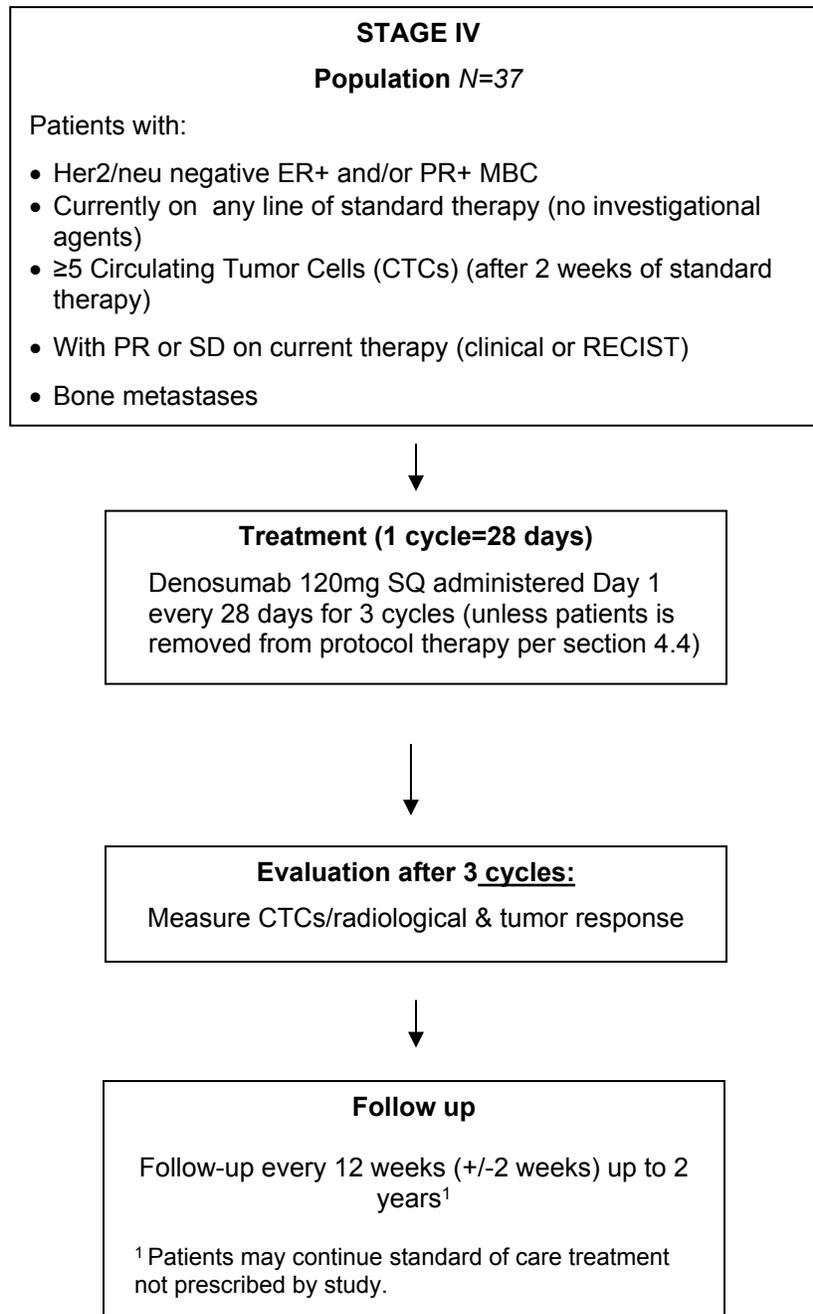
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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
ASCO	American Society of Clinical Oncology
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTC	Circulating Tumor Cells
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
ER	Estrogen Receptor
FOCBP	Female of child bearing potential
H&PE	History & Physical Exam
HER-2	Human Epidermal Growth Factor receptor-2
IV (or iv)	Intravenously
MTD	Maximum Tolerated Dose
MBC	Metastatic Breast Cancer
NCI	National Cancer Institute
ORR	Overall Response Rate or Objective Response Rate
OS	Overall Survival
ONJ	Osteonecrosis of the Jaw
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
PO (or p.o.)	Per os/by mouth/orally
PR	Partial Response
RANKL	Receptor activator of nuclear factor kappa-B ligand
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SRE	Skeletal-related events
SPGT	Serum Glutamic Pyruvic Transaminase
TNF	Tumor Necrosis factor
TRSAE	Treatment Related Serious Adverse Event
WBC	White Blood Cells

STUDY SCHEMA



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STUDY SUMMARY

Title	A Phase II, Open label Study to evaluate Denosumab in patients with ER and/or PR-positive, HER2-negative Metastatic Breast Cancer (MBC) with bone metastases and detectable circulating tumor cells (CTCs).
Short Title	Denosumab therapy for Metastatic Breast Cancer.
Version	8.15.16
Study Design	A single arm, two-stage Phase II study.
Study Center(s)	Robert H. Lurie Comprehensive Cancer Center of Northwestern University (RHLCCC).
Objectives	<p>Primary Objective</p> <ul style="list-style-type: none"> To assess the effect of Denosumab in Her2/neu negative ER+ and/or PR+ metastatic breast cancer patients who are in PR or SD after starting systemic therapy, with bone metastases and ≥ 5 CTCs, by measuring the fraction of patients with reduction in CTC quantity after 3 cycles of denosumab. <p>Secondary Objectives</p> <ul style="list-style-type: none"> To assess the effect of denosumab on CTCs enumeration considered as a continuous variable. To evaluate median progression-free survival(m-PFS) time.
Sample Size	Maximum accrual limit: 42 patients Evaluable patients: 37
Diagnosis & Key Eligibility Criteria	<ul style="list-style-type: none"> Patients with a histologically or cytologically confirmed HER-2/neu negative breast cancer and pathologic or radiographic evidence of bone metastases. Must have ≥ 5 CTCs, measured at time of registration. (Note: FDA approved commercial testing should be used). Patients may have either measurable or non-measurable disease per RECIST 1.1. Patients must have a serum calcium or albumin-adjusted serum calcium ≥ 2.0mmol/L (8.0mg/dL) and ≤ 2.9 mmol/L (11.5mg/dL) and cannot have any pre-existing uncorrected hypocalcemia (serum calcium <2.0mmol/L or <8.0mg/dL) at time of registration. Subjects cannot have prior or current history of osteonecrosis or osteomyelitis of the jaw, untreated local gum disease and non-healed dental or oral surgery at the time of registration. Subjects cannot have a history of clinically symptomatic brain metastases or who required treatment for brain metastases within 4 weeks of registration.

<p>Treatment Plan</p>	<ul style="list-style-type: none"> • Patients will be treated with Denosumab 120mg Subcutaneous at Day 1(+/-7 days) of each 28 day cycle for a total of 3 cycles. • Prior to each dose, patients will be assessed for AEs • Overall response will be assessed after cycle 3. In those with at least stable disease, patient will be considered off study treatment after 3 cycles (however, patients may continue Denosumab as standard therapy) (<i>Note: Patients will still be considered on study but in follow-up for purpose of survival endpoint.</i>).
<p>Statistical Methodology</p>	<p>We will conduct a single arm, two-stage Phase II study to assess the efficacy of a combination therapy including Denosumab in patients with bone metastatic breast cancer and detectable circulating tumor cells (CTCs). <i>The primary outcome is evidence of reduction in the number of CTCs.</i> A patient will be defined as a “success” if the number of CTCs at end of study treatment is less than the number observed at baseline. Patients who do not experience a decrease in CTC count at follow-up or are missing follow-up CTC count measurements will be defined as “failures”. The new combined treatment would be of interest if the proportion (p) of successes is at least 70%. A success proportion of less than 50% will be of no interest. We will test the null hypothesis that the success proportion is 0.5 versus the alternative that this proportion is 0.7. After testing the combination therapy on 23 patients in the first stage, the trial will be terminated if there are 12 or fewer successes. If the trial goes on to the second stage, a total of 37 patients will be studied. If the total number of successes is less than or equal to 23, the combination therapy will be rejected. The type I error for this Simon minimax design (Simon, 1989) is 4.8% and the power is 80%. The probability of early termination under the null hypothesis (i.e., p=0.5) is 66.1%.</p> <p>To assess the effect of denosumab on CTCs enumeration considered as a continuous variable, the percent change in CTCs from baseline (i.e., $100 \times [\text{number of CTCs at follow-up} / \text{number of CTCs at baseline}]$) will be calculated and compared to zero using either a one sample t-test or a Wilcoxon signed rank test.</p> <p>To evaluate median progression-free survival (PFS) time for the entire cohort, Kaplan-Meier curves will be used. Cox proportional hazards models with time dependent covariates will be used to assess the relationship between longitudinal CTC counts (as defined by 2 or more CTCs evaluation in the course of treatment) and PFS.</p>

1.0 INTRODUCTION – BACKGROUND & RATIONALE

1.1 Disease background

Breast Cancer Incidence And Subtypes

Breast cancer is the most common non-dermatological malignancy in women with an estimated 232,670 new diagnoses in 2014; and is the second leading cause of cancer death in women with an estimated 40,000 women in the United States succumbing to the disease in 2014(1). Breast cancer is a heterogeneous disease comprised of several molecular subtypes, which are commonly extrapolated into clinical subtypes based on receptor status(2). The specific receptors which are assessed in standard clinical practice are the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2-neu (HER2) receptor. Patients with ER-, PR-, and HER2-negative tumors, the so-called triple negative breast cancers (TNBC), biologically tend to display an aggressive phenotype, currently do not have targeted therapy options as a standard of care, and only have a limited amount of cytotoxic agents available to treat their disease(3).

Bone Metastasis in Breast Cancer

Metastatic colonization involves reciprocal interactions between tumor cells and a foreign microenvironment. Microenvironments consist of extracellular matrix and normal cells such as fibroblasts, endothelial cells and infiltrating inflammatory cells. Products of these resident and transient cells include growth factors, chemokines, cytokines and proteases. Microenvironments that contain tumor cells are distinct from normal tissues. Differences include the presence of hypoxia that drives angiogenesis and invasion, low pH, low glucose concentrations, alterations in extracellular matrix proteins and liberation of previously bound growth factors. There are differences in the distribution of site specific metastasis in advanced breast cancer. Bone metastasis are the most frequent site of recurrence particularly in the luminal subtypes. Moreover, the issue of site-specific metastasis has provided the opportunity to investigate the potential role of chemokines, particularly CXCR4 and RANKL.

