**The University of Arizona Cancer Center**
**Hematology Oncology Section**
**Breast Cancer Program**

# A single arm Phase II trial to assess association of BRCA1 protein expression with overall response rate in patients with metastatic breast cancer on pegylated liposomal doxorubicin

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**Title of Study:** A single arm Phase II trial to assess association of BRCA1 protein expression with overall response rate in patients with metastatic breast cancer on pegylated liposomal doxorubicin

**Primary Objective:** To evaluate if low BRCA1 protein expression has a preferential effect on response when metastatic breast cancer patients are treated with a DNA damaging chemotherapy agent.

**Secondary Objective:** To evaluate if low BRCA1 protein expression has a preferential effect on tumor progression when metastatic breast cancer patients are treated with a DNA damaging chemotherapy agent.

**Primary Endpoint:** To correlate BRCA1 protein expression, as measured by IHC with overall response rate for metastatic breast cancer patients treated with pegylated liposomal doxorubicin. The overall response rate is defined as the percentage of evaluable patients who achieve complete response (CR) or partial response (PR) as measured by RECIST 1.1 on CT or PET/CT as the best overall response

**Secondary Endpoint:** To correlate BRCA1 protein expression, as measured by IHC with median progression free survival for metastatic breast cancer patients treated with pegylated liposomal doxorubicin. The progression free survival is measured as the times from the start of pegylated liposomal doxorubicin to the time the patient is first recorded as having disease progression or die

**Background information:**

It is well known that women with germline mutations in BRCA1 or BRCA2 are at increased risk for developing various malignancies[1]. Defective homologous recombination, which occurs as a result of these mutations, impairs the normal repair mechanism for DNA double stranded breaks (DSB)[2]. Alternative error-prone, potentially mutagenic DNA repair mechanisms like non-homologous end joining (NHEJ) and single stranded annealing (SSA) take over and lead to genomic instability[3]. This "homologous recombination deficiency" (HRD) may be a critical pathway in development of cancers, and also make the affected cells more susceptible to DNA damaging drugs like alkylators, anthracyclines and platinum agents[3]. Somatic mutations in BRCA1 and BRCA2 do not occur frequently in sporadic breast cancer[4] but potentially any (somatic) inactivation of the genes could result in loss of their phenotypic expression[5]. A phenomenon called "BRCAness" has been reported in sporadic cancers that do not have the germline mutations in BRCA1 or BRCA2, but display similar inactivation of the BRCA related genes and defective HRD. The reported incidence in the literature ranges from 15% to 50%[6, 7]. The incidence varies with the histology, with higher percentage of triple negative tumors expressing "BRCAness" signature (30-60%)[8] compared to hormone receptor positive tumors (5-20%)[6, 7]. Detecting this underlying tumor cell defects in effective DNA repair could provide crucial information in attempting to "personalize" treatment for breast cancer patients, directing DNA targeted therapy to such patients.

There have been published studies assessing HRD (primarily involving BRCA1 and BRCA2) in breast cancer and associating it with response to neoadjuvant chemotherapy. Rodriguez et al reported higher sensitivity to anthracycline-based therapies in patients whose tissue samples had a defective DNA repair gene signature[9]. In a study with similar design by Lips et al[10], about half of triple negative tumors were found to have BRCA1 inactivation but it was only modestly predictive of response. However, in ER positive tumors a BRCA2 like-aCGH

was found in a surprising proportion of this neoadjuvant population (43%) and was strongly predictive of excellent response to chemotherapy. Vollenbergh et al reported association of BRCA1 signature and benefit from high dose platinum-based chemotherapy[11]. They reported improved recurrence free survival and overall survival for patients who had BRCA1 like aCGH when they received high-dose platinum treatment. Currently, there is no standard assay to detect HRD. Some early efforts have been reported in the literature[3, 13, 14, 15] including array comparative genomic hybridization (aCGH), RT-PCR, multiplex ligation-dependent probe amplification and immunohistochemistry (IHC). The most frequently used and reported assay is DNA based aCGH to assess copy number variation of BRCA1 or BRCA2 or both. All the published studies have defined "BRCA1 like signature" independently and currently, there is no standardized definition. Moreover, DNA or RNA based approaches require fresh frozen tissue for optimal results, expertise in genomic analysis and interpretation (which is not readily available in non-academic setting) and time for tissue analysis and interpretation. To date, those techniques are not yet ready for utilization on a day to day basis where decisions regarding treatment are time-sensitive. IHC can be rapidly translated into current clinical practice due to the ability to use FFPE tissue which is readily available in all academic and community practices. Furthermore, after an IHC method has been validated it can be replicated in standard clinical testing laboratories and easily added to the standard breast diagnostic IHC panel (ER, PR, HER2 and Ki 67).

In collaboration with the Tissue Core and Cellular/Molecular Analysis core (TACMASS) at the University of Arizona Cancer Center we have validated a BRCA1 IHC assay, which can detect BRCA1 protein expression in breast tumors samples. Pegylated liposomal doxorubicin is frequently used in the salvage treatment of patients with metastatic breast cancer[16]. It is a DNA damaging chemotherapy agent which has been reported to have a 25% overall response rate and 2.5 months median progression free survival in the salvage treatment of metastatic breast cancer[17]. It has been shown to be safe in patients who have received prior anthracycline treatment.

As far as we are aware, there have not been any studies or reports evaluating BRCA1 expression prospectively in the context of relative benefit from DNA damaging agents. We propose such a trial in which patients with metastatic breast cancer who will receive pegylated liposomal doxorubicin as a salvage treatment. The goal is to discern whether there appear to be effects on relative benefit from this agent, which can be determined by assaying BRCA1 protein expression on tumor specimens available from the patient.

**Study Design:** This study is a prospective single arm Phase II trial to assess the association of BRCA1 protein expression with overall response rate in patients with metastatic breast cancer treated with pegylated liposomal doxorubicin

**Study Centers:** University of Arizona Cancer Center, University of New Mexico Cancer Center

**Number of Patients:** We plan to consent 50 to 60 patients with the goal of accruing a total of 50 patients into this prospective single arm, Phase II trial.

**Main Criteria for Inclusion/Exclusion:**

To be eligible for inclusion, each patient must have:

- Metastatic breast cancer and have formalin-fixed, paraffin–embedded tumor available for testing BRCA1 protein expression
- Adults over 18 years of age
- Have resolution of all acute toxic effects of any prior chemotherapy or radiotherapy to NCI CTC grade ≤ 1 prior to study registration.

