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PRINCIPAL INVESTIGATOR: Rita Nanda, M.D.

CONTRACTING ORGANIZATION: University of Chicago Medical Center
Chicago, Illinois 60637-1470

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14. ABSTRACT Several groups have demonstrated that women with BRCA1 germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling. While BRCA1 germline mutations are uncommon, and contribute to fewer than 5% of breast cancers, our lab has demonstrated that methylation occurs in up to 50% of high-grade, hormone receptor negative sporadic tumors. As promoter methylation leads to transcriptional repression, we propose that such tumors will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, given the role that BRCA1 plays in both DNA repair and cell cycle. Using the alamar blue cytotoxicity assay and five breast cancer cell lines, my laboratory has demonstrated that one of two BRCA1 methylated cell lines is significantly more sensitive to cisplatin and more resistant to paclitaxel as compared to other human breast cancer cell lines with normal BRCA1 expression. These findings are currently being translated into a clinical trial where the BRCA1 methylation status of a patient's tumor will be correlated to response of the tumor to platinum-based therapy.					
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INTRODUCTION

Several groups have demonstrated that women with *BRCA1* germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling[1, 2]. While *BRCA1* germline mutations are uncommon, and contribute to fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* occur with much greater frequency. Our lab has previously demonstrated that methylation of the *BRCA1* promoter occurs in almost 50% of high-grade, hormone receptor negative sporadic tumors[3]. Given the role of *BRCA1* in both DNA repair and cell cycle regulation, it is likely that cells deficient in *BRCA1* secondary to methylation will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, as has previously been demonstrated for cells deficient in *BRCA1* secondary to mutation. The role of *BRCA1* methylation in determining chemosensitivity is unknown. This proposal seeks to investigate the role of *BRCA1* promoter methylation in predicting response to DNA-damaging based therapy *in vitro* and *in vivo*, with the ultimate goal of identifying novel strategies for the treatment of basal-like breast cancer.

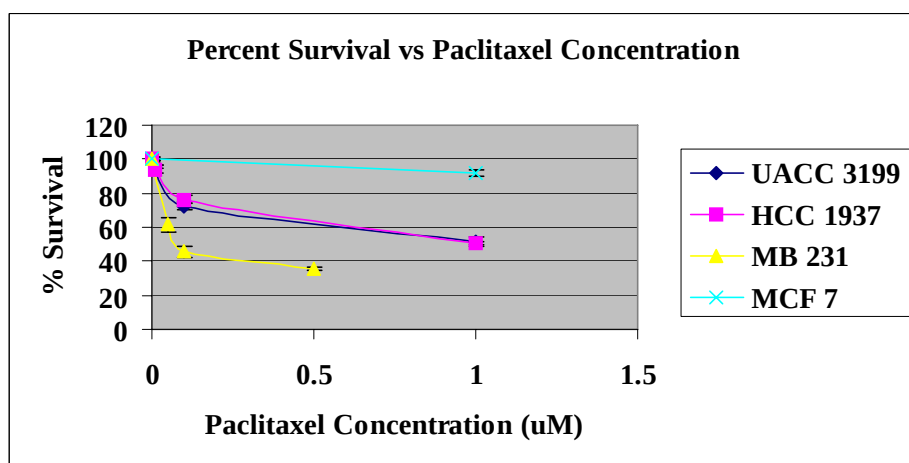
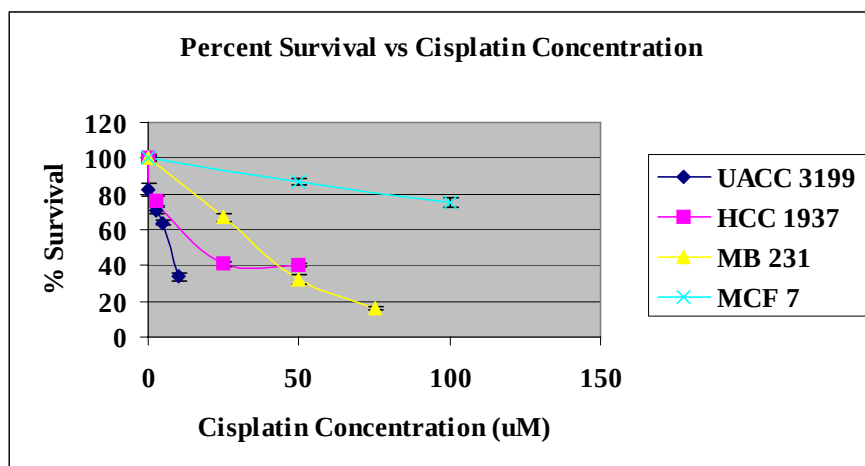
BODY

Task 1: To test *BRCA1* normal, *BRCA1* mutated and *BRCA1* methylated breast cancer cell lines *in vitro* for sensitivity to chemotherapeutic agents commonly employed in breast cancer treatment.

Flow cytometry based cytotoxicity assay

Several groups have demonstrated that cells deficient in *BRCA1* secondary to mutation are sensitive to cisplatin and resistant to paclitaxel, as compared to *BRCA1* competent cells[4, 5]. We hypothesized that cells deficient in *BRCA1* secondary to promoter methylation would also be sensitive to cisplatin and resistant to paclitaxel. To test this hypothesis, we developed an *in vitro* model consisting of four breast cancer cell lines. UACC-3199 is a breast cancer cell line that is almost completely methylated at all 30 CpG sites located at the 5' end of the *BRCA1* gene. UACC-3199 has no *BRCA1* protein expression secondary to promoter hypermethylation[6]. HCC-1937 is a breast cancer cell line derived from a patient with a germline mutation in *BRCA1*. This cell line has one mutant allele that produces a truncated form of the *BRCA1* protein[7]. Two well characterized breast cancer cell lines MCF-7 (ER positive, *BRCA1* normal) and MDA-MB-231 (ER negative, *BRCA1* normal) have been used to provide the context for determining relative sensitivity of the *BRCA1* methylated and mutated cells lines as compared to those with normal *BRCA1* expression.

To evaluate cell survival after drug exposure, exponentially growing cells were exposed to drug at escalating dose levels in triplicate. Untreated cells served as a control. Cells were exposed to drug for 24 hours. Cells were harvested 96 hours after exposure and were stained with Annexin-V-FITC and DAPI. Cell survival and apoptosis were determined by flow cytometry using FACS DiVa. FACS Flowjo analysis software (version 6.1.1) was used to generate percent apoptotic and live cells. Cells that were positive for Annexin and negative for DAPI were considered apoptotic, cells that were double negative were considered live, and cells that were double positive were considered dead. Each experiment was repeated three times. Each experiment was normalized to its own dose 0 average, and percent live versus concentration and percent apoptotic versus concentration plots were constructed. Regression methods were used to predict response with the natural logarithm of the concentration. The estimated IC_{50} and associated 95% confidence interval were obtained by projection of the fitted line and pointwise confidence bounds onto the concentration axis. The IC_{50} values for each cell line after exposure to cisplatin and paclitaxel are shown below:



Cell Line	<i>BRCA1</i> and ER Status	Cisplatin IC ₅₀ (95% CI) μ M	Paclitaxel IC ₅₀ (95% CI) μ M
UACC-3199	<i>BRCA1</i> methylated ER negative	7.39 (4.94-10.9)	1.8 (1.1-3.8)
HCC-1937	<i>BRCA1</i> mutated ER negative	14.1 (11.6-16.4)	2.5 (1.1-4.9)
MDA-MB-231	<i>BRCA1</i> normal ER negative	21.8 (18.2-30.0)	0.13 (0.09-0.16)
MCF-7	<i>BRCA1</i> normal ER positive	> 250	> 100

As demonstrated above, the *BRCA1* methylated cell line is highly sensitive to cisplatin and resistant to paclitaxel. The methylated cells are even more sensitive than the mutated cells, likely related to minimal function of the truncated *BRCA1* protein. For the *BRCA1* normal, ER negative cells, the opposite is true, demonstrating that cells with *BRCA1* promoter methylation have a unique chemosensitivity profile. These *in vitro* data support our hypothesis that *BRCA1* methylated tumors will be sensitive to DNA damaging agents and provide a strong rationale to further test this hypothesis *in vivo*. These data also raise an important concern regarding the use of paclitaxel in basal-like tumors. These results were presented at the SABCS in 2005 and the AACR annual meeting in 2006 (see appendices A and B).

AlamarBlue cytotoxicity assay

After I presented the above data at the SABCS in 2005 (see appendix A) and the AACR annual meeting in 2006 (see appendix B), I received feedback that flow cytometry is not sufficient to determine cytotoxicity, as it does not take into account inhibition of proliferation. As such, I worked with one of my co-mentors, Dr. Eileen Dolan, to optimize the AlamarBlue assay for future work. AlamarBlue is a fluorometric/colorimetric growth indicator that fluoresces and changes in color upon reduction, thus quantifying the degree of cellular proliferation. AlamarBlue interacts with metabolites produced during cellular respiration. This interaction results in the loss of oxygen and replacement with hydrogen. This substitution results in continued cell growth, accompanied by a change in the color and the ability of the media to fluoresce (Pink/red color indicates more proliferation while blue indicates no/less proliferation). AlamarBlue also has the additional benefit of being high throughput.

In addition, I received feedback that additional *BRCA1* methylated cell lines should be assayed to validate the findings observed in the UACC 3199 cell line. After screening 40 breast cancer cell lines for *BRCA1* methylation using the methylation specific PCR (MSP) assay as previously described [3], I was able to identify one additional cell line with *BRCA1* methylation (HCC 38). This cell line was obtained from ATCC.

The relative sensitivities of *BRCA1* methylated (UACC 3199 and HCC 38), mutated (HCC 1937) and competent cells (MB231 and MCF7) to cisplatin, paclitaxel, 5-fluorouracil and etoposide were determined in five representative breast cancer cell lines using the AlamarBlue cytotoxicity assay. Exponentially growing cells were treated with increasing concentrations of cisplatin, paclitaxel, 5-fluorouracil and etoposide for 72 hours. Experiments were performed in triplicate.