Understanding of the molecular pathways involved in bone metastases has involved the evaluation of key players such as RANKL (also referred to as OPGL, TRANCE or ODF) and osteoprotegerin (OPG). Receptor activator of nuclear factor kappa-B ligand (RANKL) is a member of the tumour necrosis factor (TNF) family of cytokines that binds to its receptor RANK to control osteoclast differentiation, activation and survival. OPG is a soluble decoy receptor for RANKL that blocks ligand binding to RANK, thereby preventing the signaling required for osteoclast differentiation and activation. RANK is also constitutively expressed in normal mammary gland epithelial cells, but RANKL expression is induced by sex hormones during pregnancy. Genetically, both RANKL and RANK are essential for the development of the lactating mammary gland during pregnancy and for lymph node organogenesis in mouse embryos. Furthermore, RANK has been shown to be expressed on many different epithelial tissues and epithelial tumour cells, and can activate specific downstream signaling pathways. Interestingly, RANKL also stimulates migration of primary breast epithelial cells and osteoclasts, establishing that RANKL-induced cell migration also occurs in normal, non-transformed cells. Importantly, inhibition of RANKL/RANK signaling by OPG in vivo markedly and selectively reduces bone metastasis and tumour burden in a melanoma model that does not activate osteoclasts. These data supported the evaluation of new molecular therapeutics designed to inhibit bone metastases, and these therapeutics have now entered clinical testing. Among them, the humanized monoclonal antibody denosumab (AMG 162) binds RANKL and inhibits its action.

Recently a series of investigations have suggested a potential important role of RANKL-RANK in regulating the metastatic process. Using MMTV-ErbB2 transgenic mice, investigators showed that reduced RANK gene dosage is associated with a significant decrease in the development of lung metastases. Additional data were generated by

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evaluation of the most "metastatic" form of breast cancer. Inflammatory breast cancer (IBC) is the most aggressive form of breast cancer, associated with poor survival despite appropriate multidisciplinary treatment. Patients with IBC have a predictable pattern of disease recurrence including soft tissue, bone and CNS disease. There are few cell lines and animal models currently available to evaluate the biological characteristics of IBC. MARY-X is a human xenograph model of IBC that allowed several important observations. MARY-X spheroids expressed embryonal stem cell markers including potent transcriptional factors, oct-4, nanog, and sox-2, which are associated with stem cell self-renewal and developmental potential. Most importantly, MARY-X spheroids expressed a cancer stem cell profile characterized by CD44(+)/CD24(-/low), ALDH1, and most uniquely, CD133. Furthermore, we recently showed high expression of RANK and RANKL mRNA in MARY-X cells and confirmed in tissue specimens from IBC patients suggesting association with stem cell phenotype. These data support a role of this pathway in the metastatic process and potentially as a modulator of cancer stem cell activity. Moreover, Palafox et al have recently demonstrated that RANKL expression was associated with the acquisition of stemness, EMT and capacity to invade and promote metastases. High expression of RANKL in human tumors was associated with estrogen-receptor negative (ER-)/progesterone-receptor negative (PR-) status and adverse prognosis. Furthermore, RANKL has been associated with chemotherapy resistance in preclinical models and genetic inactivation of the RANKL-R demonstrated inhibition of MPA-induced tumors.

Circulating Tumor Cells (CTCs) in Metastatic Breast Cancer

The natural history of breast cancer suggests that the disease has the capability to develop distant recurrence to specific organs and become a lethal disease. To leave the primary site and to soil in the metastatic niche, cancer cells need to disseminate through the blood and/or lymphatic system. The "seed and soil" theory, postulated by Paget in 1889 and revived fully by Hart in 1980, represents a milestone for the study of CTCs in metastatic breast cancer (MBC) patients. CTCs might represent the seed necessary for cancer dissemination and may be the cells responsible for initiating the metastatic process. In 1869, Thomas Ashworth reported that, "*Cells identical with those of the cancer itself being seen in the blood may tend to throw some light upon the mode of origin of multiple tumors existing in the same person.*" Only recent advances in detection methods have enabled their reproducible identification and further characterization.

Although CTCs provide a link between the primary tumor and metastatic sites, the factors involved with CTC survival in the blood circulation and eventual metastasis are not well understood. So far, much of what is known about CTCs in MBC patients simply involves numbering and prognostic value. Recently, advances in technology have facilitated the detection of even very small numbers of CTCs in the peripheral blood of MBC patients. Research is currently focused on specifically identifying these CTC subsets and characterizing them at the molecular level to ultimately provide a tool that would allow tailoring of treatment on an individual basis. Moreover, the biology of the metastatic process has led researchers to study the plasticity properties of tumor cells that characterize a subset that leaves the primary tumor, invades the blood stream and travels to the specific distant organs with a developed metastatic niche.

CTC identification has been based mostly on the detection of epithelial cell markers, such as the epithelial cell adhesion molecule (EpcAM), intracellular cytokeratin expression, and nuclei presence (4',6-diamidino-2-phenylindole positivity), in patient blood. Identification is further based on the contemporary exclusion of normal blood cells, for example by using WBC markers, e.g. CD45. Because CTCs are present in whole blood in such low numbers, only 1-10 CTCs per mL of whole blood, detection requires enrichment of tumor cells in blood samples. Tumor cells are enriched immunomagnetically using ferrofluids coated with antibodies targeting EpcAM. The CellSearch® system (Veridex Corporation, Warren, NJ, USA) is the only Food and Drug

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Administration-cleared CTC detection system and has the most robust clinical data with reproducible results across different laboratories. *Cristofanilli showed for the first time in 2004 that the number of CTCs detected by the CellSearch® system before starting a new line of treatment is an independent predictor of progression-free survival (PFS) and overall survival (OS) in patients with MBC.* Patients with ≥ 5 CTCs per 7.5 ml of peripheral blood have significantly inferior PFS (median PFS, 2.7 to 8.2 months) and OS (median OS, 10.1 to 21.9 months) compared to patients with < 5 CTCs (median PFS, 7 to 12 months; median OS, 18 to 40.1 months). Moreover, CTC counts at the first follow-up visit showed similar prognostic value. This prognostic utility was independently confirmed by different groups. These interesting findings suggest that CTC enumeration can be used to monitor treatments along with standard imaging modalities.

CTCs enumeration versus Standard imaging assessment

In this regard, Budd et al. compared CTC monitoring to bi-dimensional response assessment (central review) of 177 patients with MBC. Remarkably, the determination of CTCs at baseline and follow-up appeared to have superior prognostic implications in patients with measurable MBC compared to standard imaging assessment, particularly in patients with more refractory disease. Recently, De Giorgi completed a retrospective analysis of 115 MBC patients who started a new line of therapy and who had CTC counts and fluoro-deoxyglucose (FDG)-Positron Emission Tomography (PET) scans performed at baseline and at 9 to 12 weeks during therapy (midtherapy or time of planned restaging, usually around 10-12 weeks). In 102 evaluable patients, the median overall survival time was 14 months (range, 1 to > 41 months). Measurement of CTC at time of restaging demonstrated that detectable levels correlated with FDG-PET/CT response in 68 (67%) evaluable patients. In univariate analysis, midtherapy CTC counts and FDG-PET/CT response predicted overall survival ($P < .001$ and $P = .001$, respectively). FDG-PET/CT predicted overall survival ($P = .0086$) in 31 (91%) of 34 discordant patients who had fewer than five CTCs at midtherapy. Only midtherapy CTC levels remained significant in a multivariate analysis ($P = .004$), further supporting the critical importance of this test in the management of MBC. The correlation between these different monitoring modalities in advanced disease was further refined in a series of subsequent analyses from the same team of investigators with particular regard to patients with bone metastases. The largest study evaluated 195 patients with MBC who were diagnosed with relapsed/progressive MBC. These patients underwent FDG-PET/CT scans and provided blood samples for CTC analysis. One hundred seventeen (60%) patients had received prior treatment of MBC with hormonal therapy (53 cases), chemotherapy with or without hormonal therapy (48 cases), or human epidermal growth factor receptor 2 (HER2)-targeted therapies combined with chemotherapy and/or hormonal therapy (16 cases); 78 (40%) had newly diagnosed MBC. Interestingly, the analysis demonstrated that among the 137 patients with bone metastases at relapse/progression, 83 (61%) had ≥ 5 CTCs, while 54 (39%) had < 5 CTCs ($P = 0.0122$). Higher CTC numbers were detected in patients with bone metastases alone and patients with metastases in bone plus other sites relative to those with no bone metastases. Moreover, higher CTC numbers were detected in the patients with more extensive bone metastases relative to those with one or two bone lesions. With regard to the correlation with imaging findings, all but seven of the 137 patients with bone metastases had increased FDG uptake within one or more lesions. Of these seven cases, four had < 5 CTCs; of the remaining three with ≥ 5 CTCs, two also had liver metastases with elevated FDG uptake (CTCs = 143 and 25), while one presented with primary tumor with elevated FDG uptake (CTCs = 75). This represented the first demonstration of a significant association between CTC detection and bone metastases. Moreover, Giuliano et al showed that CTCs decrease to a value of < 5 CTCs has the most significant impact on PFS and OS but, any decrease can affect prognosis compared to patients with CTCs increase suggesting a possible need to evaluate CTCs decrease as a continuous variable. Patients with ≥ 5 CTCs consistently demonstrated the development of new metastatic sites at time of progression instead of the increase in the size of the original recurrent disease, indicating that measurement of CTCs can be a

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useful tool for evaluation of new anti-metastatic intervention that can affect time to new metastasis (TTNM).

1.2 Intervention background & Overview

Denosumab (AMG 162) is a fully human monoclonal IgG2 antibody to RANKL that binds with high affinity and specificity to the soluble and cell membrane-bound forms of human RANKL. Denosumab binding prevents activation of RANK and inhibits the formation, activation, and survival of osteoclasts. Consequently, bone resorption and cancer-induced bone destruction are reduced.

1.2.1 Preclinical Studies

Anti-tumor activity, toxicology, absorption, metabolism, elimination:

Preclinical studies of Denosumab have been performed in mouse models and non-human primates. Denosumab has no apparent pharmacologic effect in wild-type mice or rats. However, administration of the RANKL inhibitor OPG-Fc acts as a surrogate for denosumab in mouse models of bone metastases. In mouse models of estrogen receptor negative (ER-) human breast cancer, OPG-Fc significantly reduced skeletal tumor burden and osteolytic lesions and increased survival. Pretreatment of mice with OPG-Fc delayed and reduced the development of new bone metastases, osteolytic lesions, circulating markers of bone resorption and number of osteoclasts. Similar results have been shown in mouse models of ER+ human breast cancer, prostate cancer and non-small cell lung cancer treated with OPG-Fc.