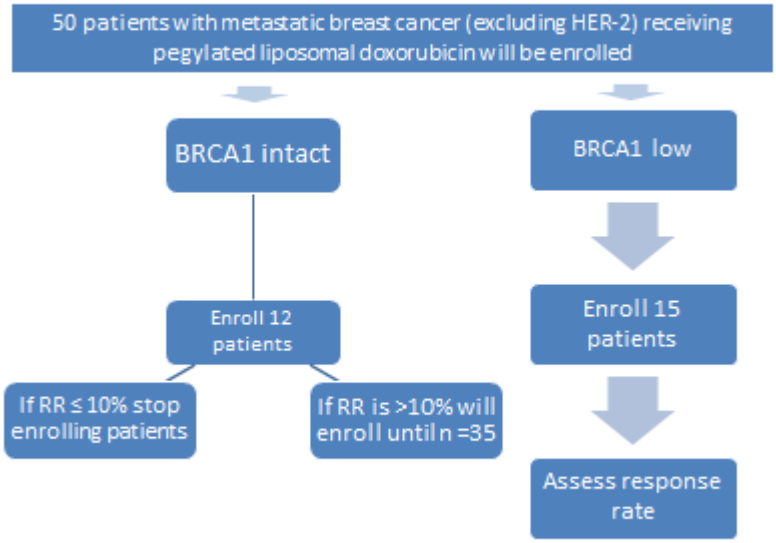
- Be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study specific screening procedures
- Be willing and able to comply with the treatment plan, scheduled clinic visits, laboratory and oncological tests and other study procedures
- Have a ECOG performance status of 0 – 2
- Measurable disease by CT by RECIST 1.1 to evaluate response. Patients with bone only lesions are also eligible to enroll. Criteria to monitor for response will be based on non-target lesions per RECIST criteria
- Adequate bone marrow function defined as platelets $\geq$ 100 X $10^9$ cells/L, neutrophils $\geq$ 1.5 x $10^9$ cells/L, white blood cells (WBC) $\geq$ 3.0 x $10^9$ cells/L and a hemoglobin $\geq$ 90 gm/L
- Liver function tests (AST and/ or ALT) should be $\leq$ 2 x upper limit of normal (ULN, defined as per laboratory where blood testing is done), total biirubin $\leq$ 1.5 x ULN (except for patients with liver metastases, ALT and/or ALT $\leq$ 5 times the upper limit of normal is accepted)
- Patients who are already on pegylated liposomal doxorubicin will be eligible to participate in this study as long as they meet the other inclusion/exclusion criteria and can sign informed consent

**Exclusion Criteria**The following will exclude patients from study participationMyocardial infarction within 6 months of registrationBrain metastases unless documented to be controlled post completion of local therapy (surgery and/or radiation therapy) for at least four weeks prior to registration Pregnant or breast feeding women. Women with child bearing potential must use effective measures to prevent pregnancy while receiving pegylated liposomal doxorubicin Have a concurrent active non-breast malignancy except for non-melanoma skin cancerHave a concurrent active non-breast malignancy except for non-melanoma skin cancerHer2 positive tumors as defined by FDA guidelines (3+ immunohistochemical staining, defined as uniform, intense membrane staining of more than 10% of invasive tumor cells, and for cases with 2+ staining showing gene amplification by FISH, expressed as a ratio of more than 2 when comparing HER-2 gene and chromosome 17 fluorescent signals)[18,19]

**Intervention:** Patients with metastatic breast cancer treated at the University of Arizona Cancer Center and University of New Mexico Cancer center will be enrolled in this trial. Patients whose treatment for their tumor includes liposomal doxorubicin are eligible to take part in this study. Their primary tumors will be obtained for evaluation of BRCA1 protein expression. When primary tumor is not available, paraffin embedded metastatic tumor will be tested for BRCA1 protein expression. Patients will receive pegylated liposomal doxorubicin intravenously at 30mg/$m^2$ every 21 days[18].

**Duration of Intervention:** Patients will get a baseline PET/CT or CT chest/abdomen/pelvis scan prior to receiving pegylated liposomal doxorubicin. We will repeat the same imaging scan at 9-12 weeks to evaluate response by RECIST 1.1. Pegylated liposomal doxorubicin will be stopped if there is evidence of disease progression. Also, it will be discontinued if there is development of persistent $\geq$ grade 3 fatigue or non-reversible $\geq$ grade 4 hematological or renal or liver abnormalities or if the patient chooses.

**Schema**




**Statistical Methods:** This study is a prospective single arm Phase II trial to assess the association of BRCA1 protein expression to overall response rate when metastatic breast cancer patients are treated with pegylated liposomal doxorubicin. We plan to consent 50 to 60 patients with the goal of enrolling a total of 50 patients to this study. It is anticipated that 30% of patients will have low BRCA1 protein expression and therefore anticipate approximately a 35 to 15 patient split for intact BRCA1 to low BRCA1 protein expression.

**Definition of primary endpoint**: Response rate is defined as the percentage of evaluable patients who achieve complete response (CR) or partial response (PR) as measured by RECIST 1.1 on CT or PET/CT as the best overall response.

**Definition of secondary endpoints**: Progression free survival will be measured as the times from the start of pegylated liposomal doxorubicin to the time the patient is first recorded as having disease progression or dies. If a patient does not progress or die while being followed via tumor assessment, progression-free survival will be censored at the time of last disease assessment.

**Sample size determination**

**Low BRCA1 protein expression**: Since it is expected only 30% of patients will have low BRCA1 protein expression, we will accrue 15 patients to this arm and estimate the response rate among these patients. With a sample size of 15 we will be able to estimate the response rate of 50% in patients with low BRCA1 protein expression with a 95% confidence interval width of ± 25%.

**Normal BRCA1 protein expression**: A Simon two-stage design will be used in which a 10% response rate is considered not promising, a 30% response rate is considered promising, and the probabilities of a type I error (falsely accepting a non-promising therapy) and type II error

(falsely rejecting a promising therapy) are set at 0.10 and 0.10, respectively[20]. In this scenario, the maximum trial size would be 35 patients. In the first stage of this design, 12 patients with intact BRCA1 expression will be accrued. If at least two responses are observed among these 12 patients, then an additional 23 patients will be accrued to the second stage. If 0 or 1 response is observed among the initial 12 patients, then accrual to this cohort will be terminated and declared negative. At the end of the study if 6 or more patients in this cohort respond then pegylated liposomal doxorubicin will be considered worthy of further study in this cohort. This design yields at least 90% probability of a positive result if the true response rate is at least 30% and a 90% probability of a negative result if the true response rate is 10%.

**Analytic plan for primary objective**:

**Low BRCA1 protein expression**: The number and percentage of patients falling into response category will be tabulated. The estimate of the response rate will be presented with 2-sided 95% exact binomial confidence intervals. We anticipate this data to be hypothesis generating and will form the basis for a future prospective clinical trial enrolling larger number patients to evaluate if low BRCA1 expression, as determine by IHC, is predictive of response to treatment with DNA damaging agents.

**Normal BRCA1 protein expression**: The estimate of the response rate will be presented with 2-sided 95% exact binomial confidence intervals. If 5 or fewer patients with response are observed among the 35 patients, then there will be no further investigation of the treatment. If 6 responses are observed out of 35 patients, there is 80% confidence that the true response rate >11.2%.

**Analytic plan for secondary objective**:
The progression free survival in patients with low BRCA1 protein expression and intact BRCA1 protein expression will be analyzed by Kaplan-Meier methodology. The results will be summarized by 25th, 50th (median), and 75th percentiles with associated 2-sided 95% confidence intervals,

**Interim Analysis**

**Low BRCA1 protein expression:** No interim analysis will be conducted

**Normal BRCA1 protein expression:** An interim analysis for futility will be conducted in this group. The response rate will be used as the endpoint for the interim analysis for this group. The interim analysis will be performed when the first 12 treated patients are accrued. Based on Simon's 2-stage design, if less than 2 responders are observed out of these initial 12 evaluable patients in normal BRCA1 protein expression, patient enrollment will be discontinued into this arm. Otherwise patient enrollment will continue until a total of 35 patients evaluable for response are enrolled. If only 1 response is observed out of 12 patients, there is 80% confidence that the true response rate <22.1%.

**Feasibility Issues:** Patients with metastatic breast cancer (exclude Her-2/neu patients) whose primary tumor tissue is available for assessing defective DNA repair will be identified by the medical oncologists at the University of Arizona Cancer Center and the University of New Mexico to be included and treated with pegylated liposomal doxorubicin in the salvage setting. During the past year we have treated more than 25 patients with metastatic breast cancer with liposomal doxorubicin. We anticipate to complete accrual of 50 patients in 2 years. If we do not

accrue as expected after year 1, we plan to expand enrollment to other institutions we are currently collaborating (University of Washington and University of New Mexico).