Sigmoid dose-response curves were used to model the relationship between cell survival and concentrations of tested drugs. First, percent cell survival was calculated as the number of cells at different doses of drugs divided by the number of cells without drug. Second, log transformation was applied to doses of drugs. Third, a least squares nonlinear regression was used to fit percent cell survival to a 3-parameter sigmoid dose-response curve of the form:

$$\%survival = \alpha + \frac{100 - \alpha}{1 + 10^{(\log \beta - \log dose)\gamma}}$$

Where, α is the bottom parameter, indicating the maximum response; β is the location parameter, indicating the dose required to provoke response halfway between baseline and maximum responses; γ is the hill slope parameter, indicating the steepness of the dose-response curve. The IC_{50} , defined as the dose required to kill 50% of the cells, was also calculated from the sigmoid dose response model. SAS 9.1 Proc NLIN was used to fit the models.

Table 1. IC₅₀ values and 95% CIs using the sigmoidal dose response model

Drug and cell line	IC ₅₀ (95% CI), μ M	α , %	β , μ M	γ	MSE of fitted model	MSE of full model*
<i>Cisplatin</i>						
HCC1937	4.8 (4.4-5.3)	0	4.8	-0.47	26.4	24.9
HCC38	11.3 (10.9-11.7)	0	11.3	-0.78	10.6	10.7
MB231	7.0 (6.4-7.7)	0	7.0	-0.64	38.2	37.7
MCF-7	11.0 (10.0-11.9)	0	11.0	-0.64	12.4	12.8
UACC3199	3.0 (2.8-3.3)	0	3.0	-0.90	68.7	62.9
<i>Paclitaxel</i>						
HCC1937	0.026 (0.019-0.037)	32.3	0.015	-0.76	68.7	62.9
HCC38	0.034 (0.029-0.040)	33.7	0.026	-1.78	10.6	10.7
MB231	0.023 (0.021-0.025)	18.3	0.017	-0.75	12.4	12.8
MCF-7	0.024 (0.012-0.048)	38.3	0.014	-1.16	38.2	37.7
UACC3199	>1	59.7	0.019	-1.72	26.4	24.9
<i>5-FU</i>						
HCC1937	>500	74.3	22.3	-0.56	27.3	28.9
HCC38	>500	78.7	21.4	-0.46	150.0	160.8
MB231	43.5 (37.3-50.9)	37.1	13.6	-0.50	19.1	16.5
MCF-7	32.7 (23.7-45.1)	38.4	8.6	-0.48	68.5	55.0
UACC3199	>500	67.1	60.2	-0.64	19.5	18.6
<i>Etoposide</i>						
HCC1937	10.2 (8.9-11.7)	22.4	4.8	-0.35	16.2	15.5
HCC38	1.4 (1.2-1.8)	15.1	1.0	-0.48	77.8	68.4
MB231	2.3 (1.9-2.7)	19.3	1.5	-0.47	40.5	32.5
MCF-7	20.9 (4.9-89.6)	47.3	0.9	-0.41	102.3	104.1
UACC3199	8.0 (7.3-8.9)	24.7	4.2	-0.46	14.1	12.0

*equivalent to model with empirical means

Only one of the two *BRCA1* methylated cell lines studied (UACC3199) was sensitive to cisplatin and resistant to paclitaxel, as hypothesized. While both cell lines are methylated, western blot analysis revealed that both express *BRCA1*, but to a lesser degree than unmethylated cells. *BRCA1* methylation, as assessed by non-quantitative MSP, does not correlate with sensitivity to cisplatin and resistance to paclitaxel. Quantification of *BRCA1* promoter methylation may better predict chemosensitivity. Identification of the degree of *BRCA1* methylation which does correlates with sensitivity to cisplatin and resistance to paclitaxel could improve treatment selection for patients with breast cancer.

These results, in part, were submitted for presentation at the 2007 ASCO annual meeting (see appendix C).

Task 2: To explore the role of *BRCA1* methylation in hormone receptor negative tumors and how it might affect response to chemotherapy *in vivo*.

Secondary to poor accrual, the gemcitabine/cisplatin trial initially proposed was closed. The trial was open for eight months, and did not accrue any patients. The main reasons for poor accrual were that gemcitabine and cisplatin are both commercially available, and many patients opted for treatment off protocol through their local oncologists. While the trial was also open to other centers that are part of the University of Chicago Phase II Network, many of the physicians reported that they also did not enroll patients to this trial as they did not feel that this regimen offered their patients a “novel” treatment. Furthermore, many community oncologists were reluctant to give cisplatin, given the length of time the treatment takes (1 hour or pre-treatment hydration and 1 hours of post-treatment hydration).

To address the issue which resulted in our inability to accrue to the previous study, another trial was designed to replace it. The goal is the same: to explore the role of *BRCA1* methylation in predicting response to DNA-damaging based chemotherapy. Given the concerns regarding cisplatin, carboplatin will be used instead to avoid the prolonged infusion time. To address the concerns that the trial did not use novel agents, bevacizumab has been added. In this trial, I have proposed to study carboplatin and bevacizumab combination therapy in a cohort of patients with hormone receptor and HER2/*neu* negative (basal-like) metastatic breast cancer. Because these patients are likely to have *BRCA1* deficiency secondary to *BRCA1* promoter methylation (and hence be more sensitive to a DNA damaging agent) and tumors with high levels of VEGF expression, we hypothesize that they are likely to respond to the combination of carboplatin and bevacizumab.

The concept for the protocol was developed and written at the AACR/ASCO Methods in Clinical Cancer Research Workshop, and at the time of the second annual report, I was in negotiation with Genentech to supply bevacizumab. I have been successful in this negotiation and the protocol has been approved by Genentech and is currently under review by the Clinical Trials Review Committee and the Institutional Review Board at the University of Chicago. Please see attached protocol and consent forms in appendix C.

Task 3: To correlate *BRCA1* methylation with response to chemotherapy *in vivo*.

Unfortunately because of the failure of the initially proposed clinical trial to accrue, and the difficulty in obtaining bevacizumab to improve the potential to accrue, I have been unable to perform task 3. As platinum agents are not commonly used in the front line treatment of metastatic breast cancer, it was not possible to substitute archival specimens as recommended by the review of the second annual report. I do hope to pursue this correlation, but unfortunately will be unable to do so until the planned clinical trial has been performed.

KEY RESEARCH ACCOMPLISHMENTS

- One of the two human breast cancer cells studied with *BRCA1* promoter methylation (UACC 3199) is relatively sensitive to cisplatin and resistant to paclitaxel, as compared to other cell lines studied (see appendices A, B and C)
- The *BRCA1* methylated cell line, UACC-3199, appears to be more sensitive to cisplatin than the *BRCA1* mutated cell line, possibly related to low level activity of the truncated *BRCA1* protein in HCC-1937 cells
- The ER positive cell line, MCF-7, is relatively resistant to both cisplatin and etoposide
- *BRCA1* methylation confers relative resistance to paclitaxel *in vitro*, and may represent a potential mechanism of acquired paclitaxel resistance
- Cells with either *BRCA1* methylation or mutation were significantly resistant to 5-fluorouracil.

- The methylated cell lines behave differently from one another, which suggests that *BRCA1* methylation is not sufficient for predicting drug sensitivity.

REPORTABLE OUTCOMES

- Based on my ability to secure research funding from the Department of Defense, I have been able to secure a faculty position at the University of Chicago (effective July 1, 2005). Based on the funding from this grant, I have been given 90% protected time to focus on developing a translational research program in breast cancer. The University has also given me a modest start up package (\$100,000 for two years) to support my research while I apply for additional funding.
- The preliminary data generated from this proposal has been the basis of a funded Doris Duke Clinical Scientist Development Award. I will also use the preliminary data generated from this award to apply for a K22 NIH Faculty Transition Award in October 2007.
- The data that has been presented here is currently being written up for publication and will be submitted in the next month. I will notify the Department of Defense once it has been accepted for publication.
- Abstracts for the 2005 SABCS and the 2006 AACR meetings were submitted and accepted. Both abstracts were accepted for poster presentations, giving me the opportunity to present my research at national meetings. For abstracts and posters, see appendices A, B and C.

CONCLUSIONS

Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are sensitive to cisplatin and resistant to paclitaxel, as compared to *BRCA1* competent cells. We have demonstrated that some cells deficient in *BRCA1* secondary to promoter methylation are also highly sensitive to cisplatin and resistant to paclitaxel. As almost 50% of high-grade hormone receptor negative tumors have *BRCA1* promoter methylation, and while *BRCA1* methylation may represent both a novel therapeutic target for platinum-based chemotherapy and a mechanism for acquired resistance to paclitaxel chemotherapy, it is likely not sufficient to predict chemosensitivity. A clinical trial investigating the role of platinum based therapy in basal-like breast cancer is scheduled to begin once CTSC and IRB approval are obtained. This trial will correlate response of basal-like metastatic breast cancer to the *BRCA1* methylation status of the tumor. Other markers of chemotherapy response are being investigated and will be incorporated into the correlative section of the proposed clinical trial.

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APPENDIX A

SAN ANTONIO BREAST CANCER SYMPOSIUM 2005 ANNUAL MEETING ABSTRACT

BRCA1 Promoter Methylation Confers Sensitivity to Cisplatin in vitro

Rita Nanda, MD¹, James J Dignam, PhD², Cindy Collins¹, and Jinhua Xu, PhD¹, M Eileen Dolan, PhD¹, Olufunmilayo I Olopade, MD¹.

¹Department of Medicine, Section of Hematology/Oncology and the ²Department of Health Studies, University of Chicago, Chicago, IL, United States, 60637.

Background: Women with *BRCA1* mutations are more likely to have breast cancers that are hormone receptor and HER2/*neu* negative. We have previously demonstrated that *BRCA1* promoter methylation occurs to some degree in 20-30% of all sporadic tumors, and up to 50% of high-grade hormone receptor negative tumors, making it much more common than germline mutation (Wei et. al., in press). Given the role of *BRCA1* in DNA repair, it is likely that cells deficient in *BRCA1* secondary to promoter methylation will have increased sensitivity to DNA damaging agents, as has previously been demonstrated in cells deficient in *BRCA1* secondary to mutation. The role of *BRCA1* methylation in determining chemosensitivity is not yet known.