Denosumab has been studied in cynomolgus monkeys. Monkeys who received a single dose of denosumab had an acute and significant reduction in bone resorption as assessed by levels of urine N-telopeptide adjusted for urine creatinine (uNTx/Cr). Older monkeys without ovaries who were given long-term (16 months) denosumab had significant gains in mass and density of cancellous and cortical bone and significant reductions in markers of bone turnover (serum C-telopeptide-I [CTX-I], serum bone-specific alkaline phosphatase, and uNTx/Cr).

Single-dose pharmacokinetics (PK) and multiple-dose toxicokinetics of denosumab following intravenous (IV) or subcutaneous (SC) administration have been evaluated in mice, rats, and cynomolgus monkeys. In mice and rats, terminal half-life values were 19 and 11 days, respectively. Maximum serum concentrations after SC doses of 1 mg/kg occurred at 72 hours post-dose in both species. Bioavailability was 86% in mice and 56% in rats.

In cynomolgus monkeys, linear PK was observed at higher doses (1-3 mg/kg). These data led to the estimation that denosumab rapidly and potently suppresses bone resorption in monkeys with a 50% effective concentration of 464 ng/mL. In all species, volume of distribution at steady-state indicated lack of extensive extravascular distribution. Studies using ¹²⁵I-labeled denosumab found that approximately 76-95% of administered radioactivity was recovered in urine and 1-3% was recovered in feces.

Preclinical toxicity studies were conducted in cynomolgus monkeys. No toxicologically significant effects were observed in a study of denosumab administered to monkeys over the course of a year. No treatment-related effects were noted on the cardiovascular or respiratory system. Denosumab showed no adverse effects on embryo-fetal development and no maternal toxicity when given during organogenesis. Effects observed in mothers and infants were consistent with the pharmacological action of denosumab as a monoclonal antibody against RANKL and inhibitor of osteoclastic bone resorption.

1.2.2 Clinical Studies

Phase I Studies, Pharmacokinetics, Anti-Tumor Activity, Dosing, Toxicology, Absorption, Metabolism, Elimination:

Phase I, placebo-controlled studies in healthy volunteers were conducted to evaluate the safety, tolerability, and pharmacodynamic effects of denosumab. Two studies of particular interest evaluated denosumab in subjects with cancer-related bone metastases. In all of the phase I studies, single and multiple doses of denosumab were well-tolerated and effectively reduced bone resorption as measured by uNTx/Cr and CTX-I. Studies of subjects with renal insufficiency indicate that renal impairment does not affect the pharmacokinetics or pharmacodynamics of the drug, but patients with chronic kidney disease or end-stage renal disease appear to be at higher risk of hypocalcemia following treatment.

The pharmacodynamic profile of denosumab was consistent across all populations studied, including subjects with advanced cancer and bone metastases. SC administration of 60mg of denosumab causes a rapid, approximately 70%, reduction in bone resorption within 6 hours as measured by CTX1, and an 85% reduction by 3 days. The pharmacokinetic profile is not affected by age, weight, body mass index, sex, race or renal function. Following SC administration, denosumab exhibits dose-dependent, nonlinear PK over a wide dose range. The maximum serum concentrations (C_{max}) are typically seen 1 to 4 weeks postdose. After C_{max} , serum levels decline over 4 to 5 months with a mean half-life of 25 to 30 days. Bioavailability is approximately 60% after SC dosing. After repeated doses of 120mg every 4 weeks, steady-state was achieved by 6 months.

Phase II studies, studies with same diagnosis as being studied, safety, and anticipated adverse events:

Denosumab has been studied in phase II and III trials evaluating its role as a potential treatment for postmenopausal osteoporosis or male osteoporosis; bone loss due to hormone-ablation therapy in subjects with cancer; rheumatoid arthritis; prevention of bone metastases in at-risk populations; prevention of skeletal-related events in patients with advanced malignancies involving bone; multiple myeloma; giant cell tumor of bone; and hypercalcemia of malignancy. Studies evaluating the role of denosumab in treating or preventing bone malignancies or metastases are of particular relevance.

1.2.3 Rationale for dose selection:

The selection of the denosumab dose and schedule for the phase 3 oncology studies was based on safety, pharmacokinetic, and pharmacodynamic data obtained from phase 1 and 2 studies. Five SC denosumab dose regimens of 30, 120, or 180 mg every four weeks (q4w) and 60 or 180 mg every 12 weeks (q12w), were compared with an IV bisphosphonate in 255 patients with breast cancer and bone metastases. The q12w dosing schedules did not maintain high serum concentrations over the entire dosing interval. Ultimately, the 120 mg q4w dose was selected as the phase 3 dosing regimen because it was well tolerated and achieved maximal suppression of uNTx/Cr over the entire dosing interval in most subjects. On November 18, 2010, the FDA approved denosumab (Xgeva™) for the prevention of skeletal-related events (SREs) in patients with bone metastases from solid tumors. The approval is based on results from three international randomized (1:1) double-blind double-dummy trials in patients with bone metastases comparing denosumab to zoledronic acid. Trial 20050103 enrolled 1,901 patients with hormone-refractory prostate cancer, Trial 20050136 enrolled 2,046 patients with breast cancer, and Trial 20050244 enrolled 1,776

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patients with advanced multiple myeloma or solid tumors other than breast or prostate cancer. In all three trials, patients were randomly assigned to receive either 120 mg denosumab subcutaneously every 4 weeks or 4 mg zoledronic acid intravenously every 4 weeks (dose-adjusted for reduced renal function). Patients with creatinine clearances less than 30 mL/min were excluded, and prior intravenous bisphosphonate therapy was not permitted. The primary outcome measure in each trial was the determination of non-inferiority in time-to-first SRE in patients treated with denosumab as compared to patients treated with zoledronic acid. An SRE was defined as a pathologic fracture, radiation therapy to bone, surgery to bone, or spinal cord compression due to cancer. Other outcome measures included superiority of time-to-first SRE and superiority of time-to-first and subsequent SRE. Denosumab therapy resulted in a statistically significant delay in the time-to-first SRE and in the time-to-first and subsequent SRE compared with zoledronic acid in patients with breast cancer (Trial 20050136) or hormone-refractory prostate cancer (Trial 20050103). In Trial 20050244, denosumab was not less effective than zoledronic acid in delaying the time-to-first SRE and did not demonstrate superiority.

1.2.4 Documented Adverse Events:

The most common adverse reactions in patients receiving denosumab (in at least 25 percent of patients) were fatigue or asthenia, hypophosphatemia, and nausea. The most common serious adverse reaction in patients receiving denosumab was dyspnea. The most common adverse reactions resulting in denosumab discontinuation were osteonecrosis and hypocalcemia.

Hypocalcemia:

Denosumab can cause severe symptomatic hypocalcemia, and fatal cases have been reported from the post-marketing setting based on a recent review of cases. All subjects will be advised to take calcium supplements (at least ≥ 500 mg of elemental calcium) and vitamin D (≥ 400 IU) daily during the study. All subjects will be recommended to receive vitamin D and calcium supplementation unless documented hypercalcemia (albumin-adjusted serum calcium > 2.9 mmol/L [11.5 mg/dL] or ionized calcium > 1.5 mmol/L) develops on study. If documented hypocalcemia occurs, additional short-term calcium supplementation may be necessary.

Osteonecrosis of the Jaw:

Osteonecrosis of the jaw (ONJ) can occur in subjects receiving denosumab. The risk of ONJ is associated with the length of exposure and risk factors such as dental extractions for ONJ. Patients should be monitored for ONJ as part of routine clinical practice. Patients should be instructed to maintain good oral hygiene and avoid invasive dental procedures, if possible.

A dental examination with appropriate preventive dentistry should be considered prior to treatment with denosumab in patients with risk factors for ONJ. Patients who are suspected of having or who develop ONJ while on denosumab should receive care by a dentist or an oral surgeon.

A temporary interruption of treatment is strongly recommended for patients who develop ONJ during treatment with XGEVA based on individual patient's risk/benefit assessment. For patients in whom invasive dental procedures cannot be avoided, the clinical judgment of the treating physician should guide the management plan of each patient based on individual benefit/risk assessment.

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In three phase III active-controlled clinical trials in patients with advanced malignancies involving bone, ONJ was confirmed in 1.8% of patients in the denosumab group treated with 120 mg subcutaneously every 4 weeks. In patients receiving zoledronic acid 4mg every 4 weeks (median exposure of 12.0 months; range 0.1 – 40.5) from the same clinical trials, ONJ was found in 1.3% of patients. The trials in patients with breast or prostate cancer included a denosumab extension treatment phase (median overall exposure of 14.9 months; range 0.1 – 67.2). The patient-year adjusted incidence of confirmed ONJ was 1.1% during the first year of treatment and 4.1% thereafter. The median time to ONJ was 20.6 months (range: 4 - 53).

Atypical femoral fracture:

Atypical femoral fracture has been reported with denosumab. These fractures can occur anywhere in the femoral shaft from just below the lesser trochanter to above the supracondylar flare and are transverse or short oblique in orientation without evidence of communication. Atypical femoral fractures most commonly occur with minimal or no trauma to the affected area. They may be bilateral and many patients report prodromal pain in the affected area, usually presenting as dull, aching thigh pain, weeks to months before a complete fracture occurs. During denosumab treatment, patients should be advised to report new or unusual thigh, hip, or groin pain. Patients presenting with such symptoms should be evaluated for an incomplete femoral fracture, and the contralateral femur should also be examined.

Hypersensitivity:

Clinically significant hypersensitivity including anaphylaxis has been reported with use of denosumab. Reactions may include hypotension, dyspnea, upper airway edema, lip swelling, rash, pruritus, and urticaria. Clinically significant hypersensitivity has been identified as a contraindication for treatment with denosumab. If an anaphylactic or other clinically significant allergic reaction occurs, appropriate therapy will be initiated and denosumab therapy permanently discontinued.