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# 1. Introduction

## 1.1. Breast Cancer

Breast cancer is a heterogeneous disease with different clinico-pathological features, responses to treatment and prognoses. Progress has been made in the development of targeted therapies (for example HER-2/neu targeting monoclonal antibodies and small molecule inhibitors) that have made a substantial improvement in both response rates and survival. Despite these clinical advances there are still 40,000 women who die in the US of breast cancer each year.[1] Therefore, there is a continuing need to search for additional therapeutic strategies. All patients with metastatic breast cancer eventually receive treatment with chemotherapy. In the available menu of chemotherapy agents, clinicians frequently select therapy based on the patient's treatment history, prior response rates to similar classes of drugs, toxicity profiles, patient preference, etc. Currently, there is no proven clinical predictive marker for selecting the most efficacious chemotherapy agent outside the setting of the targeted therapies i.e. HER2 or ER targeted antitumor agents. Better methods for choosing the most efficacious treatment would spare patients undue toxicity and lead to improved response rates.

## 1.2. Background

It is established that women with germline mutations in breast cancer 1, early onset gene (BRCA1) or breast cancer 2, early onset gene (BRCA2) are at increased risk for developing breast and ovarian cancers.[2] In addition, there is a higher risk for pancreatic, prostate and male breast cancer.[2] This is thought to be related to the roles BRCA1 and BRCA2 genes play in DNA repair. DNA damage activates cell cycle check points and recruitment of DNA repair machinery. In cells deficient in the BRCA1 or BRCA2 genes there is defective DNA repair of double-stand DNA breaks (DSB) through homologous recombination (HR), a conservative DNA repair mechanism with a high degree of fidelity. Alternative error-prone, potentially mutagenic DNA repair mechanisms like non-homologous end joining (NHEJ) and single stranded annealing (SSA) compensate for this loss, but lead to genomic instability.[3] The relative roles of BRCA1 and BRCA2 in repair of DNA DSB have been explored and better defined over the past two decades. BRCA1 is a critical organizing molecule that has been linked to a range of cellular processes beyond DNA repair, including transcriptional regulation and chromatin remodeling. BRCA2 function in HR is primarily via regulation of RAD51 activity.[4] BRCA2 regulates RAD51 recombinase, which is a critical step in strand invasion and homology directed repair.[4]

Germline mutation in one BRCA1or BRCA2 allele is sufficient to predispose for cancer development.[5] There is a loss of heterozygosity, with loss of the normal allele while retaining the mutant allele, in the tumor tissue of susceptible individuals, suggesting that the genes may have a role as tumor suppressors.[6,7] Somatic mutations in BRCA1 or BRCA2 do not occur frequently in sporadic (or non-familial) breast cancer[8] but potentially any (somatic) inactivation of the genes could result in phenotypic suppression of BRCA, [9] a phenomenon called "BRCAness" (or more properly "BRCAlessness"). This phenomenon has been reported in sporadic cancers which do not have germline mutations in BRCA1 or BRCA2, but display similar inactivation of the BRCA related genes and consequently have defective HR.[3]

**Preferential effect of chemotherapy in relation to BRCA1 or BRCA2 expression**

The key roles BRCA1 and BRCA2 genes play in DNA repair are through HR, thus cells deficient in these proteins could be more sensitive to chemotherapy agents which produce DNA damage. DNA injury can be induced by strand breaks through failure to reseal cleavable complexes in strand passage or by intercalation with base pairs (i.e. anthracyclines) or by DNA adduct formation (i.e. alkylators and platinating agents) with subsequent intra- or interstrand DNA crosslinks and resultant double strand breaks.[10] Most published clinical studies assessing homologous recombinant deficiency (HRD) involve BRCA1 signatures and are retrospective.

Rodriguez et al reported higher sensitivity to anthracycline-based therapies in patients whose tumor samples had a defective DNA repair gene signature (determined by quantitative RT-PCR in a 69 gene low density array).[11] In a study with similar design by Lips et al, approximately 50% of triple negative tumors were found to have BRCA1 inactivation (determined by aCGH, mRNA levels, or BRCA1 promoter methylation) but it was only modestly predictive of response.[12] Vollenbergh et al reported association of BRCA1 like signature (determined by aCGH) to be associated with a higher recurrence free survival and overall survival after high dose platinum-based chemotherapy.[13] One reported prospective trial of 28 patients reported higher pathological complete responses after neoadjuvant cisplatin, in patients with BRCA1 mutations, low BRCA1 mRNA levels, or BRCA1-promoter methylation.[14]

DNA damaging chemotherapy can potentially influence expression of BRCA1 protein in treated tumor cells as a potential mechanism of resistance. There is currently only 1 report in the literature assessing change in expression of BRCA1 in primary and metastatic tumors.[21] This was assessed in 9 BRCA1 germline mutation patients with ovarian tumors who were treated with a platinum-containing chemotherapy regimen. Their primary and metastatic tumor specimens were evaluated for BRCA1 mutations by DNA sequencing and BRCA1 protein expression by IHC. Four of the specimens showed reversal of mutations by DNA sequencing however clinical implication of that change was unclear. In addition this data only included patients with germline mutations and not in sporadic tumors which have an acquired loss of BRCA1. Currently, there is no data suggesting that acquired BRCA1 loss can be reversed with treatment. We propose to determine if reversal of BRCA1 protein expression is observed after DNA damaging chemotherapy as a secondary aim.

**Detecting "BRCAness"**

One area of controversy is the best method for detecting "BRCAness" in tumor samples. Several efforts to identify a "BRCAness" signature have been reported using different methodologiesincluding array comparative genomic hybridization (aCGH), quantitative real time- polymerase chain reaction (RT-PCR), multiplex ligation-dependent probe amplification (MPLA) and immunohistochemistry (IHC).[13-15]Techniques have been reported using frozen or formalin-fixed tumor tissue. As all the published studies have defined a "BRCA1 like signature" independently there is currently no standardized definition. Moreover, DNA or RNA based approaches require fresh frozen tissue for optimal results, expertise in those fields for interpretation (which is not readily available in non-academic setting) and significant time for tissue analysis and interpretation. Consequently, none of these methodologies are readily conducive for utilization by the clinician who is required to make time-sensitive treatment decisions. IHC can be rapidly translated into current clinical practice as both community

and academic pathologists utilize IHC on a routine basis from FFPE tissue and are competent in performing the assays and interpreting the results. Furthermore, IHC methods can be readily validated and replicated in standard clinical laboratories and added to the standard breast cancer diagnostic IHC panel (ER, PR, HER2, and Ki 67).

**Preliminary data**:

In collaboration with the Tissue Core and Cellular/Molecular Analysis core (TACMASS) at the University of Arizona Cancer Center (UACC) we have validated a BRCA1 IHC assay in breast tumor samples. Antigen retrieval techniques and antibody titers have been optimized using the Genscript A00490 Rb polyclonal antibody. We retrospectively analyzed 45 breast tumor samples, 37 of which were triple negative tumors. Low BRCA1 protein expression was found in 43% of the triple negative tumors. This result is in concordance with other reported series of BRCA1 signatures using DNA and/or RNA based approaches. Clinical information was available in 37 patients. A total of 21 patients received anthracycline based chemotherapy of which 13 had intact BRCA1 and 8 had low BRCA1 protein expression. Fewer breast cancer recurrences were seen in patients with low BRCA1 protein expression (2/8) compared to those with intact BRCA1 protein expression (6/13). However, in this small sample of assessed tumors, chemotherapy was administered at different time points (neoadjuvantly or adjuvantly) and follow up was not standardized in all patients. Nevertheless, these results support our hypothesis that low BRCA1 protein expression predicts increased sensitivity to treatment with DNA damaging chemotherapy.