Methods: Using an *in vitro* model, the relative sensitivity of *BRCA1* methylated, mutated and competent cells was determined in four representative breast cancer cell lines: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with doses of cisplatin between 0.25 μ M and 350 μ M. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival and apoptosis were determined by flow cytometry using FACS DiVa. FlowJo FACS analysis software (version 6.1.1) was used to generate percent apoptotic and live cells. IC₅₀ values and 95% confidence intervals were calculated from dose response curves.

Results: The IC₅₀ values for the UACC-3199 and HCC-1937 cells were 12.2 μ M (95% CI 9.49-14.88) and 60.3 μ M (95% CI 40.45-94.63), respectively. The IC₅₀ values for MCF-7 and MDA-MB-231 cells were not reached, even at a dose of 350 μ M. Peak percentage of apoptotic cells observed for the UACC-3199 was 38% at a cisplatin concentration of 50 μ M. Peak percentage of apoptotic cells observed for the HCC-1937, MCF-7 and MDA-MB-231 cells were 20%, 18.7% and 20% at cisplatin concentrations of 100 μ M, 350 μ M, and 350 μ M, respectively.

Discussion: Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are more sensitive to cisplatin than *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary to promoter methylation are also highly sensitive to cisplatin. As varying degrees of *BRCA1* methylation occurs in a significant proportion of high-grade hormone receptor negative tumors, it represents a potential therapeutic target in the treatment of a subset of sporadic breast cancers.



The Role of *BRCA1* Promoter Methylation in Determining Chemosensitivity *in vitro*

Rita Nanda, James J. Dignam, Cindy Collins, Jinhua Xu, M. Eileen Dolan, Olufunmilayo I. Olopade
The University of Chicago, Chicago, IL



Abstract

Background: Several groups have demonstrated that women with *BRCA1* germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling. While *BRCA1* germline mutations are uncommon, and contribute to fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* occur with much greater frequency. Our lab has previously demonstrated that methylation of the *BRCA1* promoter occurs in almost 50% of high-grade, hormone receptor negative sporadic tumors. Given the role of *BRCA1* in both DNA repair and cell cycle regulation, it is likely that cells deficient in *BRCA1* secondary to methylation will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, as has previously been shown for cells deficient in *BRCA1* secondary to mutation. The role of *BRCA1* methylation in determining chemosensitivity is unknown.

Methods: Using an *in vitro* model, the relative sensitivity of *BRCA1* methylated, mutated and competent cells was determined using four representative breast cancer cell lines: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with cisplatin (CDDP) and paclitaxel. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival was determined by flow cytometry using FACS Diva. FlowJo FACS analysis software was used to generate percent apoptotic and live cells. IC₅₀ values and 95% confidence intervals were calculated from dose response curves.

Results: The IC₅₀ values and 95% confidence intervals for CDDP for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 7.4 μM (4.94-10.9), 14.1 μM (11.6-16.4), and 21.8 μM (18.2-30.0), respectively. The IC₅₀ value for CDDP for MCF-7 was not reached, even at a dose of 250 μM. The IC₅₀ values and 95% confidence intervals for paclitaxel for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 1.8 μM (1.1-3.8), 2.5 μM (1.1-4.9) and 0.13 μM (0.09-0.16), respectively. Despite dose escalation to a paclitaxel concentration of 100 μM, the IC₅₀ value for MCF-7 was not reached.

Discussion: Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are sensitive to cisplatin and resistant to paclitaxel, as compared to *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary to promoter methylation are also highly sensitive to cisplatin and resistant to paclitaxel. As almost 50% of high-grade hormone receptor negative tumors have *BRCA1* promoter methylation, *BRCA1* methylation may represent both a novel therapeutic target for platinum-based chemotherapy and a mechanism for acquired resistance to paclitaxel chemotherapy.

Background

- Women with *BRCA1* germline mutations are more likely to have cancers that are ER/PR and HER2/*neu* negative
- While germline *BRCA1* mutations account for fewer than 5% of breast cancers, *BRCA1* promoter methylation is much more common
- We have previously demonstrated that *BRCA1* promoter methylation occurs to some degree in 20-30% of sporadic breast tumors, and up to 50% of high-grade hormone receptor negative tumors
- Given the role of *BRCA1* in the DNA damage response pathway, cells deficient in *BRCA1* secondary to promoter methylation are likely to have increased sensitivity to DNA damaging agents
- Given the role of *BRCA1* in cell cycle, cells deficient in *BRCA1* are likely to be resistant to paclitaxel



Olopade and Wei, Cancer Cell, 2003

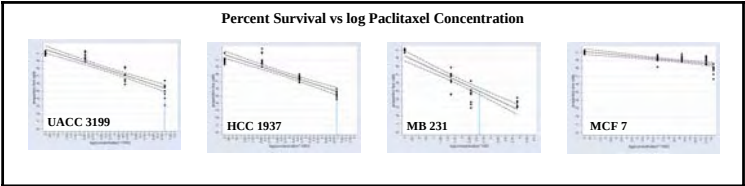
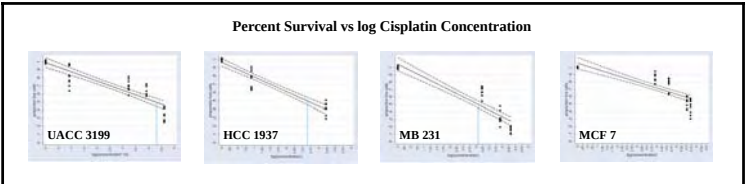
Methods

Cell Lines: The HCC-1937, MDA-MB-231 and MCF-7 cell lines were purchased from ATCC (Rockville, MD). The UACC-3199 cell line was purchased from the University of Arizona Cancer Center (Tucson, AZ). The MCF-7 cells were cultured in DMEM (Fisher Scientific, Hanover Park, IL) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD). The UACC-3199, HCC-1937 and MB-231 cells were maintained in RPMI 1640 (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum. All cell lines were grown with the addition of 1% penicillin/streptomycin (Fisher Scientific, Hanover Park, IL) at 37°C and 5% CO₂.

Survival and Apoptosis Assay: To evaluate cell survival after drug treatment, approximately 500,000 cells were plated in six-well plates and allowed to attach overnight. Exponentially growing cells were treated with cisplatin and paclitaxel (Sigma Aldrich, St. Louis, MO). Each cell line was exposed to escalating doses of drug in triplicate three times. Untreated cells served as a control. Cells were harvested 36 hours after drug exposure and stained with Annexin-V (Molecular Probes, Eugene, OR) and DAPI (Sigma Aldrich, St. Louis, MO). Cell survival and apoptosis were determined by flow cytometry using FACS Diva. FlowJo FACS analysis software (version 6.1.1) was used to generate percent apoptotic and live cells. Cells that were negative for both Annexin-V and DAPI were considered live, and cells that were positive for Annexin-V and negative for DAPI were considered apoptotic. Each experiment was normalized to its own dose 0 average, and percent live and percent apoptotic vs dose curves were constructed.

Statistical methods: Regression methods were used to predict response (proportion live cells remaining or proportion of cells apoptotic) with the natural logarithm of concentration. In all cell lines, response appeared approximately linear in log(concentration). The estimated IC50 and associated 95% confidence interval were obtained by projection of the fitted line and pointwise confidence bounds onto the concentration axis.

Results



Cisplatin IC ₅₀ Values				Paclitaxel IC ₅₀ Values			
Cell Line	IC ₅₀ (μM)	95% CI (μM)	% Apoptosis	Cell Line	IC ₅₀ (μM)	95% CI (μM)	% Apoptosis
UACC-3199	7.4	4.94-10.9	13%	UACC-3199	1.8	1.1-3.8	12%
HCC-1937	14.1	11.6-16.4	17%	HCC-1937	2.5	1.1-4.9	20%
MB-231	21.8	18.2-30.0	23%	MB-231	0.13	0.09-0.16	26%
MCF-7	> 250	N/A	N/A	MCF-7	>100	N/A	N/A

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1. Hedenfalk, L. et al., Gene-expression profiles in hereditary breast cancer. *N Engl J Med*, 2001. 344(8): p. 539-48.
2. Fushien, W.D., et al., The prognostic implication of the basal-like (cyclin E high/p53+/igf1-overexpressing) phenotype of BRCA1-related breast cancer. *Cancer Res*, 2004. 64(3): p. 830-5.
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5. Quinn, J.E., et al., BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. *Cancer Res*, 2003. 63(19): p. 6221-8.
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8. Fisher, M.L., et al., Enhancement of platinum-induced cytotoxicity by O6-benzylguanine. *Mol Cancer Ther*, 2003. 2(7): p. 633-40.

Summary

- Human breast cancer cells with *BRCA1* promoter methylation are sensitive to CDDP and resistant to paclitaxel as compared to ER negative cells with normal *BRCA1* expression
- BRCA1* methylated cells, are even more sensitive to CDDP than *BRCA1* mutated cells, likely related to low level activity of the truncated *BRCA1* protein
- As expected, the ER positive cell line, MCF-7 is resistant to both CDDP and paclitaxel
- As *BRCA1* promoter methylation occurs to some degree in almost 50% of high-grade, hormone receptor negative breast tumors, it represents a potential therapeutic target for a subset of hormone receptor negative breast cancers
- As *BRCA1* methylation confers relative resistance to paclitaxel *in vitro*, it may represent a potential mechanism of acquired paclitaxel resistance

Future Directions

- Investigate the potential role of demethylating agents as a mechanism of reversing resistance to paclitaxel *in vitro*
- Investigate the role of *BRCA1* promoter methylation in determining sensitivity to DNA damaging agents *in vivo*
- Investigate the role of *BRCA1* methylation in determining chemosensitivity to other chemotherapeutic agents used in the treatment of breast cancer, with the goal of identifying promising regimens to use in the management of *BRCA1* methylated tumors *in vivo*

Funding

This work was supported by the US Army Department of Defense Grant W81XWH-04-1-0545.