Musculoskeletal pain:

A safety assessment was performed on a cumulative review of clinical data and study serious adverse event and non-study adverse event reports of musculoskeletal pain. Based on this assessment, musculoskeletal pain, including severe cases, has been identified as an adverse drug reaction based on data from post-marketing setting.

1.3 Study Rationale

1.3.1 Rationale for study design and endpoints:

The presence of circulating tumor cells (CTCs) in patients with metastatic breast cancer (MBC) has been associated with shorter survival time than when CTCs are absent. Unlike soluble circulating tumor markers, such as CA15-3 and CA27-29, the number of CTCs seems not to simply reflect tumor bulk. A detailed analysis of tumor burden by the bidimensional sum of the metastatic lesions showed limited correlation between CTC levels and radiographic measurement of tumor load. However, the detection of CTCs in patients with evidence of nonvisceral disease (including metastases in chest wall, lymph nodes, and bone) was of higher prognostic significance than in those with visceral disease. In a retrospective analysis performed at the University of Texas, M. D. Anderson Cancer Center, 195 patients with MBC were evaluated; 103 (53%) had <5 CTCs at relapse/progression and 92 (47%) had >5 CTCs. Patients treated with HER2-

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targeted therapies (15 trastuzumab and one lapatinib) had lower CTC counts with only one case with >5 CTCs at progression during trastuzumab therapy (number of CTCs = 17). Of the 92 patients with >5 CTCs, 83 (90%) presented with bone metastases; furthermore, of 50 cases with 21 CTCs, 48 (96%) had bone metastases. Of 137 patients with bone metastases at relapse/progression, 83 (61%) had >5 CTCs, while 54 (39%) had <5 CTCs ($P = 0.0122$). Higher CTC numbers were detected in patients with bone metastases alone and patients with metastases in bone plus other sites relative to those with no bone metastases. Of the 137 patients with bone metastases, higher CTC numbers were detected in the patients with more extensive bone metastases relative to those with one or two bone lesions. Moreover, patients with ≥ 5 CTCs and bone metastases have a higher chance of developing additional metastatic disease in distant sites compared to a) patients with bone metastases and < 5 CTCs or b) patients with only visceral metastases. Moreover, chemotherapy has been shown to minimally affect CTC values after first-line therapy (approximately 10-15% decline in second-line treatment), suggesting a limited therapeutic benefit of standard therapies. Furthermore, all these patients were receiving zoledronic acid as part of their medical treatment in consideration of their documented metastatic disease to the bone. In summary, detection of CTCs remains a strong prognostic factor in MBC but particularly in patients with bone metastases. Those patients appear to have higher levels of circulating epithelial cells in spite of continuous treatment with bisphosphonates. These observations raise questions about some of the molecular factors that may be responsible for the mobilization of cancer cells in those patients, including the role of RANKL. That subset of patients with more aggressive disease (bone mets and detectable CTCs) could potentially benefit from the development of an agent with CTC-targeting properties.

In summary, we hypothesize that use of the RANKL inhibitor denosumab will decrease the number of CTCs measured in patients with MBC and bone metastases.

We plan to address the change in CTCs as a continuous variable considering the potential benefit associated with any decrease in CTCs but, we will use the $CTC \geq 5$ as a reference cutoff for maximum benefit. We further hypothesize that administration of denosumab will increase the time to progression of disease (new site vs. progression of lesions in previous site), which would be an indicator of the antimetastatic effect of RANKL inhibition.

1.3.2 Rationale for dose selection:

We plan to use the standard dosing regimen of 120mg SC q4w, as established in phase II studies and used in phase III studies. This dose was well-tolerated without significant adverse effects and should pose minimal risk to our patients. (refer section 1.2.3.)

1.3.3 Rationale for study patient population:

Beyond the enumeration of CTCs, molecular characterization provides a key to demonstrating their cellular origin from primary and metastatic tumor deposits, and may also provide clues to their evolution during the course of cancer treatment. B. Aktas et al. studied the ER, PR, and HER2 expressions on CTCs from 87 MBC patients. Blood samples were analyzed for CTCs with the AdnaTest BreastCancer™ (AdnaGen AG, Langenhagen, Germany), which enables the immunomagnetic enrichment of tumor cells via epithelial and tumor-associated antigens. The authors showed that CTCs were ER-negative in 48/62 (77%) patients with ER-positive tumors, and that 46/53 (87%) patients with PR-positive tumors did not express PR on CTCs. Primary tumors and CTCs

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displayed a concordant ER and PR status in only 41% ($p=0.260$) and 45% ($p=0.274$) of cases, respectively. Moreover, regarding the immunohistochemical subtype, most CTCs were triple-negative (39 of 86 patients, 45%) or HER2-positive (27 of 86 patients, 32%). More recently Nole' et al reported that the detection of CTCs is higher in patients with luminal A and B (HER-2) and significantly in patients with bone metastases. The data support the value of CTCs for prognostic and biomarker analysis particularly in patients with HER-2 negative disease and demonstrated ER+ in the primary tumors. Those patients have the highest incidence of bone metastases and a higher percentage of disease presenting with CTC > 5 making them ideal candidates for studies using CTCs as surrogate endpoint of efficacy.

1.3.4 Rationale for exploratory objectives:

We will evaluate gene expression profiles of circulating tumor cells. We will analyze ER and PR expression, of the CTCs. We will also analyze stem cell characteristics of these cells by studying epithelial and tumor associated antigens associated with epithelial/mesenchymal transition. We will also perform Receptor activator of nuclear factor kappa-B ligand (RANKL) analysis.

1.4 Pregnancy and Risk to fetus

Denosumab falls in Pregnancy Category D:

(http://pi.amgen.com/united_states/xgeva/xgeva_pi.pdf)

Defined as “Drugs which have caused, are suspected to have caused or may be expected to cause, an increased incidence of human fetal malformations or irreversible damage. These drugs may also have adverse pharmacological effects.”

Risk Summary:

Xgeva can cause fetal harm when administered to a pregnant woman based on findings in animals. In utero denosumab exposure in cynomolgus monkeys resulted in increased fetal loss, stillbirths, and postnatal mortality, along with evidence of absent lymph nodes, abnormal bone growth, and decreased neonatal growth. There are no adequate and well-controlled studies with Xgeva in pregnant women. Women should be advised not to become pregnant when taking Xgeva.

Clinical Considerations:

The effects of denosumab on the developing human fetus at the recommended therapeutic dose are unknown. The effects of Xgeva are likely to be greater during the second and third trimesters of pregnancy. Monoclonal antibodies are transported across the placenta in a linear fashion as pregnancy progresses, with the largest amount transferred during the third trimester.

In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential. The extent to which denosumab is present in seminal fluid is unknown. There is potential for fetal exposure to denosumab when a male treated with Xgeva has unprotected sexual intercourse with a pregnant partner. Hence, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) to study entry, for the duration of treatment, and for at least 5 months after the completion of treatment.

If the patient becomes pregnant during Xgeva therapy, consider the risks and benefits in continuing or discontinuing treatment with Xgeva.

Animal Data: The effects of denosumab on prenatal development have been studied in both cynomolgus monkeys and genetically engineered mice in which RANK ligand (RANKL) expression was turned off by gene removal (a “knockout mouse”). In cynomolgus monkeys dosed subcutaneously with denosumab throughout pregnancy at a

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pharmacologically active dose, there was increased fetal loss during gestation, stillbirths, and postnatal mortality. Other findings in offspring included absence of axillary, inguinal, mandibular, and mesenteric lymph nodes; abnormal bone growth, reduced bone strength, reduced hematopoiesis, dental dysplasia, and tooth malalignment; and decreased neonatal growth. At birth out to one month of age, infants had measurable blood levels of denosumab (22-621% of maternal levels). Following a recovery period from birth out to 6 months of age, the effects on bone quality and strength returned to normal; there were no adverse effects on tooth eruption, though dental dysplasia was still apparent; axillary and inguinal lymph nodes remained absent, while mandibular and mesenteric lymph nodes were present, though small; and minimal to moderate mineralization in multiple tissues was seen in one recovery animal. There was no evidence of maternal harm prior to labor; adverse maternal effects occurred infrequently during labor. Maternal mammary gland development was normal. There was no fetal NOAEL (no observable adverse effect level) established for this study because only one dose of 50 mg/kg was evaluated.

In RANKL knockout mice, absence of RANKL (the target of denosumab) also caused fetal lymph node agenesis and led to postnatal impairment of dentition and bone growth. Pregnant RANKL knockout mice showed altered maturation of the maternal mammary gland, leading to impaired lactation.

2.0 OBJECTIVES

2.1 Primary Objectives

To assess the effect of denosumab in Her2/neu negative ER+ and/ or PR+ metastatic breast cancer patients who are in PR or SD after starting systemic therapy with bone metastases and ≥ 5 CTCs by measuring the fraction of patients with reduction in CTCs after 3 cycles of denosumab.

2.2 Secondary Objectives

- To assess the effect of denosumab on CTCs enumeration considered as a continuous variable (percent change from baseline) in this population.
- To evaluate median Progression-free survival (m-PFS).

2.3 Exploratory objective

- CTC enumeration after enrichment.
- To assess the effect on CTC profiling and characterization of stem cell phenotype (CTC-EMT).
- To evaluate the type of progressive disease (new site vs. progression of lesions in previous sites).
- To analyze the expression of RANKL.

3.0 PATIENT ELIGIBILITY

The target population for this study is in patients with Her2/neu negative ER+ and/ or PR+ Metastatic Breast Cancer (MBC) with bone metastases and detectable circulating tumor cells (CTCs). This will be a single-center trial conducted at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

A total of 42 patients can be enrolled in order to obtain 37 evaluable subjects needed for this trial. Approximately 5 potentially eligible patients are seen per month, and it is anticipated that at least 2 per month will be accrued. Potential patients may be referred to the Principal Investigator (PI) at Northwestern University, Dr. Massimo Cristofanilli (312) 695-0990.

Eligibility will be evaluated by the study team according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be

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registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

3.1 Inclusion Criteria

- 3.1.1** Patients must have histologically or cytologically confirmed ER and/or PR positive, HER-2/neu negative metastatic breast cancer. They can be enrolled in any line of therapy without investigational agents and should have stable disease or a partial response (which can be determined clinically) on current systemic treatment. Patients must also have pathologic OR radiographic evidence of bone metastases and ≥ 5 CTCs.

(Note: the pathology report that is used by the physician to determine diagnosis, will be used to determine patient eligibility. ER and PR status should be available at the time of registration).