DNA damage leads to the early activation of BRCA1 where it plays a vital role in the homologous recombination (HR) repair of double stranded DNA. Unlike other mechanisms of DNA repair, BRCA1 mediated HR tends to be conservative and error free. Thus cells deficient in BRCA1 protein are uniquely susceptible to agents/mechanisms which cause DNA damage due to their inability to repair through HR. We propose to test the clinical utility of BRCA quantification by immunohistochemical (IHC) analysis for predicting response to chemotherapy in the prospective setting. Our **hypothesis** is that low BRCA1 protein expression in primary breast tumors of metastatic breast cancer patients predicts improved response to DNA damaging chemotherapy in the metastatic setting.

**Methodology:**

**IHC staining for BRCA1**: Tumor samples will be reviewed to confirm adequate sample for staining. Tissues sections will be stained for BRCA1 by Genscript A00490 Rb polyclonal clone antibody using the Benchmark Ultra automated stainer (Ventana Medical Systems Inc, Tucson, Arizona). Low BRCA1 protein expression will be defined as ≤5% of cells staining with 1+ intensity of the tumor nucleus (i.e. nuclear long score ≤ 5)[16]. Intact BRCA1 protein expression will be defined as ≥10% of cells staining with 1+ intensity or ≥5% of cells staining with 2+ intensity of their nucleus (i.e. nuclear long score of ≥10). Nuclear long score of 6-9 i.e. 5-10% of cells staining with 1+ or <5% of cells stain with 2+ intensity, will be defined as indeterminate status.

**Quantitation of IHC**: Two Board Certified pathologists (Raymond B. Nagle,MD, PhD and Lauren Lebeau, MD) will evaluate each case using a semi quantitative histologic scoring method as previously described.[19] Briefly, staining intensity for neoplastic cell's nucleus will be scored as: 0 negative, 1 weak, 2 moderate and 3 intense. In addition,

the percentage of positive neoplastic cells will be evaluated. The overall scores will be calculated by multiplying the intensity by the corresponding percentage of positive cells, resulting in a values ranging from 0 to 300. Low BRCA1 protein expression will be defined as nuclear long score of ≤ 5, intact BRCA1 as nuclear long score of ≥ 10, indeterminate BRCA1 expression as nuclear long score of 6-9.

**Chemotherapy agent**: Pegylated liposomal doxorubicin is frequently used in the salvage treatment of patients with metastatic breast cancer. It is a DNA damaging chemotherapy agent which has been reported to have a 25% overall response rate and 2.5 months median progression free survival in the salvage treatment of metastatic breast cancer[16]. It has been shown to be safe in patients who have received prior anthracycline treatment[17].

1.3. **Study Population**

This study will enroll patients with HER2 negative metastatic breast cancer whose treatment includes liposomal doxorubicin.. The enrolled patients will be treated with pegylated liposomal doxorubicin at 30mg/m$^2$ every 21 days

## 2. Trial Objectives

**Primary Objective:** To evaluate if low BRCA1 protein expression has a preferential effect on response when metastatic breast cancer patients are treated with DNA damaging chemotherapy agent.

**Secondary Objective:** To evaluate if low BRCA1 protein expression has a preferential effect on tumor progression when metastatic breast cancer patients are treated with DNA damaging chemotherapy agent.

## 3. Trial Design

### 3.1. Study Endpoints

**Primary Endpoint:** To correlate BRCA1 protein expression, as measured by IHC with overall response rate for metastatic breast cancer patients treated with pegylated liposomal doxorubicin. . The overall response rate is defined as the percentage of evaluable patients who achieve complete response (CR) or partial response (PR) as measured by RECIST 1.1 on CT or PET/CT as the best overall response

**Secondary Endpoint:** To correlate BRCA1 protein expression, as measured by IHC with median progression free survival for metastatic breast cancer patients treated with pegylated liposomal doxorubicin. The progression free survival is measured as the times from the start of pegylated liposomal doxorubicin to the time the patient is first recorded as having disease progression or dies.

3.2. **Study Design** This study is a prospective single arm Phase II trial to assess the association of BRCA1 protein expression with overall response rate in patients with HER2 negative metastatic breast cancer treated with pegylated liposomal doxorubicin. Patients who qualify for enrollment into the study will receive pegylated liposomal doxorubicin as a single agent intravenous at 30mg/m$^2$ every 21 days

3.3. **Study Duration and Follow up**

All patients will be treated with pegylated liposomal doxorubicin until evidence of tumor progression. Patients will get a baseline CT or PET/CT scan prior to receiving pegylated liposomal doxorubicin. Repeat imaging at week 9 -12 (± 7 days) will be performed to evaluate response. Subsequent imaging schedule will be left to the treating physician's discretion. All patients will be treated until documented disease progression, intolerable toxicity or withdrawal of consent.

## 4. Selection and Withdrawal of Patients

### 4.1. Inclusion Criteria:

To be eligible for inclusion, each patient must have:

4.1.1 Metastatic breast cancer and have formalin-fixed, paraffin–embedded tumor available for testing BRCA1 protein expression

4.1.2 Adults over 18 years of age

4.1.3 Have resolution of all acute toxic effects of any prior chemotherapy or radiotherapy to NCI CTC grade ≤ 1 prior to study registration.

4.1.4 Be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study specific screening procedures

4.1.5 Be willing and able to comply with the treatment plan, scheduled clinic visits, laboratory and oncological tests and other study procedures

4.1.6 Have a ECOG performance status of 0 – 2

4.1.7 Measurable disease by CT by RECIST 1.1 to evaluate response. Patients with bone only lesions are also eligible to enroll. Criteria to monitor for response will be based on non-target lesions per RECIST criteria

4.1.8 Adequate bone marrow function defined as platelets $\geq$ 100 X $10^9$ cells/L, neutrophils $\geq$ 1.5 x $10^9$ cells/L, white blood cells (WBC) $\geq$ 3.0 x $10^9$ cells/L and a hemoglobin $\geq$ 90 gm/L

4.1.9 Liver function tests (AST and/ or ALT) should be $\leq$ 2 x upper limit of normal (ULN, defined as per laboratory where blood testing is done), total bilirubin $\leq$ 1.5 x ULN (except for patients with liver metastases, ALT and/or ALT $\leq$ 5 times the upper limit of normal is accepted)

4.1.10 Patients who are already on pegylated liposomal doxorubicin will be eligible to participate in this study as long as they meet the other inclusion/exclusion criteria and can sign informed consent

### 4.2. Exclusion Criteria

The following will exclude patients from study participation:

4.2.1. Myocardial infarction within 6 months of registration

4.2.2. Brain metastases unless documented to be controlled post completion of local therapy (surgery and/or radiation therapy) for at least four weeks prior to registration

4.2.3. Pregnant or breast feeding women. Women with child bearing potential must use effective measures to prevent pregnancy while receiving pegylated liposomal doxorubicin

4.2.4. Have a concurrent active non-breast malignancy except for non-melanoma skin cancer

4.2.5. Her2 positive tumors as defined by FDA guidelines(3+ immunohistochemical staining, defined as uniform, intense membrane staining of more than 10% of invasive tumor cells, and for cases with 2+ staining showing gene amplification by FISH, expressed as a ratio of more than 2 when comparing HER-2 gene and chromosome 17 fluorescent signals)[18,19]

## 4.3. **Study Exit**

Patients will continue with study visits until study exit. Patients will exit the study at tumor progression or withdrawal. Each patient has the right to withdraw from the study at any time without prejudice. Should a patient withdraw from the study prior to disease progression, the reason(s) must be stated on the case report form. All procedures (vital signs, laboratory evaluations, physical examination, tumor response, adverse events and concomitant medications) should be performed at the study exit visit. All enrolled patients will be followed for 30 days after week 9 visit.