APPENDIX B

AMERICAN ASSOCIATION OF CANCER RESEARCH 2006 ANNUAL MEETING ABSTRACT

The Role of *BRCA1* Promoter Methylation in Determining Chemosensitivity *in vitro*

Rita Nanda¹, Cindy Collins¹, James J Dignam², Jinhua Xu¹, M Eileen Dolan¹, and Olufunmilayo I Olopade¹.

¹Department of Medicine and ²Department of Health Studies, University of Chicago, Chicago, IL, United States, 60637.

Background: Several groups have demonstrated that women with *BRCA1* germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling. While *BRCA1* germline mutations are uncommon, and contribute to fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* occur with much greater frequency. Our lab has previously demonstrated that methylation of the *BRCA1* promoter occurs in almost 50% of high-grade, hormone receptor negative sporadic tumors. Given the role of *BRCA1* in both DNA repair and cell cycle regulation, it is likely that cells deficient in *BRCA1* secondary to methylation will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, as has previously been shown for cells deficient in *BRCA1* secondary to mutation. The role of *BRCA1* methylation in determining chemosensitivity is unknown.

Methods: Using an *in vitro* model, the relative sensitivity of *BRCA1* methylated, mutated and competent cells was determined using four representative breast cancer cell lines: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with cisplatin (CDDP) and paclitaxel. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival was determined by flow cytometry using FACS DiVa. FlowJo FACS analysis software was used to generate percent apoptotic and live cells. IC₅₀ values and 95% confidence intervals were calculated from dose response curves.

Results: The IC₅₀ values and 95% confidence intervals for CDDP for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 7.4 μ M (4.94-10.9), 14.1 μ M (11.6-16.4), and 21.8 μ M (18.2-30.0), respectively. The IC₅₀ value for CDDP for MCF-7 was not reached, even at a dose of 250 μ M. The IC₅₀ values and 95% confidence intervals for paclitaxel for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 1.8 μ M (1.1-3.8), 2.5 μ M (1.1-4.9) and 0.13 μ M (0.09-0.16), respectively. Despite dose escalation to a paclitaxel concentration of 200 μ M, the IC₅₀ value for MCF-7 was not reached.

Discussion: Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are sensitive to cisplatin and resistant to paclitaxel, as compared to *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary to promoter methylation are also highly sensitive to cisplatin and resistant to paclitaxel. As almost 50% of high-grade hormone receptor negative tumors have *BRCA1* promoter methylation, *BRCA1* methylation may represent both a novel therapeutic target for platinum-based chemotherapy and a mechanism for acquired resistance to paclitaxel chemotherapy. This work was supported by the US Army Department of Defense Grant W81XWH-04-1-0545.



The Role of *BRCA1* Promoter Methylation in Determining Chemosensitivity *in vitro*

Rita Nanda, Cindy Collins, James J. Dignam, Jinhua Xu, M. Eileen Dolan, Olufunmilayo I. Olopade
The University of Chicago, Chicago, IL



Abstract

Background: Several groups have demonstrated that women with *BRCA1* germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling^{1,2}. While *BRCA1* germline mutations are uncommon, and contribute to fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* occur with much greater frequency. Our lab has previously demonstrated that heterogeneous methylation of the *BRCA1* promoter occurs in almost 50% of high-grade, hormone receptor negative sporadic tumors³. Given the role of *BRCA1* in both DNA repair and cell cycle regulation, it is likely that cells deficient in *BRCA1* secondary to methylation will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, as has previously been demonstrated for cells deficient in *BRCA1* secondary to mutation^{4,5}. The role of *BRCA1* methylation in determining chemosensitivity is unknown.

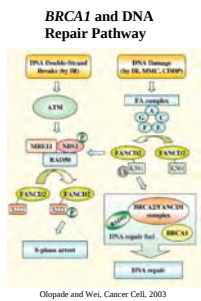
Methods: Using an *in vitro* model, the relative sensitivity of *BRCA1* methylated, mutated and competent cells was determined using four representative breast cancer cell lines: UACC-3199 (methylated *BRCA1*), HCC-1937 (mutated *BRCA1*), MCF-7 (wildtype *BRCA1*, ER positive) and MDA-MB-231 (wildtype *BRCA1*, ER negative). Exponentially growing cells were treated with cisplatin and paclitaxel. Cells were harvested 96 hours after drug exposure and stained with Annexin-V and DAPI. Cell survival was determined by flow cytometry using FACS Diva. FlowJo FACS analysis software was used to generate percent apoptotic and live cells. IC₅₀ values and 95% confidence intervals were calculated from dose response curves.

Results: The IC₅₀ values and 95% confidence intervals for cisplatin for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 7.4 μM (4.94-10.9), 14.1 μM (11.6-16.4), and 21.8 μM (18.2-30.0), respectively. The IC₅₀ value for cisplatin for MCF-7 was not reached, even at a dose of 250 μM. The IC₅₀ values and 95% confidence intervals for paclitaxel for the UACC-3199, HCC-1937 and MDA-MB-231 cells were 1.8 μM (1.1-3.8), 2.5 μM (1.1-4.9) and 0.13 μM (0.09-0.16), respectively. Despite dose escalation to a paclitaxel concentration of 100 μM, the IC₅₀ value for MCF-7 was not reached.

Discussion: Previous studies have demonstrated that cells deficient in *BRCA1* secondary to mutation are sensitive to cisplatin and resistant to paclitaxel, as compared to *BRCA1* competent cells. We have demonstrated for the first time that cells deficient in *BRCA1* secondary to promoter methylation are also highly sensitive to cisplatin and resistant to paclitaxel. As almost 50% of high-grade hormone receptor negative tumors have some degree of *BRCA1* promoter methylation, *BRCA1* methylation may represent both a novel therapeutic target for platinum-based chemotherapy and a mechanism for acquired resistance to paclitaxel chemotherapy.

Background

•Women with *BRCA1* germline mutations are more likely to have cancers that are ER, PR and HER2/*neu* negative
•Germline *BRCA1* mutations account for fewer than 5% of breast cancers, but *BRCA1* promoter methylation is much more common
•We have demonstrated that *BRCA1* promoter methylation occurs to some degree in almost 50% of high-grade hormone receptor negative tumors
•Given the role of *BRCA1* in DNA repair, cells deficient in *BRCA1* secondary to promoter methylation are likely to have increased sensitivity to DNA damaging agents
•Given the role of *BRCA1* in the G₂/M checkpoint, cells deficient in *BRCA1* are likely to be resistant to paclitaxel, as paclitaxel sensitivity is dependent on an intact G₂/M checkpoint



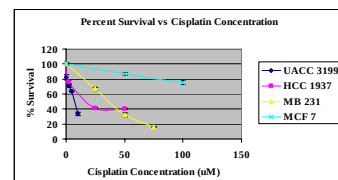
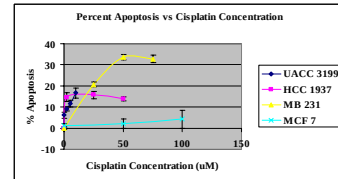
Methods

Cell Lines: The HCC-1937, MDA-MB-231 and MCF-7 cell lines were purchased from ATCC (Rockville, MD). The UACC-3199 cell line was purchased from the University of Arizona Cancer Center (Tucson, AZ). The MCF-7 cells were cultured in DMEM (Fisher Scientific, Hanover Park, IL) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD). The UACC-3199, HCC-1937 and MDA-MB-231 cells were maintained in RPMI 1640 (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum. All cell lines were grown with the addition of 1% penicillin/streptomycin (Fisher Scientific, Hanover Park, IL) at 37°C and 5% CO₂.

Survival and Apoptosis Assay: To evaluate cell survival after drug treatment, approximately 500,000 cells were plated in six-well plates and allowed to attach overnight. Exponentially growing cells were treated with cisplatin and paclitaxel (Sigma Aldrich, St. Louis, MO). Each cell line was exposed to escalating doses of drug in triplicate three times. Untreated cells served as a control. Cells were harvested 36 hours after drug exposure and stained with Annexin-V (Molecular Probes, Eugene, OR) and DAPI (Sigma Aldrich, St. Louis, MO). Cell survival and apoptosis were determined by flow cytometry using FACS Diva. FlowJo FACS analysis software (version 6.1.1) was used to generate percent apoptotic and live cells. Cells that were negative for both Annexin-V and DAPI were considered live, and cells that were positive for Annexin-V and negative for DAPI were considered apoptotic. Each experiment was normalized to its own dose 0 average, and percent live and percent apoptotic vs dose curves were constructed as previously described⁶.

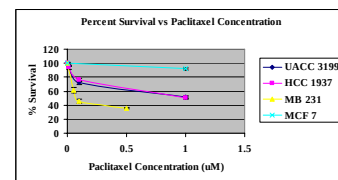
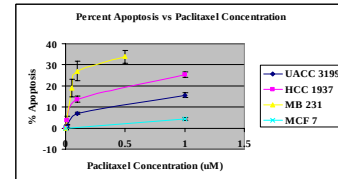
Statistical methods: Regression methods were used to predict response (proportion live cells remaining or proportion of cells apoptotic) with the natural logarithm of concentration. In all cell lines, response appeared approximately linear in log(concentration). The estimated IC₅₀ and associated 95% confidence interval were obtained by projection of the fitted line and pointwise confidence bounds onto the concentration axis.

Results



Cisplatin IC₅₀ Values

Cell Line	IC ₅₀ (μM)	95% CI (μM)
UACC-3199	7.4	4.94-10.9
HCC-1937	14.1	11.6-16.4
MB-231	21.8	18.2-30.0
MCF-7	> 250	N/A



Paclitaxel IC₅₀ Values

Cell Line	IC ₅₀ (μM)	95% CI (μM)
UACC-3199	1.8	1.1-3.8
HCC-1937	2.5	1.1-4.9
MB-231	0.13	0.09-0.16
MCF-7	>100	N/A

References

- Hendelink, L. et al., *Gene-expression profiles in hereditary breast cancer*. N Engl J Med, 2001. 344(8): p. 539-48.
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- Grushko, T.A., et al., *Molecular-cytogenetic analysis of HER-2/*neu* gene in BRCA1-associated breast cancers*. Cancer Res, 2002. 62(5): p. 1481-8.
- Fisher, M.L., et al., *Enhancement of platinum-induced cytotoxicity by O6-benzylguanine*. Mol Cancer Ther, 2003. 2(7): p. 633-40.