- 3.1.2** Patients may have either measurable or non-measurable within 30 of days of registration. (Lesions treated with radiation therapy must not be used as a target lesion).

(Note: Per RECIST criteria v. 1.1, Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension.

Non-measurable disease is defined as All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with P10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, and inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses /abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.[see Appendix 3]).

- 3.1.3** Patients may be enrolled in any line of standard treatment (without investigational agents). The start date of current treatment should be at least two 2 weeks or more prior to registration.

(Note: Patients will continue to receive the planned active treatment with chemotherapy or endocrine therapy (standard of care) and initiate denosumab at the recommended dose for this protocol.)

- 3.1.6** Age ≥ 18 years. Both men and women are eligible for this study.

- 3.1.7** ECOG performance status 0- 2 (see Appendix 1).

- 3.1.8** Patients must have normal organ and marrow function as defined below within 30 days of registration;

- Leukocytes $\geq 3,000/\text{mcL}$ (without growth factor)
- Platelets $\geq 100,000/\text{mcL}$ (with or without transfusion)
- Hemoglobin ≥ 8 (with or without transfusion)
- AST&ALT (SGOT&SGPT) ≤ 2.5 times institutional upper limit of normal (For patients with liver metastasis up to ≤ 5 times of ULN is allowed)
- Bilirubin ≤ 1.5 ULN

(For patients with liver metastasis up to ≤ 5 times of ULN is allowed)

- Serum Creatinine ≤ 1.5 ULN

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- Creatinine clearance ≥ 60 mL/min/1.73 m² for patients with creatinine levels above institutional normal (creatinine clearance should be calculated per institutional standard)

3.1.9 Patients must have a serum calcium of ≥ 2.0 mmol/L (8.0mg/dL) or albumin-adjusted serum calcium ≤ 2.9 mmol/L (11.5mg/dL) within 30 days of registration.
(Note: If patients are undergoing treatment for hypocalcemia and the serum calcium value at screening is >8.0 mg/dl, then the patient will be eligible for this study).

3.1.10 Females of child-bearing potential (FOCBP) and males with his or her partner must agree to use two acceptable methods of effective contraception (see Appendix 2), at study entry, for the duration of study participation, and for 5 months following completion of therapy. Subjects who are surgically sterile (e.g., history of bilateral tubal ligation, hysterectomy) or whose sexual partner is sterile (e.g., history of vasectomy) are not required to use additional contraceptive measures. Should a female patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Likewise, if a male patient impregnates his female partner, he should inform the treating physician immediately.

NOTE: A FOCBP is *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- *Has not* undergone a hysterectomy or bilateral oophorectomy
- *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.11 FOCBP must have a negative serum OR urine pregnancy test ≤ 7 days prior to registration.

3.1.12 Ability to understand and willingness to sign a written informed consent and HIPAA consent document prior to registration.

3.1.13 Willingness and ability of subject to comply to study requirements.

3.2 Exclusion Criteria

3.2.1 Patients may not be receiving any other investigational agents. A 2 week wash-out period for investigational agents is required before registration.

3.2.2 Patients with clinically symptomatic brain metastases or who required treatment for brain metastases within 4 weeks of registration (Stable sequelae acceptable if treatment has been completed. These lesions cannot be used as target lesions).

3.2.3 Patients who have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to Denosumab are not eligible (i.e. same class of drugs)

(Note: Prior bisphosphonates are allowed. Patients could have received bisphosphonates or be bisphosphonate-naïve. Patients who were previously on bisphosphonates can be enrolled in the study, as long as they have a wash-out period of 2 weeks prior to registration).

3.2.4 Patients who are on corticosteroids or immunosuppressant's are not eligible. A 2 week wash-out period for is required before registration.

- 3.2.5** Patients who have a known additional malignancy that is progressing or requires active treatment are not eligible. Patients who have had a prior diagnosis of cancer and if it has been < 3 years since their last treatment are also not eligible. NOTE: Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
- 3.2.6** Patients who have an uncontrolled intercurrent illness including, but not limited to any of the following, are not eligible:
- Hypertension (defined as 160/90 mmHg for 3 consecutive readings 2-5 mins apart) that is not controlled on medication.
 - Symptomatic congestive heart failure.
 - Unstable angina pectoris.
 - Psychiatric illness/social situations that would limit compliance with study requirements.
 - Any other illness or condition that the treating investigator feels would interfere with study compliance or would compromise the patient's safety or study endpoints.
- 3.2.7** Subject is pregnant or breast feeding, or planning to become pregnant within 5 months after the end of treatment.
- 3.2.8** Known HIV-positive patients who are on combination antiretroviral therapy. (This is because of the potential for pharmacokinetic interactions with denosumab.)
- 3.2.9** No known prior history or current evidence of osteonecrosis/osteomyelitis of the jaw.
- 3.2.10** No known prior history or current evidence of untreated local gum or oral infection.
- 3.2.11** No known/planned active dental or jaw condition which requires oral surgery, including tooth extraction.
- 3.2.12** No known non-healed dental/oral surgery, including tooth extraction.
- 3.2.13** Patients have planned invasive dental procedures during the course of the study.

4.0 TREATMENT PLAN

4.1 Overview

This is a Phase II targeted to enroll a maximum of 42 patients to get 37 evaluable patients with confirmed ER and/or PR positive, HER-2/neu negative metastatic breast cancer receiving any line of standard therapy, for their disease. Patients will continue to receive the planned active treatment with chemotherapy or endocrine therapy (standard of care) and initiate denosumab (XGeva®) at 120 mg SQ every 4 weeks x 3 doses. Patients will have repeated measurement of CTCs and be re-evaluated by standard imaging (CT of metastatic sites or PET/CT) at study enrollment and again after 3 cycles of denosumab. At that time we will determine that fraction of patients with decrease in CTCs. In this cohort, a decrease of CTCs during standard treatment is expected in 40-50% and a normalization of CTCs (< 5 CTCs) is expected only in 10-15% of patients; a decrease and a normalization higher than expected from historical cohorts will indicate benefit of the current treatment. In the absence of treatment delays due to adverse events, treatment may continue for 3 cycles on study or until one of the criteria listed in Section 4.4. Patients will be off the treatment phase of the study, but will continue the

same treatment (e.g. chemotherapy with denosumab), as standard of care, under their treating physician. Patients will still be considered on study but in follow-up for purpose of survival endpoints.

4.2 Treatment Administration

All subjects will be treated in cycles of 28 days (4 weeks). There is a window of +/-7 days for scheduling a visit. Treatment will continue until disease progression, unacceptable toxicity, patient withdrawal or death. Missed doses will not be made up. If treatment delays are > 14 days, patients will be taken off study. Patients will still be followed up for survival.

All study treatment visits should be scheduled to coincide with standard of care visits or treatment visits. Patients do not need to come in separately for research treatment/evaluation. They will continue to receive their standrd of care treatment with chemotherapy or endocrine therapy, and the study drug Denosumab will be initiated. Denosumab will be administered on Day 1 of each 28 day cycle, at the dose of 120mg subcutaneously for a total of 3 cycles. There are no required premedications.

Regimen description					
Agent	Premeditations, precautions	Dose	Route	Schedule	Cycle Length
Denosumab	None	120mg	Subcutaneous	Day 1 (+/-7 days) for 3 cyclesd	4 weeks (28 days)

4.2.1 Investigational Agent: Denosumab

Denosumab 120mg to be given Subcutaneous (SQ) in clinic on Day 1 of each cycle for a total of 3 cycles. One cycle is defined as 28 days.

Note: Patients will continue to receive the planned active treatment with chemotherapy or endocrine therapy (standard of care) and initiate denosumab (XGeva®) Patients will be admitted as out patients in the Lurie Cancer Center, and drug will be administered by nursing staff. All treatment visits will coincide with standard of care visits.

Risks (local) associated with subcutaneous injection of Denosumab

- red, dry, or itchy skin
- oozing or crusty blisters on skin
- peeling skin

4.2.2 Missed or skipped doses

Administration of Denosumab may be done in a +/-7 days window due to weather emergencies, missed appointments, holiday or other unplanned events not related to toxicity. Missed doses will not be made up. If treatment delays are > 14 days, will be taken off study. Patients will still be followed up for survival.

4.2.3 Supportive Care therapy and pre-medications:

Patients should receive appropriate supportive care therapies and pre-medications that would otherwise be given with the chemotherapy or endocrine therapy regimen they are getting concurrently with Denosumab, including the recommended Vitamin D and calcium as outlined below. Patients should also receive full supportive care therapies concomitantly during the study including transfusions of blood and blood products, antibiotics when appropriate. Palliative radiation therapy is permitted for irradiating areas of painful bony metastases that cannot be managed adequately using systemic or local analgesics, as long

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as the presence of these osseous metastases was recorded at baseline prior to study initiation and there is no definite evidence of objective disease progression at the site based on RECIST1.1 criteria (Appendix 3). These sites cannot be used as target lesions or as part of the patient's response.

4.2.4 Recommended Supplemental therapy:

It is strongly recommended that all patients receive daily supplements of at least 500 mg calcium and at least 400 IU of vitamin D, unless documented hypercalcemia (albumin-adjusted serum calcium >2.9mmol/L [11.5mg/dL] or ionized calcium > 1.5mmol/L [6.0mg/dL] develops on study.

4.2.5 Concomitant therapies requiring special attention

The use of erythropoietin, colony stimulating factors (per ASCO guidelines), antiemetic agents, nonsteroidal anti-inflammatory drugs (Ibuprofen (400 mg qid) can be administered in patients with normal renal function (CrCl >60 mL/min). Patients with mild to moderate renal insufficiency (CrCl from 45 to 59 mL/min) should avoid taking salicylates or NSAIDs with long elimination half-lives.

4.2.6 Permitted concomitant medications

Glucocorticosteroids may be used as anti-emetics and megestrol acetate may be used for appetite.

4.2.7 Prohibited concomitant medications:

Use of bisphosphonates are not permitted while participating in this study. However, patients who were previously on bisphosphonates can be enrolled in the study, as long as they have a wash-out period of 2 weeks prior to registration. Use of concurrent investigational agents is not permitted.

4.3 Toxicity Management & Dose Delays/Modifications

4.3.1 General Principles

Dose modifications will not be permitted with denosumab. Concomitant chemotherapy or hormonal therapy doses may be modified as per the primary treating oncologist.