Patients may be withdrawn from the study early due to:

a. Development of toxicity which in the Investigator's judgment precludes further study participation
b. Significant protocol violations or noncompliance on the part of the patient or Investigator
c. Discontinuation, in the judgment of the Investigator, is in the patient's best interest
d. The patient is beginning another treatment
e. Refusal of the patient to continue treatment or follow-up
f. Loss to follow-up
g. Pregnancy

## 4.4. **Patient Registration**

Data for patients enrolled on interventional trials must be entered per current practices into the Oncore electronic clinical trials management system.

University of Arizona Cancer Center

Patients must be registered prior to initiation of treatment. Patients will be registered through a Breast Team Clinical Research Coordinator (CRC) from 8:00 a.m. to 5:00 p.m., Mountain Standard Time, Monday through Friday, (excluding holidays)

University of New Mexico Cancer Center

Patients must be registered prior to initiation of treatment. Prior to registration, eligibility criteria must be confirmed with the UNM CC Clinical Research Office. If the patient meets the eligibility criteria he/she will be assigned a **unique patient study number** by the Clinical Research Office as further detailed below.
The patient will be registered in the Oncore System using the study number.

## 4.5. **Study Patient Identification**

Patients who have been consented and are undergoing study screening will be identified on study-related documentation and forms by their initials (first/middle and last name initials). All patients treated on this trial will be identified by their study initials and a unique study identification number. A unique study number will only be assigned to patients who meet the eligibility requirements and have completed the screening visit and are registered for treatment. The unique number will begin with the following prefix: BD. The prefix will be followed by the patient identification number beginning with # 001 in each cohort. These numbers will be issued to patients sequentially and no patient identification numbers will be re-assigned in the event that the subject withdraws from protocol treatment.

Patients enrolled at University of New Mexico Cancer Center will be identified by prefix NM to help identify patients enrolled at that site. The prefix will be followed by the patient identification number beginning with # 001 in each cohort. These numbers will be issued to patients sequentially and no patient identification numbers will be re-assigned in the event that the subject withdraws from protocol treatment

## 5. Study Procedures

### 5.1. Pretreatment

Patients will be consented and evaluated for participation based on the following procedures which should be performed within 28 days of registration

- Physical exam
- Complete blood count with absolute neutrophil count and differential
- Liver function panel to include total bilirubin
- Serum Creatinine
- Staging studies by the same method that will be used throughout the study (per treating physician discretion):
    - Such as CT of the chest, abdomen and pelvis or
    - PET/CT or
    - Bone scan

### 5.2. Patient Registration

See section 4.4 above

**Registration Guidelines**

Before a subject participates in the trial, the investigator or delegate is responsible for obtaining written informed consent after adequate explanation of the aims, methods, anticipated benefits, subject responsibilities and potential hazards of the study and before any protocol-specific screening procedures or any study required medications are administered. All patients must be registered before the start of treatment.

### 5.3. Treatment Phase

The following tests and observations will be performed during the treatment phase of

the study.

| Test | Interval |
|---|---|
| Physical exam | No less than every 8 weeks |
| Complete blood counts and ANC and platelet count | No less than every 4 weeks |
| Liver function panel to include total bilirubin | No less than every 4 weeks |
| Serum creatinine | No less than every 4 weeks |
| Repeat disease assessment with physical exam and repeat imaging test that showed measured pretreatment | No less than every 9 weeks |

5.4. **Follow Up**

Patients will be followed according to the institutional standard guidelines. We will only follow patients who have experienced grade 3 or 4 adverse event or serious adverse event that are possible/probably or definitely related to the study required medication for 30 days after the week 9 visit.

## 6. Criteria for Evaluation

The primary endpoint is the clinical response rate (complete response + partial response, see 6.3.4.1). Other efficacy measures are progression-free survival.

### Evaluation Criteria Definitions

6.1. **Treatment related toxicity** is an adverse effect that is clearly related to the chemotherapy regimen.

6.2. **Clinical response**: Will be evaluated using the following definitions:

6.2.1. **Baseline assessment**:

To assess response, it is necessary to estimate the tumor burden at baseline before the start of treatment. Subsequent measurements will be compared to this. Measurable disease is defined by the presence of at least one lesion that can be accurately measured in at least one dimension (longest diameter recorded) as $\geq$ 2.0 cm with conventional techniques or as $\geq$ 1.0 cm by spiral CT scan. All lesions that do not fit these criteria are considered to be non-measurable disease. All measurable lesions up to a maximum of five lesions per organ and ten lesions in total should be identified as target lesions and be recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with longest diameter) and their suitability for accurate repeated measurements. A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum. This will be used as the reference for characterizing the objective tumor response

6.2.2. **Tumor assessments** will be made during screening and then at 9 (± 7 days) weeks to evaluate response rate. There after the use of further imaging for tumor assessment will be left to the treating physicians discretion

6.2.3. **Evaluation of target lesions**

Tumor response will be evaluated as follows (see appendix 2, Recist 1.1)

| Target lesions | Non-target lesions | New lesions | Overall response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Non-CR/non-PD | No | PR |
| CR | Not evaluated | No | PR |
| PR | Non-PD or not evaluated | No | PR |
| SD | Non-PD or not evaluated | No | SD |
| Not all evaluated | Non-PD | No | NE |
| PD | Any | Yes or no | PD |
| Any | PD | Yes or no | PD |
| Any | Any | Yes | PD |

**6.2.4 Evaluation of best overall response**: The best response is recorded from the start of treatment and repeat imaging done at 9 -12 weeks (± 7 days)

6.2.5 **Response rate:** The clinical response rate is the percentage of evaluable patients who achieve complete response (CR) or partial response (PR) as the best overall response.

6.2.6 **Progression-free survival**: will be measured as the time from the start of pegylated liposomal doxorubicin to the time the patient is first recorded as having disease progression or dies. If a patient does not progress or die while being followed via tumor assessment, progression-free survival will be censored at the time of last disease assessment.

## 7. Study Drug Information

### Pegylated Liposomal Doxorubicin (Doxil)

Doxil® (doxorubicin HCl liposome injection) is doxorubicin hydrochloride (HCl) encapsulated in STEALTH® liposomes forintravenous administration. Doxorubicin is a cytotoxic anthracycline antibiotic isolated from *Streptomyces peucetius* var. *caesius.* Doxorubicin HCl, is the established name for (8*S*,10*S* )-10-[(3-amino-2,3,6-trideoxy-_-L-*lyxo*-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione. Doxil will be obtained from commercial sources and administered on days 1 of 21 day cycle (this will be done as standard of care). This drug will be administered as directed by their package inserts or standard of care. Doxil will be administered intravenously at 30mg/m$^2$.