Summary

•Human breast cancer cells with *BRCA1* promoter methylation are relatively more sensitive to cisplatin and more resistant to paclitaxel, as compared to ER negative cells with normal *BRCA1* expression

•The *BRCA1* methylated cell line, UACC-3199, appears to be more sensitive to cisplatin than the *BRCA1* mutated cell line, possibly related to low level activity of the truncated *BRCA1* protein in HCC-1937 cells

•The ER positive cell line, MCF-7, is relatively resistant to both cisplatin and paclitaxel

•*BRCA1* methylation confers relative resistance to paclitaxel *in vitro*, and may represent a potential mechanism of acquired paclitaxel resistance

•*BRCA1* promoter methylation occurs to some degree in a significant proportion of high-grade, hormone receptor negative breast tumors, and represents a potential therapeutic target

Future Directions

•Investigate the role of promoter demethylation as a mechanism of reversing resistance to paclitaxel *in vitro*

•Investigate the role of *BRCA1* promoter methylation in determining sensitivity to DNA damaging agents *in vivo*

•Investigate the role of *BRCA1* methylation in determining chemosensitivity to other chemotherapeutic agents used in the treatment of breast cancer, with the goal of identifying promising regimens to use in the management of *BRCA1* methylated tumors *in vivo*

Funding

This work was supported by the US Army Department of Defense Grant W81XWH-04-1-0545.

APPENDIX C

AMERICAN SOCIETY OF CLINICAL ONCOLOGY 2007 ANNUAL MEETING ABSTRACT

Relationship between *BRCA1* promoter methylation and sensitivity of breast cancer cell lines to cisplatin and paclitaxel

C. Collins¹, D. Huo², J. Xu¹, W. K. Bleibel¹, M. E. Dolan¹, O. I. Olopade¹, R. Nanda¹

¹Section of Hematology/Oncology, University of Chicago, Chicago, IL, ²Department of Health Studies, University of Chicago, Chicago, IL.

Background: While *BRCA1* germline mutations are uncommon, and contribute to fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* occur more frequently. *BRCA1* promoter methylation has been detected in 10-30% of breast tumors. Given the role of *BRCA1* in DNA repair and cell cycle regulation, we hypothesize that cells with decreased expression of *BRCA1* secondary to promoter methylation will be sensitive to DNA damaging agents and resistant to microtubule inhibitors, as has previously been shown for cells deficient in *BRCA1* secondary to mutation.

Methods: *BRCA1* methylation was determined using methylation specific PCR (MSP) as previously described (Wei et al, Cancer Research 2005). The relative sensitivities of *BRCA1* methylated, mutated and competent cells to cisplatin and paclitaxel were determined in five representative breast cancer cell lines using the AlamarBlue cytotoxicity assay. Exponentially growing cells were treated with increasing concentrations of cisplatin and paclitaxel for 96 hours. IC₅₀ values and 95% confidence intervals (CI) were calculated from sigmoidal dose response curves fitted with SAS 9.1 Proc NLIN. Western blot analysis for *BRCA1* was performed on each cell line.

Results:

Cell Line	BRCA1 status	ER status	Cisplatin IC ₅₀ (95%CI) μ M	Paclitaxel IC ₅₀ (95% CI) μ M
HCC38	methylated	neg	11.3 (10.9-11.7)	0.049 (0.041-0.057)
HCC1937	mutated	neg	4.8 (4.4-5.3)	0.057 (0.027-0.120)
MB231	wildtype	neg	7.0 (6.4-7.7)	0.032 (0.028-0.037)
MCF7	wildtype	pos	11.0 (10.0-11.9)	0.048 (0.011-0.214)
UACC3199	methylated	neg	3.0 (2.8-3.3)	> 1.0

Conclusions: Only one of the two *BRCA1* methylated cell lines studied (UACC3199) was sensitive to cisplatin and resistant to paclitaxel, as hypothesized. While both cell lines are methylated, western blot analysis revealed that both express *BRCA1*, but to a lesser degree than unmethylated cells. *BRCA1* methylation, as assessed by non-quantitative MSP, does not correlate with sensitivity to cisplatin and resistance to paclitaxel. Quantification of *BRCA1* promoter methylation may better predict chemosensitivity. Identification of the degree of *BRCA1* methylation which does correlates with sensitivity to cisplatin and resistance to paclitaxel could improve treatment selection for patients with breast cancer.

This work was supported by the US Army Grant W81XWH-04-1-0545.

APPENDIX D

PROTOCOL AND CONSENT

**“A PHASE II STUDY OF CARBOPLATIN AND BEVACIZUMAB
(AVASTIN[®]) COMBINATION THERAPY FOR BASAL-LIKE
METASTATIC BREAST CANCER”**

University of Chicago IRB #:

A Phase II Study of Carboplatin and Bevacizumab (Avastin®) Combination Therapy for Basal-like Metastatic Breast Cancer

Institution: University of Chicago

Principal Investigator: Rita Nanda, M.D.
University of Chicago Medical Center
5841 S. Maryland Ave, MC 2115
Chicago, IL 60637
Phone: 773-834-2756
Fax: 773-834-3834

Co-Investigators: Funmi Olopade, M.D.
Gini Fleming, M.D.

Statistician: Dezheng Huo, Ph.D.

Responsible Research Nurse: Gina Menyah, R.N.

Responsible Data Manager: Bernadette Libao

Version Date: 03/26/07

SCHEMA

Patient Eligibility	Required Laboratory Data
Pathologically confirmed metastatic breast cancer. ER, PR and HER2/ <i>neu</i> negative tumor. Measurable disease. ECOG PS 0-1. Age ≥ 18 years. Non-pregnant and not breast feeding. No prior therapy for metastatic disease. No prior therapy with platinum agents, bevacizumab, or other VEGF inhibitors. No evidence of CNS disease, including primary brain tumor. No currently active secondary malignancy. No prior history of significant bleeding (see section 3.2.9). Tumor block must be available for correlative studies Peripheral neuropathy $\leq$ grade 1 No other serious medical or psychiatric disease. No serious or non-healing wound, ulcer or bone fracture. No serious active infection (viral, fungal, bacterial). No infection requiring parenteral antibiotics at the time of registration. No clinically significant cardiac disease (see section 3.2.5). No inadequately controlled hypertension (see section 3.2.10)	Absolute neutrophils $\geq 1,500/\mu\text{L}$ Platelets $\geq 100,000/\mu\text{L}$ Bilirubin $\leq \text{ULN}^1$ AST and ALT $\leq 2.5 \text{ ULN}$ Creatinine (Cr) $\leq \text{ULN}$ Creatinine Clearance $\geq 60 \text{ mL/min/1.73m}^2$ (if Cr above ULN) PT INR $\leq 1.5^2$ Urine protein $\leq 1+^3$

¹ULN = upper limits of normal

²Unless patient is on anticoagulation. Pt must be on a stable dose of warfarin or LMWH and have an in-range INR (usually between 2-3).

³For $\geq 2+$ proteinuria on urine dipstick, if a 24 hour urine collection demonstrates $\leq 1\text{gm/dL}$ of protein/24 hours the patient would be eligible.

TREATMENT PLAN

Patients with ER, PR and HER2/neu negative metastatic disease who have had no prior therapy for metastatic disease will be treated with:

Carboplatin AUC 6 IV every 3 weeks
Bevacizumab 15 mg/kg IV every 3 weeks

Primary Endpoint: Time to progression

Secondary Endpoints: Response rate, duration of response, correlation of response to *BRCA1* promoter methylation

Correlative Studies: Tumor blocks from the primary tumor will be collected on all women and examined for *BRCA1* promoter methylation. Tissue Microarrays (TMA) will be constructed for further immunohistochemical study of markers of interest.

Potential Toxicity: Nausea, vomiting, fatigue, dyspnea, neurotoxicity, ototoxicity, renal dysfunction, electrolyte wasting, rash, bleeding, thrombotic events, cardiotoxicity, hypertension, cytopenias, fever/infection.

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1. OBJECTIVES

1.1. Primary Objective

To determine the time to disease progression of metastatic ER, PR and HER2/neu negative breast cancers to the combination of carboplatin and bevacizumab therapy.

1.2. Secondary Objectives

1.2.1. To determine response rate and duration of response.

1.2.2. To perform a preliminary analysis exploring whether tumors that have *BRCA1* promoter hypermethylation have a response rate that differs from those tumors without *BRCA1* promoter hypermethylation.

2. BACKGROUND

2.1 Metastatic Breast Cancer

Breast cancer is the second highest cause of cancer death in women in the United States¹. While breast cancer is being diagnosed at earlier stages because of mammography, approximately 20-85% of patients go on to develop distant metastases. About 6-10% of women in the U.S. present with metastatic disease. Chemotherapy and hormone therapy have both been used in the treatment of metastatic disease. Most patients experience an objective response, with responses lasting anywhere from 8-14 months. Unfortunately, progression of disease is inevitable, and response to second-line therapy is less likely. While a few trials for certain first-line regimens are associated with improved survival, chemotherapy beyond first-line treatments has not been shown to improve overall survival². Metastatic breast cancers that are negative for ER, PR and HER2/*neu* negative (basal-like) are aggressive and confer a poor prognosis. There is an urgent need to identify better therapies for this aggressive and difficult to treat disease.

2.2 Role of *BRCA1* in Sporadic Breast Cancers

Several groups have demonstrated that women with *BRCA1* germline mutations are more likely to have breast cancers that are basal-like by gene expression profiling. While germline mutations in *BRCA1* are uncommon and account for fewer than 5% of breast cancer cases, epigenetic alterations in *BRCA1* are much more common. Our laboratory has demonstrated that hypermethylation of the *BRCA1* promoter occurs in almost 50 % of high-grade hormone receptor negative sporadic tumors, making promoter methylation much more common than germline mutation³.