4.3.2 Specific Toxicities and Dose Delays

Denosumab dosing will be held only for the study treatment-related toxicities listed below. Please note that there is a dosing window of +/- 7 days for all other delays due to logistical or administrative issues.

- The dose of denosumab should be held in the event of hypocalcemia < Institutional lower limit of normal (LLN). In the event of clinically significant hypocalcemia, correction may be achieved with intravenous calcium repletion.
- In the event of a hypersensitivity reaction, subsequent doses of denosumab will not be given and the patient will be removed from the study.
- The dose of denosumab should be held and permanently discontinued in the event of development of Osteonecrosis of the jaw(ONJ)
- Any patient who receives at least one dose of study therapy will be evaluable for toxicity endpoints. Each patient will be assessed for the development of toxicity according to the timeframe referenced in the Schedule of Events table. Toxicity will be assessed according to NCI CTCAE version 4.03.

4.4 Duration of Therapy and Criteria for Study Termination

In the absence of treatment delays due to adverse events, study treatment may continue for 3 cycles or until one of the following criteria applies:

- Disease progression

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- Patients with clinical or radiological evidence of progressive disease per RECIST 1.1 (Appendix 3)
- Inter-current illness that prevents further administration of treatment
- Prolonged delay of treatment administration related to concurrent illness or toxicity would be an indication for study termination. If treatment delays are > 14 days, study therapy will be discontinued.
- Adverse Events- Administration of investigational product (denosumab) will be withheld for any subject who experiences:
- A grade 3 or 4 adverse event per NCI CTCAE version 4.03 related or possibly related to investigational product, and/or any AEs in the treating physician's discretion that would affect the safety of the patient.
- Any grade of osteonecrosis of the jaw (ONJ).
- Patient becomes pregnant (in case of females).
- Patient decides to withdraw from the study.
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.

4.5 Duration of Follow up

Follow-up beyond the 30-day post-last-dose visit will be done approximately every 12 weeks (+/-2 weeks) for a maximum of 2 years. These follow-up visits will be done at the same time as a standard of care visit. Alternatively, it can also be done by phone calls.

4.6 Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted).
- Patient withdraws consent (no follow-up permitted).
- Patient is unable to comply with protocol requirements.(follow-up permitted)
- Patient demonstrates disease progression. follow-up permitted
- Patient experiences unacceptable toxicity. follow-up permitted
- Treating physician determines that continuation on the study would not be in the patient's best interest. follow-up permitted
- Patient becomes pregnant. follow-up permitted
- Patient develops a second malignancy that requires treatment which would interfere with this study. follow-up permitted
- Patient becomes lost to follow-up (LTF). This is defined as 3 attempts, made 1 week apart, to contact patient by preferred method of contact.

4.7 Patient Replacement:

If a patient is enrolled in the study but comes off study before cycle 1 day 1 of treatment, the patient may be replaced by the DMC. Request should be made to the QA monitor for replacement by DMC.

5.0 STUDY PROCEDURES

Assessments	Baseline (-30 days)	W1 (+/-7 days)	W5 (+/-7 days)	W9 (+/-7 days)	Off study treatment (W 13 +/-7 days)	Follow-up ⁸
Informed Consent ¹	X					
Medical History & eligibility	X					
Physical Exam ²	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X
Vitals ³	X	X	X	X	X	
ECOG ⁵	X	X	X	X	X	
CBCw/diff, plts	X					
Serum Chemistry ⁴	X	X	X	X	X	
AE/Toxicity Assessment	X	X	X	X	X	X
Tumor measurements ⁷	X ⁷	X ⁷			X ⁷	X ⁷
Pregnancy test (urine or serum) ¹¹	X					
CTC Measurement ⁶	X ⁶					
Denosumab administration ⁹		X	X	X		
Research blood tests	X ¹⁰				X ¹⁰	
Survival ⁸					X	X

1 cycle= 4 weeks (28 days)

¹ Informed consent must be signed within 30 days of registration. If signature is outside that window the patient must sign a new consent.

²A full physical exam will be done as indicated.

³Vitals include temperature, blood pressure, pulse rate, and weight. Height will be taken only at baseline.

⁴Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

⁵ See Appendix 1 for ECOG performance status table.

⁶Standard CTC measurement to be done at baseline. Two CellSave tubes will be used to collect approximately 20 ml of peripheral blood. Tubes will be kept at ambient temperature (22-24 degrees C).

(Note: This baseline sample will be a standard of care procedure and will be sent to QUEST diagnostics through the hospital.)

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⁷Tumor measurements are by CT. PET-CT can be done as an adjunct per investigator discretion. Tumor measurements are required at baseline/screening. It does not need to be repeated unless it is >6 weeks from Day 1 of study treatment. It will be repeated after 3 cycles. Documentation (radiologic or clinical) must be provided for patients removed from study for progressive disease. After 3 cycles, CT will be done per physician discretion as a standard of care procedure. In the follow up phase, patients will have scans every 12 weeks (+/- 2 weeks) up to 2 years for PFS data.

⁸Follow up for *PD*, resolution of treatment related toxicities, and survival should be every 12 weeks (+/- 2 weeks) up to 2 years (or more frequently if clinically indicated) for PFS data. Survival will be assessed during standard clinic visit or by phone call. *Note: Any labs or scans relating to a toxicity or disease measurement should be conducted at an interval determined by the treating investigator and results should be recorded on the applicable long term follow up case report forms.*

⁹ Denosumab administration is standard of care. Doses may be given after completion of study therapy, but not as part of the study.

¹⁰ Blood will be collected for correlative studies to analyze the expression of RANKL, and additional CTC analyses including enrichment, enumeration and CTC profiling and characterization of stem cell phenotypes. Blood samples for these research tests will be collected at baseline and at 3 months, coinciding with Denosumab administration.

- i) 8ml in 1 10ml Cellsave tube (for RANKL expression).
- ii) 7.0 ml each in 2 Adna Collect syringes (for CTC enrichment, enumeration and CTC profiling and characterization of stem cell phenotypes-)
- iii) 20ml in 2 CellSave tubes (for CTC measurement **after 3 cycles of Denosumab** [wk13];

(Note: Tubes will be kept at ambient temperature [22-24 degrees C])

(Note; these tests will be only be for research purposes and will be conducted at the CTC lab at northwestern university [see section 9.0 and the study lab manual for details]).

¹¹Pregnancy test (urine or serum) is only required for FOCBP (refer to section 3.1.10)

6.0 ENDPOINT ASSESSMENT

6.1 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.

The Response Evaluation Criteria in Solid Tumours (RECIST 1.1, Refer Appendix 3) criteria will be used for objective tumour response assessment.

Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension.

Non-measurable disease is defined as All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with P10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, and inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses /abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques

Assessments will be performed after three cycles of treatment. Once protocol treatment has been completed subjects will be assessed every three months or sooner as indicated by treating physicians.

6.2 Primary Objectives

To assess the effect of denosumab in in Her2/neu negative ER+ and/ or PR+ metastatic breast cancer patients who are in PR or SD after starting systemic therapy with bone metastases and ≥ 5 CTCs (who are on standard systemic therapy) by measuring the fraction of patients with reduction in CTCs after 3 cycles of denosumab.

Only those patients who have received *at least one cycle of therapy*, and have had their disease re-evaluated will be considered evaluable for response.

6.3 Secondary Objectives

- To assess the effect of denosumab on CTCs enumeration considered as a continuous variable (percent change from baseline).
- To evaluate median Progression-free (m-PFS) survival time for the entire cohort.

Only those patients who, have received *at least one cycle of therapy*, and have had their disease re-evaluated will be considered evaluable for response.

6.4 Exploratory objective

- The correlative study outcome will be CTC enumeration after enrichment.
- To assess the effect on CTC profiling and characterization of stem cell phenotype (CTC-EMT).
- To evaluate the type of progressive disease (new site vs. progression of lesions in previous site)
- To analyze the expression of RANKL.

CTCs are present in whole blood in low numbers (only 1-10 CTCs per mL of whole blood). Therefore, enrichment of tumor cells in peripheral blood samples is required for their detection and enumeration. The correlative study outcome will be CTC enumeration after enrichment. Refer to Section 9.0 for methodology.

Enumeration of CTCs will be every 3 months while patients remain on study. Enumeration is standard of care and is ordered as a clinical CTC test.

To analyze the expression of RANKL, approximately 8ml of blood will be collected in a 10 ml CellSave tube at the same time as when the CTC sample is collected (at baseline and at 3 months, coinciding with Denosumab administration).

(Note; this test will be only be for research purposes and will be conducted at Dr. Zhaomei Mu's laboratory at northwestern university[see section 5.0 and 9.0 for details]).

7.0 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (<http://cancer.northwestern.edu/CRO/data/DataandSafetyMonitoringPlanMay2014.pdf>). The level of risk attributed to this study requires high as outlined in the DSMP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (see Section 5 for time points). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event once consent is signed, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Definitions & Descriptions**7.2.1 Adverse Event**

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment 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

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associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

7.2.2 Severity of AEs

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v4 is available at <http://ctep.cancer.gov/reporting/ctc.html>

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 30 days after the last administration of study drug, must be reported upon discovery or occurrence.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **Results in death.**
If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- **Is life-threatening.**
The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- **Requires in-patient hospitalization or prolongation of existing hospitalization for \geq 24 hours.**
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly/birth defect.**

- **Is an *important medical event*.**

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".

For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.2.4 Unanticipated Problems Involving Risks to Subject or Others

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is *unanticipated* in terms of nature, severity, or frequency
- Places the research subject or others at a different or *greater risk of harm*
- Is deemed to be *at least possibly related* to participation in the study.

7.3 Adverse Event Reporting

7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF according to the time intervals noted in the appendices. Routine AEs will be reviewed by the Data Monitoring Committee (DMC) according to the study's phase and risk level, as outlined in the DSMP.

7.3.2 Determining if Expedited Reporting is Required

This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

- 1) Identify the type of adverse event using the NCI CTCAE v 4.03.
- 2) Grade the adverse event using the NCI CTCAE v 4.03.
- 3) Determine whether the adverse event is related to the protocol therapy. Attribution categories are as follows:
 - Definite: AE is clearly related to the study treatment.
 - Probable: AE is likely related to the study treatment.
 - Possible: AE may be related to the study treatment.
 - Unlikely: AE not likely to be related to the study treatment.
 - Unrelated: AE is clearly NOT related to the study treatment.
- 4) Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:
 - the current protocol
 - the drug package insert
 - the current Investigator's Brochure

7.3.3 Expedited Reporting of SAEs/Other Events

7.3.3.1 Reporting to the Northwestern University QAM/DMC

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Completion of the NU CRO SAE Form is required.