### Dose Modification Guidelines

Patients will be carefully monitored for toxicity. Adverse reactions, such as Hand Foot Syndrome (HFS), hematologic toxicities, and stomatitis will be managed by dose delays and adjustments. Following the first appearance of a Grade 2 or higher adverse reactions, the dosing will be adjusted or delayed as described in the following tables. Once the dose has been reduced, it will not be increased at a later time. After a dose modification, if treatment is continued, it will return to the original dose interval of every 21 days.

**Recommended Dose Modification Guidelines**

**Table 1: Dose Adjustment for Hand-Foot Syndrome (HFS)**

| Toxicity Grade | Dose Adjustment |
|---|---|
| 1-mild erythema, swelling, or desquamation not interfering with daily activities | Redose unless patient has experienced previous Grade 3 or 4 HFS. If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval. |
| 2- erythema, desquamation, or swelling interfering with, but not precluding normal physical activities; small blisters or ulcerations less than 2 cm in diameter | Delay dosing up to 2 weeks or until resolved to Grade 0-1. If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks, and there are no prior Grade 3-4 HFS, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval. |
| 3- blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing | Delay dosing up to 2 weeks or until resolved to Grade 0-1. Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued. |
| 4- diffuse or local process causing infectious complications, or a bedridden state or hospitalization | Delay dosing up to 2 weeks or until resolved to Grade 0-1. Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued. |

**Table 2: Dose Adjustments for Stomatitis**

| Toxicity Grade | Dose Adjustment |
|---|---|

| | |
|---|---|
| 1-painless ulcers, erythema, or mild soreness) | Redose unless patient has experienced previous Grade 3 or 4 HFS. If so, delay up to 2 weeks and decrease dose by 25%. Return to original dose interval. |
| 2-painful erythema, edema, or ulcers,but can eat | Delay dosing up to 2 weeks or until resolved to Grade 0-1. If after 2 weeks there is no resolution, DOXIL should be discontinued. If resolved to Grade 0-1 within 2 weeks, and there are no prior Grade 3-4 HFS, continue treatment at previous dose and return to original dose interval. If patient experienced previous Grade 3-4 toxicity, continue treatment with a 25% dose reduction and return to original dose interval. |
| 3-painful erythema, edema, or ulcers, and cannot eat | Delay dosing up to 2 weeks or until resolved to Grade 0-1. Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued. |
| 4-requires parenteral or enteral support) | Delay dosing up to 2 weeks or until resolved to Grade 0-1. Decrease dose by 25% and return to original dose interval. If after 2 weeks there is no resolution, DOXIL should be discontinued. |

**Table 3: Dose Adjustments for Hematological Toxicity**

| Situation | Doxil Dose |
|---|---|
| First episode of febrile neutropenia (grade 3 or 4) | 75% |
| Second episode of febrile neutropenia (grade 3 or 4) | Discontinue |
| Grade 4 thrombocytopenia or bleeding associated with thrombocytopenia | 75% |

In addition to the above dose adjustments, subjects experiencing febrile neutropenia secondary to Doxil may receive G-CSF therapy in association with subsequent chemotherapy doses.

**Table 4: Dose Adjustments for Other Toxicities**

| **NCI CTC** Grade | Doxil Dose |
|---|---|
| 0 - 2 | 100% |
| 3 (except alopecia) | 75% |
| 4 | Hold until resolution of toxicity |

8. **Study Statistics**

8.1. **Statistical Methods:** This study is a prospective single arm Phase II trial to assess association of BRCA1 protein expression with overall response rate in patients with metastatic breast cancer on pegylated liposomal doxorubicin in patients with metastatic breast cancer. We plan to consent 50 to 60 patients with the goal of enrolling a total of 50 patients, with adequate primary tumor tissue for BRCA1 protein expression, into this study. It is estimated that 30% of patients will have low BRCA1 protein expression[19] and therefore anticipate approximately a 35 to 15 patient split for normal BRCA1 to low BRCA1 expression status.

Recruitment and tissue analysis: Patients will be consented and after enrollment of the first 12 patients, a batch analysis of their tumors will be performed to analyze tumor BRCA1 protein expression. This will allow us to assign the patients to their respective cohorts (Normal BRCA1 protein expression and Low BRCA1 protein expression). This will be done until we have 12 patients in the normal BRCA1 protein arm. After that, an interim analysis will be performed for the normal BRCA1 arm and stopping rule will be applied as mentioned in the study design

8.2. **Analytic plan for primary objective**:

**Definition of primary endpoint**: Response rate is defined as the percentage of evaluable patients who achieve complete response (CR) or partial response (PR) as measured by RECIST on imaging as the best overall response.

**Low BRCA1 protein expression**: The number and percentage of patients falling into response category will be tabulated. The estimate of the response rate will be presented with 2-sided 95% exact binomial confidence intervals. We anticipate this data to be hypothesis generating and will form the basis for a future prospective clinical trial enrolling larger number patients to evaluate if low BRCA1 expression, as determine by IHC, is predictive of response to treatment with DNA damaging agents.

**Normal BRCA1 protein expression**: The estimate of the response rate will be presented with 2-sided 95% exact binomial confidence intervals. If 5 or fewer patients with response are observed among the 35 patients, then there will be no further investigation of the treatment. If 6 responses are observed out of 35 patients, there is 80% confidence that the true response rate >11.2%.

Interim analysis:

**Low BRCA1 protein expression:** No interim analysis will be conducted

**Normal BRCA1 protein expression:** An interim analysis for futility will be conducted for this arm. The response rate will be used as the endpoint for the interim analysis. The interim analysis will be performed when the first 12 treated patients are accrued to normal BRCA1 protein expression arm. Based on Simon's 2-stage design, if less than 2 responders are observed out of these initial 12 evaluable patients, patient enrollment will be discontinued into this arm. Otherwise patient enrollment will continue until a total of 35 patients evaluable for response are enrolled. If only 1 response is observed out of 12 patients, there is 80% confidence that the true response rate <22.1%.

If the normal BRCA1 protein expression arm is closed to accrual, we will continue to consent patients who are being treated with pegylated liposomal doxorubicin. As the expression of BRCA1 protein is anticipated to be low in only 30% of patients, we will continue to consent patients and analyze their tumors and only those with low BRCA1 protein expression will be enrolled into the arm. We anticipate consenting total of 50-60 patients to enroll 15 patients into the low BRCA1 protein expression arm.

8.3. **Analytic plan for secondary objective**:

**Definition of secondary endpoints**: Progression free survival will be measured as the times from the start of pegylated liposomal doxorubicin to the time the patient is first recorded as having disease progression or dies. If a patient does not progress or die while being followed via tumor assessment, progression-free survival will be censored at the time of last disease assessment.

We will calculate the median progression free survival in patients with low BRCA1 and normal BRCA1 protein expression. Median progression free survival will be estimated by Kaplan-Meier methodology.

9. **Data and Safety Monitoring Plan**

**Data and Safety Monitoring Plan for Patients Enrolled at the University of Arizona**

The University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) reviewed the protocol and determined this study does not require DSMB oversight or routine monitoring by the QA/QC program. Principal investigator will be responsible for monitoring and reporting any changes/ adverse events to IRB. PI will be responsible all the study related activities and will report annually to IRB.

The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF). Source documentation supporting the CRF data will indicate the subject's participation in the trial and will document the dates and details of study procedures, adverse events, and patient status.

Case report forms, which include the inclusion/exclusion criteria form, adverse event forms and serious adverse event forms should be completed with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All subject forms and study files will be stored in a secure area limited to authorized staff.