A great deal about the biochemical function of the *BRCA1* gene is known. Substantial evidence exists supporting the role of *BRCA1* in the cellular response to DNA damage. In response to various DNA-damaging agents, *BRCA1* is phosphorylated and subsequently colocalizes with RAD51 to the site of DNA damage and initiates repair⁴. Lafarge and colleagues demonstrated that decreased expression of *BRCA1* in the HBL100 breast cancer cell line led to increased sensitivity to the DNA damaging agent cisplatin⁵. It has been suggested that this

increased platinum sensitivity accounts for the increased survival of ovarian cancer patients with *BRCA1* germline mutations, as most ovarian cancer patients are treated with platinum-based regimens⁶. A number of studies have also correlated *BRCA1* deficiency with defects in cell cycle checkpoints. Tumors lacking functional *BRCA1* protein demonstrate a high frequency of chromosomal aneuploidy, characteristic of a defective G₂/M checkpoint⁷. Sudo and colleagues have demonstrated that paclitaxel sensitivity is dependent on an intact checkpoint function, thus implying that any interference with the spindle assembly checkpoint would generate paclitaxel resistance⁸. Several groups have convincingly demonstrated that *BRCA1* deficient cells secondary to mutation are relatively resistant to paclitaxel^{5, 9, 10}. Put together, these data suggest that loss of normal *BRCA1* expression confers a unique chemosensitivity profile. We hypothesize that *BRCA1* deficiency secondary to promoter methylation represents a novel therapeutic target for the management of a subset of basal-like breast cancers.

My laboratory has generated preliminary *in vitro* data that demonstrate breast cancer cells with *BRCA1* promoter methylation and low expression levels are three-fold more sensitive to cisplatin and ten times more resistant to paclitaxel, as compared to cells with normal *BRCA1* expression¹¹.

2.3 **Platinum agents and Breast Cancer**

As a single agent, carboplatin has been reported to have about a 30% response rate in previously untreated breast cancer and a response rate of less than 10% in women with previous therapy¹²⁻¹⁴. However, there has recently been increased interest in using platinum agents for women with breast cancer because of the marked synergy suggested *in vitro* with carboplatin and trastuzumab, and promising preliminary results with cisplatin and trastuzumab in heavily pretreated breast cancer patients¹⁵. A cisplatin/docetaxel regimen and a carboplatin/paclitaxel regimen have both been shown to have good activity as first-line treatment for breast cancer and the carboplatin /docetaxel regimen is currently being tested in the adjuvant setting. Cisplatin causes more nausea, vomiting, and neuropathy than carboplatin, but less myelotoxicity¹⁴. Overall, carboplatin appears to be at least as effective as cisplatin, and much better tolerated.

2.4 **VEGF Inhibitors and Breast Cancer**

In addition to *BRCA1* methylation, angiogenesis has also been shown to be a potential therapeutic target for hormone receptor negative breast cancer. Hormone receptor negative tumors are highly angiogenic as measured by intratumoral microvessel density¹⁶. Furthermore, Chang and colleagues demonstrated that the “wound response” signature, which includes genes involved in angiogenesis and matrix remodeling, was remarkably similar to the basal-like signature, further suggesting a role for anti-angiogenic agents in these tumors¹⁷. VEGF is a key molecule involved in both angiogenesis and endothelial cell survival¹⁸. For solid tumors to grow, they must induce new blood vessel formation. VEGF appears to be the single most important factor in the formation of new blood vessels, and VEGF secreted by tumor cells appears to stimulate the

growth of endothelial cells and increases microvascular permeability^{19, 20}. Anti-angiogenic therapy as monotherapy, however, has not been very effective in breast cancer²¹. Bevacizumab, a VEGF inhibitor, was recently shown to have a survival advantage for women with metastatic breast cancer when given in combination with paclitaxel chemotherapy as compared to those who were treated with paclitaxel alone²². Furthermore, VEGF inhibitors are an attractive new class of agents, as they act synergistically with chemotherapy without increasing toxicity.

2.5

Rationale for Study

In this trial, we propose to study carboplatin and bevacizumab combination therapy in a cohort of patients with hormone receptor and HER2/*neu* negative (basal-like) metastatic breast cancer. Because these patients are likely to have BRCA1 deficiency secondary to *BRCA1* promoter methylation (and hence be more sensitive to a DNA damaging agent) and tumors with high levels of VEGF expression, we hypothesize that they are likely to respond to the combination of carboplatin and bevacizumab.

3. PATIENT SELECTION

3.1 Eligibility Criteria

- 3.1.1 Patients must have pathologically confirmed ER, PR and HER2/*neu* negative (FISH ratio of <2.0 or IHC $\leq 1+$) metastatic breast cancer.
- 3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan. See section 10 for the evaluation of measurable disease.
- 3.1.3 Patients must not have received prior chemotherapy for metastatic breast cancer (not including adjuvant therapy). Patients should be ≥ 4 weeks from their most recent chemotherapy or radiation therapy treatment.
- 3.1.4 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of carboplatin in combination with bevacizumab in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 2 combination trials.
- 3.1.5 ECOG performance status ≤ 1 (Karnofsky $\geq 80\%$; see Appendix A).
- 3.1.6 Patients must have normal organ and marrow function as defined below:
 - absolute neutrophil count $\geq 1,500/\mu\text{L}$
 - platelets $\geq 100,000/\mu\text{L}$
 - total bilirubin within normal institutional limits

- AST(SGOT)/ALT(SGPT) ≤ 2.5 X institutional upper limit of normal
 - creatinine within normal institutional limits
- OR
- creatinine clearance ≥ 60 mL/min/1.73 m² for patients with creatinine levels above institutional normal
 - PT INR $\leq 1.5^*$
 - urine protein $\leq 1^{+**}$

* Unless patient is on anticoagulation, in which case the patient should be on a stable dose of warfarin or LMWH, with an INR in the desired range (usually between 2-3).

**For patients with proteinuria on urine dipstick $\geq 2^+$, if a 24 hour urine collection demonstrates ≤ 1 gm/dL of protein, the patient is eligible.

3.1.7 Tissue from the primary tumor must be available for correlative studies.

3.1.8 The effects of bevacizumab and carboplatin on a developing human fetus at the recommended therapeutic dose are unknown. For this reason women of child-bearing potential and men must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Nursing patients must discontinue breast feeding prior to the initiation of therapy.

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

3.2 Exclusion Criteria

3.2.1 Patients who have had prior therapy with platinum agents or a VEGF inhibitor are not eligible.

3.2.2 Patients may not be receiving any other investigational agents.

3.2.3 Patients with known brain metastases will be excluded from this clinical trial because of the risk of CNS bleeding in patients receiving bevacizumab.

3.2.4 Patients may have had prior radiation therapy, provided the patient has measurable disease and there has been clear progression since the completion of radiation therapy. Patients who have had radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events due to therapy administered more than 4 weeks earlier will be excluded.

- 3.2.5 Patients with significant cardiac dysfunction will be excluded from this trial as bevacizumab is associated with an increase in the risk of cardiac dysfunction. This includes patients with an ejection fraction below institutional limits of normal, myocardial infarction or unstable angina within 6 months of registration, New York Heart Association grade II or greater CHF, or grade II or greater peripheral vascular disease.
- 3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.2.7 Pregnant women are excluded from this study because carboplatin, and bevacizumab have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued.
- 3.2.8 Because patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy, HIV-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with carboplatin or the other agents administered during the study.
- 3.2.9 Patients with evidence of bleeding diathesis or coagulopathy. If a patient is on full-dose anticoagulation, the patient must be on a stable dose of warfarin or LMWH and have an in-range INR (usually between 2-3).
- 3.2.10 Patients with inadequately controlled hypertension will be excluded from this trial as bevacizumab has been associated with hypertensive crisis. Inadequately controlled hypertension is defined as blood pressure > 150/100 on medication.
- 3.2.11 Patients who have had a stroke or TIA within 6 months of registration will be excluded.
- 3.2.12 Patients with a history of hypertensive crisis or hypertensive encephalopathy will be excluded.
- 3.2.13 Patients with a history of abdominal fistula, GI perforation, or intra-abdominal abscess within 6 months of registration.
- 3.2.14 Patient with history of serious non-healing wound, ulcer or bone fracture.
- 3.2.15 Patients with major surgery, open biopsy, or significant traumatic injury within 28 days of registration or anticipated need for surgery during course of study treatment.

3.2.16 Patients with a history core biopsy or other minor surgery, excluding venous access device (VAD) placement, within 7 days of registration.

3.2.17 Patients with active second malignancy.

3.2.18 Known hypersensitivity to any component of bevacizumab.

3.2.19 Peripheral neuropathy > Grade 1.

3.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. However, because of the rarity of breast cancer in males, coupled with the fact that most male breast cancers are hormone receptor positive, it is expected that only female patients will be enrolled. The proposed study population is illustrated in the table below. Figures are based on a 5-year average of University of Chicago accrual to CALGB protocol.

Race/Ethnicity

Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Hispanic	Asian or Pacific Islander	Unknown	Total
Male	0	0	0	0	0	0
Female	24	10	1	1	0	36
Total	24	10	1	1	0	36

4. SAFETY PLAN

4.1 Bevacizumab

4.1.1 In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail as follows and in the bevacizumab Investigator Brochure.

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria (see Section 3.2) and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements. Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28–42 days) after the decision to discontinue treatment.

- Hypertension will be monitored through routine evaluation of blood pressure.
- Proteinuria will be monitored by urine protein:creatinine (UPC) ratio or dipstick at least every 6 weeks.
- If patients on treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure (in the case of high risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 wk and bevacizumab no earlier than 8 wk after surgery).

Hypertension: Hypertension has been commonly seen in bevacizumab clinical trials to date and oral medications have been used to manage the hypertension when indicated. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) (Ozcan et al., 2006; Glusker et al., 2006). RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances / cortical

blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and discontinuation of bevacizumab.