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The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient’s identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the DMC at their next meeting.

7.3.3.2 Reporting to the Northwestern University IRB

The following information pertains to the responsibilities of the lead site (Northwestern University). Additional participating sites should follow their local IRB guidelines for reporting to their local IRBs.

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

7.3.3.3 Reporting to Amgen

All SUSARs, SAEs, and Aggregated reports should be reported to the Amgen as stated in the table below. The investigator or qualified designee must complete the Medwatch form) and send it electronically to Amgen contact:

Lorie B. Bruno
 Manager, Investigator-Sponsored Studies
 Non-Amgen Sponsored Clinical Research (NASCR)
 PRA Health Sciences Providing Services to Amgen
 Phone: 570-732-4059
 Cell: 570-778-8765
 Fax: 913-307-5758
 Email: lorieb@amgen.com

AGGREGATE REPORTS	
Safety Data	Timeframe for submission to Amgen
Annual Safety Report (e.g. EU Clinical trial Directive[CTD]), Annual safety report, US IND Annual report)	Annually

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<p>Other aggregate analyses (any report containing safety data that is generated during the course of the study)</p>	<p>At the time of ISS sponsor submission (Anybody governing research conduct e.g. RA, IRB etc.)</p>
<p>Final (End of study report)</p> <ul style="list-style-type: none"> • Unblinding data for blinded studies • Reports of unauthorized use of a blinded product 	<p>At the time of ISS sponsor submission (anybody governing research conduct RA, IRB etc.) but not later than one calendar year of study completion</p>

8.0 DRUG INFORMATION

8.1 Agent Denosumab

Denosumab is a human IgG2 monoclonal antibody with high affinity and specificity for RANKL. Denosumab has an approximate molecular weight of 147 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells. Denosumab is commercially available and this commercial supply will be used for this study.

8.1.1 Other names: AMG162, Xgeva

8.1.2 Classification - human IgG2 monoclonal antibody with high affinity and specificity for RANKL.

8.1.3 Mode of action: Denosumab is a human IgG2 monoclonal antibody with high affinity and specificity for RANKL

8.1.4 Storage and stability

Denosumab should be stored protected from light and according to the storage and expiration information provided in the package insert.

8.1.5 Protocol dose specifics

Denosumab 120mg to be given subcutaneous (SQ) in clinic on Day 1 of each cycle for a total of 3 cycles. One cycle is defined as 28 days.

Note: Patients will continue to receive the planned active treatment with chemotherapy or endocrine therapy (standard of care) and initiate denosumab (XGeva®) (Refer to section 4.0 for further details).

8.1.6 Preparation

Denosumab is provided as a sterile, colorless to slightly yellow, practically free from particles, preservative-free solution intended for subcutaneous injection. The vial presentations contain 60 mg/mL denosumab, 17 mM sodium acetate, and 4.7% (w/v) sorbitol, at a pH of 5.2, filled to a target deliverable volume of 1.0 mL; or 70 mg/mL denosumab, 18 mM sodium acetate and 4.6% (w/v) sorbitol, at a pH of 5.2, filled to a target deliverable volume of 1.7 mL. The prefilled syringe (PFS) drug product contains denosumab at 60 mg/mL, 17 mM sodium acetate, 4.7% (w/v) sorbitol, and 0.01% (w/v) polysorbate 20, at a pH of 5.2, filled to a target deliverable volume of 1.0 mL.

8.1.7 Route of administration for this study

Denosumab will be administered subcutaneously. The use of a 27-gauge needle is recommended for administration. A 30-gauge needle should not be used, as this may affect the quality of the product.

8.1.8 Incompatibilities: none known

8.1.9 Availability & Supply

Denosumab is commercially available and will be administered as standard of care drug. The drug will be obtained through prescription from the treating physician. The cost of the drug will be billed to the patient's insurance.

8.1.10 Side effects

Risks associated with denosumab:>10%:

- hypertension
- fatigue
- headache
- swelling in legs or ankle
- lowering of the phosphate levels
- lowering of the calcium levels
- nausea
- decreased appetite
- vomiting
- constipation or diarrhea
- anemia
- infection (influenza)
- weakness or joint pains
- limb pain or back pain
- shortness of breath or cough

1% to 10%:

- chest pain
- sciatica
- high cholesterol levels
- passing more gas
- new secondary cancer
- osteonecrosis of the jaw (infection and damage of the jaw bone)
- cataract
- upper respiratory infection

<1% (Limited to important or life-threatening):

- developing antibodies to the drug
- infection of the heart
- fracture of femur
- hypersensitivity/allergy including anaphylaxis
- low blood pressure
- pancreatitis

8.1.11 Nursing implications

All research visits will coincide with Standard of Care visits. Vitals will be monitored per Standard of Care and Institutional guidelines.

8.1.12 Return and Retention of Study Drug

Not applicable

Commercial supply of Denosumab will be used.

9.0 CORRELATIVES/SPECIAL STUDIES

9.1 Enrichment and Enumeration of CTCs

CTCs are present in whole blood in low numbers (only 1-10 CTCs per mL of whole blood). Therefore, enrichment of tumor cells in peripheral blood samples is required for their detection and enumeration.

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9.1.2 Enrichment and Molecular Characterization of CTCs using Adna Test

AdnaTest BreastCancer and AdnaTest EMT-2/StemCell allow molecular characterization of CTCs by combining AdnaTest BreastCancerSelect with

AdnaTest BreastCancerDetect and AdnaTest EMT-2/StemCellSelect with AdnaTest EMT-2/StemCellDetect. AdnaTest BreastCancerSelect and AdnaTest EMT-2/StemCellSelect are highly specific immunomagnetic cell-selection system for enriching CTCs from peripheral blood. AdnaTest BreastCancerDetect and AdnaTest EMT-2/StemCellDetect allow sensitive analysis of breast cancer-associated gene and EMT/tumor stem cell-associated gene expression in immunomagnetically enriched CTCs by reverse transcription (RT) and PCR analysis.

Please see the Laboratory Manual for detailed blood collection, processing and shipping instructions.

AdnaTest can be performed using two methods based on the gene expression profile. The assay will be performed using reagent kits (QIAGEN) according to the manufacturer's protocols.

Please see the Laboratory Manual for detailed blood collection, processing and shipping instructions.

9.1.3 AdnaTest BreastCancerSelect/Adna Test Breast Cancer Detect

Adna Test BreastCancer Select generates a magnetic bead-labeled cell lysate that is used for further analysis with the *Adna Breast Cancer Detect* kit (QIAGEN) RT-PCR with the *Breast Cancer Detect* test generates tumor gene fragments GA733-2, Muc-1, Her-2.

The test is considered positive if a PCR fragment of at least one tumor associated transcript is clearly detected. Peaks with a concentration of > 0.30 ng/μl are positive. Peaks that are not detected at the above setting are negative (i.e., concentration < 0.15 ng/μl). Peaks with an intermediate concentration of 0.15 ng/μl to 0.30 ng/μl are inconclusive and require a re-testing.

Method

Enrichment of CTCs from peripheral blood will be performed using the AdnaTest BreastCancerSelect kit (QIAGEN). The kit contains BreastSelect Beads in a solution containing sodium azide. BreastCancerSelect enables the immunomagnetic enrichment of tumor cells via epithelial and tumor associated antigens. Antibodies against epithelial and tumor associated antigens are conjugated to magnetic beads (Dynabeads) for the labeling of tumor cells in peripheral blood. The labeled cells are extracted by a magnetic particle concentrator (AdnaMag-L and MPC-S) and are subsequently lysed. The cell lysate is used for further analysis with the AdnaTest BreastCancerDetect.

AdnaTest BreastCancerDetect is used for the detection of breast cancer-associated gene expression in immunomagnetically enriched tumor cells by RT-PCR. BreastCancerDetect contains oligo (dT)₂₅-coated beads for the isolation of mRNA from the lysate of pre-enriched tumor cells. RT results in cDNA, which is the template for tumor cell detection and characterization by multiplex-PCR. With the PrimerMix BreastDetect three tumor associated antigens and one control gene are amplified.

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The primers generate fragments of the following sizes:

- GA733-2: 395bp
- Muc-1: 293bp
- Her-2: 270bp
- Actin: 114bp (internal PCR control)

In addition to the *BreastCancerDetect*, the *ER/PR-Detect* is available for the PCR-expression analysis of hormone receptor gene for estrogen and progesterone in enriched tumor cells. With the *PrimerMix ER/PR-Detect* the hormone receptor genes for estrogen, progesterone and one control gene, actin, are amplified.

The primers generate fragments of the following sizes:

- ER: 305bp
- PR: 270bp
- Actin: 119bp (internal PCR control)

Using the AdnaTest *ER/PR-Detect*, the test is considered positive for ER if a peak with a concentration of ≥ 0.15 ng/ μ l is detected and positive for PR if a peak with a concentration of > 0 ng/ μ l is detected.

9.1.4 AdnaTest EMT-2/Stem CellSelect/ AdnaTest EMT-2/Stem CellDetect:

AdnaTest EMT-2/Stem CellSelect generates a magnetic bead-labeled cell lysate that is used for further analysis with AdnaTest EMT-2/Stem CellDetect. The results of the test generate EMT associated gene fragments Akt-2, Twist 1, PI3K α , and Actin (control), and tumor stem-cell marker ALDH1

Method:

Enrichment of CTCs from peripheral blood will be performed using the AdnaTest EMT-2/Stem CellSelect kit (QIAGEN). The kit enables the immunomagnetic enrichment of tumor cells via epithelial and tumor associated antigens. Antibodies against epithelial and tumor associated antigens are conjugated to magnetic beads (Dynabeads) for the labeling of tumor cells in peripheral blood. The labeled cells are extracted by a magnetic particle concentrator and are subsequently lysed. The cell lysate is used for further analysis with the AdnaTest *EMT-2/Stem CellDetect*.