**Note:** Routine monitoring of regulatory documents and test article will be conducted at least annually.

**Data and Safety Monitoring Plan for Patients Enrolled at the University of New Mexico**

The University of New Mexico Cancer Center (UNM CC) places a high priority on ensuring the safety of patients participating in clinical trials. All clinical trials require monitoring commensurate with the degree of risk involved in participation of studies. Clinical Research

Office Standard Operating Procedures (SOP's) detail functions and processes found in this plan. SOPs are available at http://hsc.unm.edu/UNM CC/intranet/ctoforms.asp.

Data and safety monitoring activities for each study continue until all patients have completed their treatment and all patients are beyond the time point at which study-related adverse events would likely be encountered. The UNM CC has implemented a process for routine, real time data monitoring and safety review of Investigator Initiated trials which takes into account the Essential Elements of the National Cancer Institute (NCI) guidelines, the FDA monitoring regulations, Good Clinical Practice Guidelines and other DSM plans and programs approved by the NCI. These are outlined in the UNM CC Data and Safety Monitoring Plan, or DSMP.

In addition to complying with NIH/NCI guidelines, the UNM CC DSMP complies with, the University of New Mexico Health Sciences Center Human Research Protections Office (HRPO) guidelines for safety and data monitoring which can be found at: http://hsc.unm.edu/som/research/hrrc/PoliciesGuidelines.shtml).

The DSMP is distinct from, and complements, the activities of the Protocol Monitoring & Review Committee (PRMC) and the Clinical Protocol Data Management (CPDM) functions of UNM CC. The most current version is maintained on file with the Human Research Review Committee, and is therefore not included in this protocol as an Appendix.

De-identified study results, in the form of glass slides, digitized pathology and radiology images and Microsoft Word documents and Microsoft Excel databases, will be stored for 10 years. All study results will be stored in locked offices and on password protected computers. During that time, PIs at the University of Arizona and the University of New Mexico will have access to the de-identified data for the purposes of analysis and publication.

**Process to implement study closure when significant risks or benefits are identified:** Stopping rules will be based on Simon's optimal design (Simon, R. "Optimal Two-Stage Design for Phase II Clinical Trials". Controlled Clinical Trials, 101-10, 1989, Elsevier Science Publishing Company, N.Y., N.Y.) which minimizes the expected sample size under the null response rate.[20] In the patients with normal BRCA1 protein expression if at least two responses are observed among these 12 patients, then an additional 23 patients will be accrued to the second stage. If 0 or 1 response is observed among the initial 12 patients, then accrual to this cohort will be terminated and declared negative. At the end of the study if 6 or more patients in this cohort respond then pegylated liposomal doxorubicin will be considered worthy of further study in this cohort. This design yields at least 90% probability of a positive result if the true response rate is at least 30% and a 90% probability of a negative result if the true response rate is 10%.

**Description of adverse events and reporting procedures:**

**Adverse Event**

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a casual relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse events of grade 3 or 4 that are possible/probably or definitely related to the study required medication or serious adverse events will be recorded on the UMC adverse events record form and reviewed by the Principal Investigator. These same events will be reported in the case report form.

All adverse events will be classified using either the MedDRA term or NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and will address:

- Grade
- Relationship to study drug(not related, unlikely, possible, probable, definitely)
- Causality other than study drug (disease related, concomitant medication related, intercurrent illness, other)
- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, held, dose reduced, discontinued, medication given)

**Serious Adverse Events**

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of an existing hospital stay
- Results in disability persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

**Note**: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

All SAEs which meet the criteria for a reportable event will be reported in writing to the FDA and to the University of Arizona Human Subjects Protection Program within 10 working days with the exceptions of unexpected death or life-threatening even, which should be reported within 5 working days after learning of the event.
Non-local Unanticipated Problem Involving Risks to Subjects or Others, which is any information that meets **all three** of the following criteria:

i) Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent; and (b) the characteristics of the subject population being studied. A harm is “unexpected” when its specificity and severity are not accurately reflected in the consent document.
ii) Related or possible related due to participation in this research (possible related means that the outcome may have been caused by the procedures involved in the research); and
iii) Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. A harm is “at least probably related to the Human Research procedures” if in the opinion of the investigator, the research procedures more likely than not caused the harm.

Patients will be followed according to the institutional standard guidelines. We will only follow patients who have experienced grade 3 or 4 adverse event or serious adverse event that are possible/probably or definitely related to the study required medication.

**Plan for assuring data accuracy and protocol compliance:**
Routine study activity and safety information will be reported to the IRB once a year, or more frequently if requested. These reports will include:

- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies.

**Data, safety and study progress will be reported to:**

- Human Subjects Protection Program (IRB) at least annually;
- Sponsor (if applicable) at least every six months.

**Identification of the sponsor or funding agency, as applicable:**
The PI will immediately notify; in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.

10. **Data Submission Schedule**

Data forms must be completed for all subjects registered to the study.
Electronic case report forms will be completed in the OnCore system.

11. **Special instructions**

Patients will be consented for acquiring their primary tumor tissue to evaluate for BRCA1 protein expression. Formalin-fixed paraffin-embedded (FFPE) tissue will be accessed from the site where they had surgery for their primary tumor. If their primary surgery was at UMC we will request department of pathology for their tumor blocks. If they had surgery at an outside institution we will request their tumor blocks. Patients will need to sign a release of tissue form. We will use the standard tissue requisition form used for University of Arizona Cancer Center.

Patient tissue in the form of a tumor block is preferred. However, if tumor block is not available, 10 unstained slides will be submitted.

For patients participating at the University of New Mexico Cancer Center, it will be confirmed that tissue (in the form of tumor block or 10 unstained slides) is available from either primary or metastatic site prior to registration.

Once their tumor blocks / slides have been accessed they will be sent via Fed-Ex to the University of Arizona Cancer Center for further analysis.

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## 12. Ethics

The trial will be conducted in accordance with the Declaration of Helsinki for biomedical research involving human subjects and local regulatory requirements.

**Ethical Principles**
This study will be conducted in accordance with Title 21 of the Code of Federal Regulations (CFR). Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by an Institutional Review Board; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the patients will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; patients will give their informed consent and will be competent to do so and not under duress; and the study will comply with the ethical principles in Title 21 of the CFR.

**Informed Consent**
This study will be conducted in full compliance with the informed consent regulations in 21 CFR 50. The Sponsor-Investigator is responsible for ensuring that written informed consent from potential patients is obtained prior to performing any trial tests or assessments required by the protocol.
**Informed consent, University of Arizona**

A copy of the fully executed informed consent form (PHI authorization form and ancillary consent forms if applicable), is given to the subject. One copy is placed in the subject's medical record, another copy is placed in the research chart, and the originals are filed by the protocol number in room 2111 at UACC North Campus.

**Informed consent, University of New Mexico**

A copy of the fully executed informed consent form will be given to the subject. One copy is placed in the subject's medical record, another copy is placed in the research chart, and the originals are filed by protocol number in the Clinical Trails Office at the New Mexico Cancer Care Alliance, UNM Cancer Center Admin. Bldg. 2nd Floor.

**Institutional Review Board**
This study will be conducted in full compliance with the Institutional Review Board (IRB) regulations in 21 CFR 56, in accordance with the Declaration of Helsinki. This protocol will not be initiated unless it and the informed consent form have been reviewed and approved by, and remains open to continuing review by, an IRB meeting the requirements of 21 CFR 56. The IRB shall review and have the authority to approve, require modification in (to

secure approval), or disapprove the protocol. The IRB shall notify the Investigator and the institution in writing of its decision. The IRB shall require that the information given to patients as part of the informed consent is in accordance with 21 CFR 50.25. The IRB shall conduct continuing reviews of the protocol at intervals appropriate to the degree of risk, but not less than once per year. At the completion or early termination of the trial, a final report should be sent to the IRB by the Investigator. The Investigator is obligated to maintain an IRB correspondence file.