Proteinuria: Proteinuria has been commonly seen in bevacizumab clinical trials to date. The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1 or 2. In study AVF2107g, none of the 118 patients receiving bolus-IFL plus placebo, three of 158 patients (2%) receiving bolus-IFL plus bevacizumab, and two of 50 (4%) patients receiving 5-FU/LV plus bevacizumab who had a 24-hour collection experienced grade 3 proteinuria (> 3.5 g protein/24 hr). Rare events of nephrotic syndrome have occurred, and bevacizumab should be discontinued in patients with nephrotic syndrome.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials. In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of **venous TE** events that was not statistically significant in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%). There was also a higher rate of **arterial TE** events (3% vs. 1%) such as myocardial infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina. A pooled analysis of the rate of arterial TE events from 5 randomized studies (1745 patients) showed that treatment with chemotherapy plus bevacizumab increased the risk of having an arterial TE event compared with chemotherapy alone (3.8% vs. 1.7%, respectively) (Skillings et al., 2005). Furthermore, subjects with certain baseline characteristics (age ≥ 65 years and/or a history of a prior arterial TE event) may be at higher risk of experiencing such an event. See the bevacizumab Investigator Brochure for additional information on risk factors.

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal perforation Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to bevacizumab has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, and breast) and may be higher in incidence in some tumor types.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 20 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

Hemorrhage: Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-associated hemorrhage – was observed in phase I and phase II bevacizumab studies. Six serious events, of which 4 had fatal outcome, were observed in a phase II trial of patients with non-small cell lung cancer receiving bevacizumab. These events occurred suddenly and presented as major or massive hemoptysis in patients with either squamous cell histology and/or tumors located in the center of the chest in close proximity to major blood vessels. In five of these cases, these hemorrhages were preceded by cavitation and/or necrosis of the tumor. Tumor-associated hemorrhage was also seen rarely in other tumor types and locations, including central nervous system (CNS) bleeding in a patient with hepatoma with occult CNS metastases and continuous oozing of blood from a thigh sarcoma with necrosis.

Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen.

There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Congestive heart failure: CHF has been reported in bevacizumab clinical trials and may be increased in incidence in patients with prior exposure to anthracyclines or prior irradiation to the chest wall. In a phase III trial (AVF2119g) of capecitabine with or without bevacizumab for metastatic breast cancer, 7 subjects (3.1%) who received capecitabine plus bevacizumab developed clinically significant CHF compared with 2 subjects (0.9%) treated with capecitabine alone; of note, all subjects in this trial had had prior anthracycline treatment. In addition, 2 subjects had a left ventricular ejection fraction < 50% at baseline and 2 others had prior left chest wall irradiation. A recently published phase II study in subjects with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or decreases to <40% in left ventricular ejection fraction) of 48 subjects treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but one of these subjects had significant prior exposure to anthracyclines as well (Karp et al., 2004). Other studies are ongoing in this patient population. Patients receiving anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA or ECHO with a normal ejection fraction.

Additional Adverse Events: See the bevacizumab Investigator Brochure for additional details regarding the safety experience with bevacizumab.

5. TREATMENT PLAN

5.1 Agent Administration

- 5.1.1 Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for carboplatin and bevacizumab are described in Section 6. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.
- 5.1.2 Carboplatin will be administered at a dose of AUC 6 in 250 mL normal saline intravenously over 30 mins.
- 5.1.3 Bevacizumab will be administered at a dose of 15 mg/kg in 100 mL normal saline. The initial dose will be delivered over 90±15 minutes. If the first infusion is tolerated without infusion-associated adverse events (fever and/or chills), the second infusion may be delivered over 60±10 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30±10 minutes. If a subject experiences an infusion-associated adverse event, he or she may be premedicated for the next study drug infusion; however, the infusion time may not be decreased for the subsequent infusion. If the next infusion is well tolerated with premedication, the

subsequent infusion time may then be decreased by 30 ± 10 minutes as long as the subject continues to be premedicated. If a subject experiences an infusion-associated adverse event with the 60-minute infusion, all subsequent doses should be given over 90 ± 15 minutes. Similarly, if a subject experiences an infusion-associated adverse event with the 30-minute infusion, all subsequent doses should be given over 60 ± 10 minutes.

- 5.1.4 Carboplatin and bevacizumab will both be administered on day 1 of every 3 week cycle.

5.2 Supportive Care Guidelines

- 5.2.1 While the exact regimen to be used is left to the discretion of the treating physicians, all patients should receive anti-emetic therapy. A reasonable option would be a combination of dexamethasone 10-20 mg and a 5HT₃ receptor antagonist prior to carboplatin.
- 5.2.2 The use of darbopoeitin or erythropoeitin is permitted, and should be documented.
- 5.2.3 The use of granulocyte colony stimulating agents should only be used if there is persistent neutropenia despite a dose reduction in the previous course. G-CSF may also be used as clinically indicated for neutropenic infection.
- 5.2.4 The use of bisphosphonates is permitted, however in these patients bone should not be used as a site in determining progression of disease.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until:

- X Disease progression,
- X Intercurrent illness that prevents further administration of treatment,
- X Unacceptable adverse events(s),
- X Patient decides to withdraw from the study, or
- X General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Should the patient have a complete response, the carboplatin therapy should be discontinued and the bevacizumab may be continued at the physician's discretion until the time of disease progression or unacceptable toxicity.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Carboplatin Associated Toxicity

Patients who require discontinuation of carboplatin therapy prior to documented response (either partial or complete) or stable disease will be removed from study and no further carboplatin or bevacizumab will be administered. If patient has had a documented response (either partial or complete) or stabilization of disease and must discontinue carboplatin due to unacceptable toxicity, patients may continue to receive bevacizumab at the treating physician's discretion.

6.1.1 Hematologic Toxicity

Subsequent cycles of therapy (day 1) will not begin until the ANC is $\geq$ 1500 cells/uL and the platelet count is $\geq$ 100,000/uL. Initiation of a new cycle will be delayed for a maximum of two weeks until these values are achieved. Patients who fail to recover adequate counts within a two week delay will be removed from study.

In case of neutropenic fever, grade 4 thrombocytopenia, or delay of a cycle for over 7 days, the carboplatin dose will be decreased to AUC 4.5 for subsequent cycles. For a second episode of febrile neutropenia, G-CSF should be given with the next cycle. For an episode of febrile neutropenia despite dose reduction and G-CSF, protocol treatment will be discontinued.

6.1.2 Infusion Reaction

Infusion of carboplatin should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 3.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from carboplatin treatment.

6.1.3. Neuropathy

Grade 2 or greater peripheral neuropathy will require a delay in carboplatin therapy until recovery to grade 1.

6.1.4 Nephrotoxicity

If the creatinine is not within institutional normal limits on the day of a carboplatin or bevacizumab dose, both carboplatin and bevacizumab therapy will be held. It may be re-instituted at full dose when the creatinine recovers to within institutional limits of normal or $\geq$ 60 mL/min/1.73m² for patients with baseline creatinine above upper limits of normal.

6.2 Bevacizumab Associated Toxicity

For those patients who require permanent discontinuation of bevacizumab therapy, they may continue on study and continue to receive carboplatin until progression of disease or unacceptable toxicity.

There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes. Any toxicities associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice. Bevacizumab has a terminal half-life of 2 to 3 weeks; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

6.2.1 Infusion Reaction

Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 3.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from bevacizumab treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.

6.2.2 Cardiotoxicity

For congestive heart failure or LV systolic dysfunction, no dose modification for grade 1-2 events. For grade 3 events, hold bevacizumab until resolution to grade ≤ 1 . If the patient develops clinical grade 4 cardiotoxicity, bevacizumab therapy will be discontinued permanently.

6.2.3 Hypertension

Bevacizumab should be permanently discontinued in patients with hypertensive crisis, RPLS (confirmed on MRI) or hypertensive encephalopathy. No dose modifications for grade 1/2 events. For grade 3, if not controlled to 150/100 mmHg with medication, discontinue bevacizumab. For all grade 4, discontinue bevacizumab.

6.2.4 Hemorrhage

No dose modification is required for grade 1-2 non-pulmonary and non-CNS events. For pulmonary or CNS hemorrhage $\geq$ grade 2, bevacizumab will be permanently discontinued. For grade 3 non-pulmonary and non-

CNS hemorrhage, subjects who are on full anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have study treatment held until all of the following criteria are met: 1) the bleeding has resolved and hemoglobin is stable, 2) there is no bleeding diathesis that would increase the risk of therapy and 3) there is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat grade 3 hemorrhagic event will be discontinued from receiving bevacizumab. All grade 4 hemorrhage requires immediate discontinuation of bevacizumab and aggressive medical management.

6.2.5 Proteinuria

No dose modifications for grade 1/2 events. For grade 3, hold bevacizumab treatment until $\leq$ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g. For grade 4 (nephrotic syndrome) discontinue bevacizumab.

6.2.6 Arterial Thromboembolic Event (ATE)

For any ATE, as defined as angina, myocardial infarction, transient ischemic attack, cerebrovascular accident or any other ATE, bevacizumab will be discontinued for any event, grade 1-4.

6.2.7 Venous Thrombosis

No dose modification for grade 1-2 events. For grade 3 and asymptomatic grade 4, hold study drug treatment. Hold study drug treatment. If the planned duration of full-dose anticoagulation is <2 weeks, study drug should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, study drug may be resumed during the period of full-dose anticoagulation if all of the following criteria are met: 1) The patient must have an in-range stable dose of warfarin (or other anticoagulant) prior to drug treatment. 2) The subject must not have had a hemorrhagic event while on anticoagulation. 3) The subject must not have had evidence of tumor involving major blood vessels on any prior CT scan. For symptomatic grade 4 events,

the
INR on a
restarting study
grade 3 or 4
The subject must not have
vessels on any prior CT
bevacizumab will be discontinued.

6.2.8 GI Perforation

Discontinue bevacizumab.

6.2.9 Bowel Obstruction

For grade 1 partial obstruction not requiring medical intervention, continue patient on study. For grade 2 partial obstruction requiring medical intervention, hold until complete resolution. For grade 3-4, hold bevacizumab for complete obstruction. If surgery is necessary, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion.

6.2.10 Wound dehiscence
Discontinue bevacizumab.

6.2.11 Other unspecified bevacizumab-related adverse events
For grade 3 events, hold bevacizumab until recovery to less than grade 1.
For all grade 4 events, discontinue bevacizumab.

7. AGENT FORMULATION AND PROCUREMENT

7.1 Carboplatin

7.1.1 Product description: Carboplatin is a second generation tetravalent organic platinum compound. Like cisplatin, carboplatin produces predominantly interstrand DNA crosslinks rather than DNA-protein crosslinks and is cell-cycle non-specific.