The AdnaTest *EMT-2/Stem CellDetect* is used for the analysis of EMT-2/Stem Cell characteristics in immunomagnetically enriched circulating tumor cells by RT and The kit uses oligo (dT)₂₅-coated beads for the isolation of mRNA from the lysate of pre-enriched tumor cells. RT results in cDNA, which is the template for tumor cell detection and characterization by multiplex-PCR. With the *PrimerMix EMT-2* three EMT related genes and one control gene are amplified. The *PrimerMix Stem Cell* amplifies ALDH1, which is accepted as tumor stem-cell marker. The *PrimerMix Breast Detect* can be used to determine breast cancer associated gene expression.

The different primer-mixes generate the following fragments:

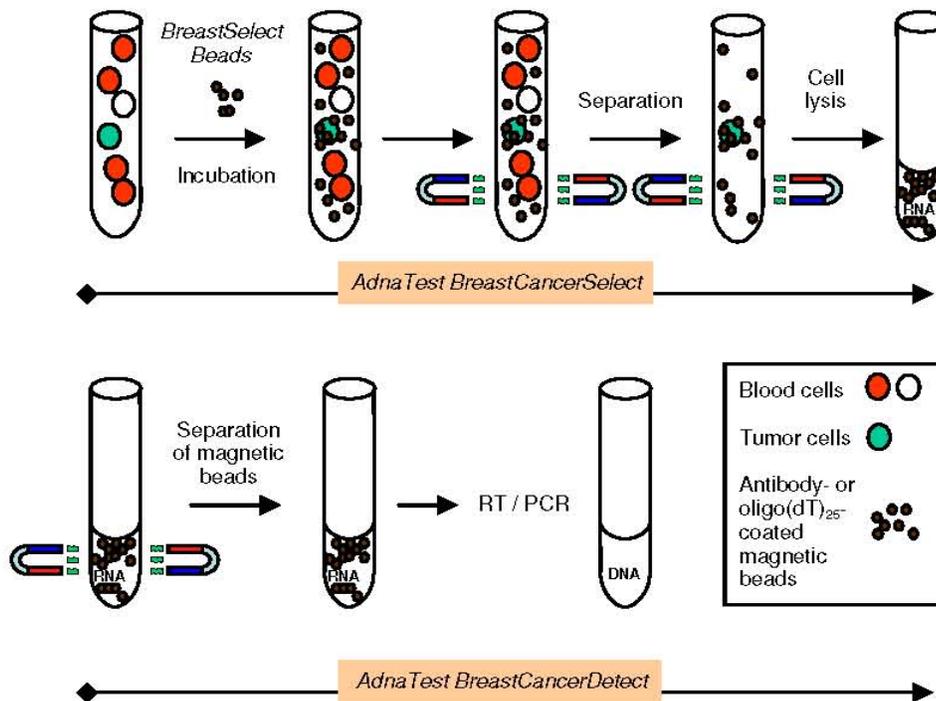
EMT-2:

- Akt-2: 309bp
- Twist 1: 201bp
- PI3K α : 551bp
- Actin: 111bp (Internal PCR Control)

Stem Cell:

- ALDH1: 161bp

9.2 Figure 1. Schematic Overview of Sample Preparation



9.2.1 Enumeration of CTCs and RANKL analysis:

Samples will be maintained at room temperature and processed within 96 h after collection. Enumeration of CTCs and RANKL expression on CTCs will be performed on CellSearch System (Janssen Diagnostics, LLC) according to the manufacturer’s standard CTC measurement protocol. Briefly, 7.5 mL of whole blood will be processed on the CellSearch system using CellSearch CXC kits (Janssen Diagnostics). The CellSearch system consists of a semiautomated system (CellPrep) for the preparation of the sample and a CellSpotter Analyzer with semi-automated fluorescence-based microscopy system for the identification of CTCs by cellular images. At the same time of CTCs enumeration, expression of RANKL on CTCs will be stained by PE (phycoerythrin)-conjugated specific RANK antibody (R&D Systems). CTCs will be identified by positive staining for cytokeratins (CK) and DAPI (double stranded DNA), and negative staining for anti-CD45 (leukocytes marker). RANK-positive CTCs will be identified by positive staining for both CK and RANK, and by negative staining for anti-CD45. Quantitative results are expressed as the total number of CTCs and RANK-positive CTCs.

(Note; this test will be only be for research purposes and will be conducted at Dr. Zhaomei Mu’s laboratory at northwestern university [also see section 5.0])

Please see the Laboratory Manual for detailed blood collection, processing and shipping instructions.

(Note: left over blood samples from all correlative tests will not be banked for future use.)

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For any question related to correlative tests, please contact:

CTC Lab at Northwestern University

Zhaomei Mu, MD
 Research Assistant Professor
 Department of Medicine-Hem/Oncology
 Feinberg School of Medicine
 710 N. Fairbanks Ct, Olson Rm: 8-523
 Chicago, IL 60611;
 Tel: **312-503-5489(primary)**
 312-503-3025(secondary)
 Email: Zhaomei.mu@northwestern.edu

10.0 STATISTICAL CONSIDERATIONS**10.1 Study Design/Endpoints**

We will conduct a single arm, two-stage Phase II study to assess the efficacy of a combination therapy including Denosumab in patients with bone metastatic breast cancer and detectable circulating tumor cells (CTCs). *The primary outcome is evidence of reduction in the number of CTCs.* A patient will be defined as a “success” if the number of CTCs at follow-up is less than the number observed at baseline. Patients who do not experience a decrease in CTC count at follow-up or are missing follow-up CTC count measurements will be defined as “failures”. The new combined treatment would be of interest if the proportion (p) of successes is at least 70%. A success proportion of less than 50% will be of no interest. We will test the null hypothesis that the success proportion is 0.5 versus the alternative that this proportion is 0.7. After testing the combination therapy on 23 patients in the first stage, the trial will be terminated if there are 12 or fewer successes. If the trial goes on to the second stage, a total of 37 patients will be studied. If the total number of successes is less than or equal to 23, the combination therapy will be rejected. The type I error for this Simon minimax design (Simon, 1989) is 4.8% and the power is 80%. The probability of early termination under the null hypothesis (i.e., $p=0.5$) is 66.1%.

Based on data from phase III studies of denosumab, we anticipate that the probability of serious adverse events related to these agents including acute reaction, renal failure or osteonecrosis of the jaw (if meet criteria for serious adverse event)) will not exceed 0.05. In this trial, accrual will be suspended if, at any point among the first 23 patients, four or more subjects are observed with a treatment related serious adverse event (TRSAE). Assuming that the true probability of a TRSAE is 5%, the probability that we will observe at least four subjects with a TRSAE among the first 23 patients is 2.58%. Similarly, accrual will be suspended if, at any point in the trial, six or more subjects are observed with a TRSAE. If the true TRSAE is 5%, then the probability that the study will be declared as too toxic at any point is 2.93%. Alternatively if the true TRSAE rate is 20%, then the probability that the study will be terminated early (i.e., ≤ 23 subjects accrued) is 70.4% and the chance of declaring the treatment as too toxic at any point in the study exceeds 82%. The population for safety analyses will include all patients who receive at least one dose of the combination therapy including denosumab.

10.2 Sample Size/Accrual Rate

We plan to enroll up to 42 patients to obtain 37 evaluable patients. We anticipate that patients will be accrued at a rate of 2-3 per month.

10.3 Stratification Factors

No stratification is planned.

10.4 Statistical Analysis

For the primary objective, the proportion of successes will be estimated using a bias-corrected estimator and its confidence interval as described by Porcher and Desseaux (2012).

To assess the effect of denosumab on CTCs enumeration considered as a continuous variable, the percent change in CTCs from baseline (i.e., $100 \times [\text{number of CTCs at follow-up} / \text{number of CTCs at baseline}]$) will be calculated and compared to zero using either a one sample t-test or a Wilcoxon signed rank test.

To evaluate median progression-free survival (PFS) time for the entire cohort, Kaplan-Meier curves will be used. Cox proportional hazards models with time dependent covariates will be used to assess the relationship between longitudinal CTC counts (as defined by 2 or more CTCs evaluation in the course of treatment) and PFS.

To detect the antimetastatic effect of denosumab, the first progression will be classified as occurring either at a new site or at a previous site. A competing risk PFS curve will be calculated with new site occurrence as the primary event and previous site as the competing event. Similarly, a competing risk PFS curve will be calculated with previous site occurrence as the primary event and new site as the competing event. These two curves will be compared descriptively to determine the recurrence pattern of both types of outcomes. Greater occurrence at previous sites would indicate an antimetastatic effect.

11.0 STUDY MANAGEMENT

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by Janssen Scientific Affairs. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

11.3 Registration Procedures

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive a subject identification number:

- Eligibility eCRF (complete in NOTIS)
- Eligibility checklist (signed and dated by the treating physician – upload in NOTIS)
- Signed and dated informed consent document (upload in NOTIS)
- Pathology Report (upload in NOTIS)

The QAM will review the registration, register the patient, assign an identification number, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.4 Data Submission

Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document). Generally, for all phase II patients, data are due at the end of every cycle.

11.5 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to Appendices for additional information). The level of risk attributed to this study requires high level of monitoring as outlined in the DSMP (<http://cancer.northwestern.edu/CRO/data/DataandSafetyMonitoringPlanMay2014.pdf>) The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the Appendices for additional data submission instructions.

11.6 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.6.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

11.6.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.
- Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation may be considered an instance of Reportable New Information (RNI) if it:

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- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.7 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

11.8 Publication Policy.

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. For trials that require high intensity monitoring, the assigned QAM will prepare a preliminary data summary (to be approved by the DMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data collection for the primary endpoint). If the investigator's wish to obtain DMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DMC at their next available meeting, and a final, DMC-approved dataset will be released along with any DMC decisions regarding publication. The investigators are expected to use only DMC-approved data in future publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers.

REFERENCES

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APPENDICES

APPENDIX 1 ECOG PERFORMANCE STATUS

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

APPENDIX 2

Barrier Methods	Intrauterine Device Methods	Hormonal Methods
<ul style="list-style-type: none"> • Male condom plus spermicide • Cap plus spermicide • Diaphragm plus spermicide 	<ul style="list-style-type: none"> • Copper T • Progesterone T • Levonorgestrel-releasing 	<ul style="list-style-type: none"> • Implants • Hormone shot or injection • Combined pill • Minipill • Patch

NOTE: choice of contraception should be discussed with primary treating oncologist to discuss the risks and benefits of different modalities of contraception.

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APPENDIX 3

Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

Can be accessed at:

http://ctep.cancer.gov/protocolDevelopment/docs/recist_guideline.pdf