**Confidentiality of Patient Data**

The investigator must ensure that patient confidentiality will be maintained. Patients will be identified by initials and a protocol-assigned patient number as described in section 4.5. Permission for direct access to patient data will be sought in writing for the patient by the investigator as part of the informed consent procedure. The patient will be informed that all clinical information is confidential, but that the IRB, and regulatory authorities may inspect these records.

## 13. Bibliography


1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA: a cancer journal for clinicians. Jan 2013;63(1):11-30
2. Wooster R, Weber BL. Breast and ovarian cancer. The New England journal of medicine. Jun 5 2003;348(23):2339-2347
3. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer. Oct 2004;4(10):814-819
4. Turner N, Tutt A, Ashworth A. Targeting the DNA repair defect of BRCA tumours. Current opinion in pharmacology. Aug 2005;5(4):388-393.
5. Venkitaraman AR. Functions of BRCA1 and BRCA2 in the biological response to DNA damage. Journal of cell science. Oct 2001;114(Pt 20):3591-3598
6. Collins N, McManus R, Wooster R, et al. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. Oncogene. Apr 20 1995;10(8):1673-1675
7. Cornelis RS, Neuhausen SL, Johansson O, et al. High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCAl-linked families. The Breast Cancer Linkage Consortium. Genes, chromosomes & cancer. Jul 1995;13(3):203-210.
8. Rahman N, Stratton MR. The genetics of breast cancer susceptibility. Annual review of genetics. 1998;32:95-121.
9. Rhiem K, Todt U, Wappenschmidt B, Klein A, Wardelmann E, Schmutzler RK. Sporadic breast carcinomas with somatic BRCA1 gene deletions share genotype/phenotype features with familial breast carcinomas. Anticancer research. Sep 2010;30(9):3445-3449.
10. Quinn JE, Kennedy RD, Mullan PB, et al. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res. Oct 1 2003;63(19):6221-6228.

11. Rodriguez AA, Makris A, Wu MF, et al. DNA repair signature is associated with anthracycline response in triple negative breast cancer patients. Breast cancer research and treatment. Aug 2010;123(1):189-196.
12. Lips EH, Mulder L, Hannemann J, et al. Indicators of homologous recombination deficiency in breast cancer and association with response to neoadjuvant chemotherapy. Ann Oncol. Apr 2011;22(4):870-876.
13. Vollebergh MA, Lips EH, Nederlof PM, et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol. Jul 2011;22(7):1561-1570.
14. Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. Mar 1 2010;28(7):1145-1153.
15. Lips EH, Laddach N, Savola SP, et al. Quantitative copy number analysis by Multiplex Ligation-dependent Probe Amplification (MLPA) of BRCA1-associated breast cancer regions identifies BRCAness. Breast Cancer Res. 2011;13(5):R107.
16. Verma S, Dent S, Chow BJ et al: Metastatic breast cancer: the role of pegylated liposomal doxorubicin after conventional anthracyclines. Cancer Treat Rev. 2008 Aug;34(5):391-406
17. O'Brien MER, Wigler N, Inbar M et al. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCL (CAELYX™/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. Annals of Oncology. 2004;15: 440-449
18. Birner P, Oberhuber G, Stani J, et al. Evaluation of the United States Food and Drug Administration–approved scoring and test system of HER-2 protein expression in breast cancer. Clin Cancer Res. 2001;7:1669-1675.
19. Jacobs TW, Gown AM, Yaziji H, et al. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration–approved scoring system. J Clin Oncol. 1999;17:1983-1987
20. Simon R, Optimal two-stage designs for phase II clinical trials, Controlled Clinical Trials, 1989, 10, 1 - 10.

**Appendix 1. Eastern Cooperative Oncology Group (ECOG) Performance Status**

| Activity Status | Description |
|---|---|
| 0 | Asymptomatic, fully active, and able to carry on all predisease performance without restrictions |
| 1 | Symptomatic, fully ambulatory but restricted in physical strenuous activity and able to carry out performance of a light or sedentary nature, e.g., light housework, office work |
| 2 | Symptomatic, ambulatory and capable of self-care but unable to carry out any work activities. Up and about more than 50% of waking hours, but not bedridden |
| 3 | Symptomatic, capable of only limited self care, confined to a bed/chair more than 50% of waking hours, but not bedridden. |
| 4 | Completely disabled. Can not carry on self-care. Totally bedridden. |

| | |
|---|---|
| 5 | Dead |

## Appendix 2. Response Evaluation Criteria in Solid Tumors (RECIST 1.1)

### Eligibility

- Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

  **Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

  **Measurable lesions** - lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm using conventional techniques or ≥10 mm with spiral CT scan.

  **Non-measurable lesions** - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and

- All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

### Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
- All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

**Response Criteria**

**Evaluation of target lesions**

| | |
|---|---|
| * Complete Response (CR): | Disappearance of all target lesions |
| * Partial Response (PR): | At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD |
| * Progressive Disease (PD): | At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions |
| * Stable Disease (SD): | Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started |

**Evaluation of non-target lesions**

| | |
|---|---|
| * Complete Response (CR): | Disappearance of all non-target lesions and normalization of tumor marker level |
| * Non-CR/Non-PD | Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits |
| * Progressive Disease (PD): | Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1) |

(1) Although a clear progression of “non target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

**Evaluation of best overall response**

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

| Target lesions | Non-Target lesions | New Lesions | Overall response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Non-CR/non-PD | No | PR |
| CR | Not evaluated | No | PR |

| | | | |
|---|---|---|---|
| PR | Non-PD or not all evaluated | No | PR |
| SD | Non-PD or not all evaluated | No | SD |
| PD | Any | Yes or No | PD |
| Any | PD | Yes or No | PD |
| Any | Any | Yes | PD |

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

**Confirmation**

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

**Duration of overall response**

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

**Duration of stable disease**

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

**Response review**

- For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

**Reporting of results**

- All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).
- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.
- All conclusions should be based on all eligible patients.

**APPENDIX 3**

**Investigator Agreement**

**Protocol No.**

**Protocol Title:** A single arm Phase II trial to assess association of defective DNA repair status with overall response rate in patients with HER2 negative metastatic breast cancer on pegylated liposomal doxorubicin

By signing below I agree:

1) That my staff and I have read, understand and will adhere to the protocol as written, and that any changes to the protocol will be agreed to and approved by the Principal Investigator and the Institutional Review Board (IRB)

2) To abide by all obligations stated on the FDA Form 1572 and other documents required by regulation;

3) To conduct this study in accordance with the current International Conference on Harmonization (ICH) guidance, the Good Clinical Practices (GCP) guidance, the Declaration of Helsinki, US FDA regulations and local IRB and legal requirements;

4) To obtain IRB approval of the protocol, any amendments to the protocol, and periodic re-approval as required, and to keep the IRB informed of adverse events as required by their guidelines report the status of the study to them;

5) To ensure that each individual enrolled into the trial, or legally authorized representative, has read, understands, and has signed the Informed Consent form;

6) To ensure that I and all persons assisting me with the study are adequately informed and trained about the study and the possible adverse events associated with the study required medication

7) To make prompt reports of SAEs and deaths to the FDA according to the regulations;

8) To prepare and maintain adequate and accurate case histories to document all observations and other data pertinent to the study for each individual enrolled in the clinical trial.

**Investigator Signature Date**

**Investigator Name (Print)**