7.1.2 Solution preparation: Add 5, 15, or 45 mL of sterile water, normal saline or 5% dextrose to the 50, 150 or 450 mg vials, respectively. The resulting solution contains 10 mg/mL. The desired dose is further diluted, usually in 5% dextrose.

7.1.3 Storage requirements: Intact vials are stored at room temperature protected from light.

7.1.4 Stability: When further diluted in glass or polyvinyl plastic to a concentration of 500 mg/mL, solutions have the following stability: in normal saline 8 hours at 25°, 24 hours at 5°.

7.1.5 Route of administration: Intravenous administration over 30 minutes on day 1 of every 3 week cycle.

7.1.6 Adverse effects:

Hematologic: thrombocytopenia, neutropenia, leukopenia, more pronounced in patients with compromised renal function

Gastrointestinal: nausea and vomiting, treatable with moderate doses of antiemetics, anorexia, diarrhea and constipation

Dermatologic: rash, urticaria

Hepatic: abnormal liver function tests, usually reversible with standard doses

Neurologic: rarely peripheral neuropathy is seen

Renal: elevations in serum creatinine, BUN; electrolyte loss

Other: asthenia, pain, flu-like symptoms

7.2 Bevacizumab

7.2.1 Product description: Recombinant humanized monoclonal antibody which binds to the vascular endothelial growth factor (VEGF).

7.2.2 Solution preparation: Opened vials must be used within 8 hours. Bevacizumab will be diluted in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP.

7.2.3 Storage requirements: Upon receipt of the study drug, vials are to be refrigerated at 2°C–8°C (36°F–46°F) and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE. Vials should be protected from light. Opened vials must be used within 8 hours. VIALS ARE FOR SINGLE USE ONLY. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.2.4 Stability: Once bevacizumab has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.2.5 Route of administration: Administered as a continuous IV infusion of 10 mg/kg every 3 weeks. Anaphylaxis precautions should be observed during study drug administration. First dose of bevacizumab should be administered over 90 minutes. If the first infusion is tolerated well, the second dose can be delivered over 60 minutes. If the second dose is tolerated well, then all subsequent doses can be delivered over 30 minutes.

7.2.6 Adverse effects:

Hematologic: arterial and venous thromboembolism, CNS hemorrhage, GI bleeding, epistaxis, pulmonary hemorrhage

Musculoskeletal: arthralgias, chest pain

Cardiovascular: hypertension (including hypertensive crisis), hypotension, decrease in cardiac function, pericardial effusion, tamponade

Renal: proteinuria, nephrotic syndrome

Reproductive: fertility impairment of unknown duration

Skin: rash, desquamation, urticaria, delay in wound healing

Gastrointestinal: nausea, colitis, intestinal obstruction, vomiting, bowel perforation

Pulmonary: dyspnea

Constitutional: fevers, chills, rigors, headaches, asthenia, infection without neutropenia

Hepatic: reversible and marked elevations of LFTs

8. CORRELATIVE/SPECIAL STUDIES

8.1 Biospecimen Processing

As part of the methylation analysis of tumor, paraffin embedded tissue will be required. Pathology reports along with either a representative block of tumor tissue or five 20 micron unstained slides and ten 5 micron slides will be collected. Specimens should be sent to:

Cindy Collins
The University of Chicago Medical Center
5841 S. Maryland Ave., MC 2115
Chicago, IL 60637-1470

8.2 Methylation Analysis

We will use Methylation Specific PCR (MSP) to assess methylation status of the tumor. This assay will be performed according to the protocol established in the laboratory of Dr. Funmi Olopade at the University of Chicago.

8.3 Immunohistochemical Analysis

VEGF expression in the tumor will be determined by immunohistochemical analysis. The antibody against VEGF is commercially available and staining will be performed as previously described. All slides will be read independently by two pathologists. Each tumor will be stained in duplicate. The tumors will be scored using a 0-3+ scale as has been previously described. The final score will be a composite of the two independent reviewers' scores.

9. CLINICAL AND LABORATORY EVALUATION

9.1 Study Calendar

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12 ¹	Off Study
Carboplatin		X			X			X			X			
Bevacizumab		X			X			X			X			
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X-----X												
Physical exam	X	X			X			X			X			X
Vital signs	X	X			X			X			X			X
Height	X													
Weight	X	X			X			X			X			X
Performance status	X	X			X			X			X			X
CBC, CMP	X	X			X			X			X			X
Urinalysis ²	X	X						X						X
Adverse event evaluation		X-----X												X
Tumor measurements	X	Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.												X
B-HCG ³	X													
ER, PR and HER2/ <i>neu</i> evaluation	X													
Tumor Block Collection	X													

¹Day 1 assessments will continue for patients remaining on study for longer than 12 weeks

²Urinalysis will be performed every other cycle while the patient is on study

³Only in women of childbearing potential

9.2 Subject Discontinuation

Subjects who meet the following criteria should be discontinued from study treatment:

- Grade 4 hypertension or reversible posterior leukoencephalopathy syndrome (RPLS)
- Nephrotic syndrome
- Grade > 2 pulmonary or CNS hemorrhage;
- Grade 4 hemorrhage
- Symptomatic grade 4 venous thromboembolic event

- Any grade arterial thromboembolic event
- Grade 4 congestive heart failure
- Gastrointestinal perforation
- Wound dehiscence requiring medical or surgical intervention
- Inability of subject to comply with study requirements
- Determination by the investigator that it is no longer safe for the subject to continue therapy.
- All Grade 4 events thought to be related to bevacizumab by the investigator

Patients who have an ongoing bevacizumab-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible (see Section 6.0).

10. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be evaluated for response after the first two cycles (six weeks) and every two to three cycles (six to nine weeks) thereafter. In addition to a baseline scan, confirmatory scans should also be obtained four to nine weeks following initial documentation of objective response.

10.1. Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable” in reference to measurability will not be used because it does not provide additional meaning or accuracy.

10.1.1 Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques (CT, MRI, x-ray) or as ≥ 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

10.1.2 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

10.1.3 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 response.

10.1.4 Non-target lesions

All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

10.2 Guidelines for Evaluation of Measurable Disease

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.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If they are used, they should have progressed since the time of radiation 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. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

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

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

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

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

standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

10.3 Response Criteria

10.3.1 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 longest diameter (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

10.3.2 Evaluation of non-target lesions

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

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 at a later time by the review panel (or study chair). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

10.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1).

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

- X 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.
- X 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) before confirming the complete response status.

10.4 Confirmatory Measurement/Duration of Response

10.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed 4-9 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks (see section 9.3.3).

10.4.2 Duration of overall response

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

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

10.4.3 Duration of Stable Disease

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

10.4.4 Progression Free Survival

Progression free survival is defined as the duration of time from start of study treatment to time of disease progression or death due to any cause, whichever comes first. If a patient has not progressed or died, progression-free survival is censored at the time of last follow-up.

11. REGULATORY AND REPORTING REQUIREMENTS

Adverse event (AE) reporting for this study is via MedWatch. The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 3.0 will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTC version 3.0. A more complete list of adverse events that have occurred or might occur can be found in Section 7.2.6. A copy of the CTC version 3.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov/reporting/ctc.html>).

11.1 Expedited Adverse Event Reporting

11.1.1 Expedited Reporting Guidelines

In the event of an adverse event the first concern will be for the safety of the subject. Investigators are required to report to Genentech Drug Safety ANY serious treatment emergent adverse event (STEAE) as soon as possible.

A STEAE is any sign, symptom or medical condition that emerges during bevacizumab treatment or during a post-treatment follow-up period that (1) was not present at the start of bevacizumab treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of Bevacizumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

UNEXPECTED EVENT		EXPECTED EVENT	
GRADES 2 – 3 Attribution of Possible, Probable or Definite	GRADES 4 and 5 Regardless of Attribution	GRADES 1 - 3	GRADES 4 and 5 Regardless of Attribution
Expedited report within 10 working days. (Grade 1 Adverse Event Expedited Reporting NOT required.)	Report by phone to UCCRC Clinical Trials Office within 24 hrs. Expedited report to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.	Adverse Event Expedited Reporting NOT required.	Expedited report, within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days. Grade 4 Myelosuppression not to be reported, but should be submitted as part of study results. Other Grade 4 events that do not require expedited reporting would be specified in the protocol.

- For **Hospitalization** only – Any medical event equivalent to CTC Grade 3, 4, or 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.
- Telephone reports to the University of Chicago Cancer Center Clinical Trials Office (CCTO) (773-834-0357) by the end of the business day when investigator and/or research study nurse becomes aware of the event. Events occurring after business hours will be reported to the CCTO by 12 pm (noon) the next business day.
- The following information is required when calling in the event:
 - Reporter's Name and Telephone Number
 - Patient Initials and Medical Record Number
 - IRB Protocol Number
 - PI of Study
 - Attending Physician
 - Date of Event
 - Description of Event (including grade of the event and if the event required hospitalization)
- E-mail is sent to the research nurse, attending physician and PI of the study informing them that ADR notification has been received.
- A completed MedWatch form (FDA form 3500A) must be sent to the University of Chicago Cancer Center Clinical Trials along with the University of Chicago's IRB Adverse Event Form within **5 working days of event occurrence**. The UC IRB Adverse Event form is available on-line at: <http://ors.bsd.uchicago.edu/HS/newirbforms>. This form must be typed. Once the forms are completed forward the original to the study PI in the pink SAE folder. The PI will then review, sign and place folder in QA coordinator's box. A weekly report of delinquent or pending documents will be forwarded to Denise Friesema, RN. All delinquent reporting (greater than 10 days from event occurrence) must include documentation of reason for delinquency and may require implementation of an action plan.
- Once the appropriate AE documents have been received, the University of Chicago Cancer Center Clinical Trials forwards these to the IRB and a copy will be forwarded to the appropriate Research Nurse.

11.1.2 Reporting of Serious Treatment Emergent Adverse Events

All STEAEs should be recorded on a MedWatch 3500a Form and faxed to:
 Genentech Drug Safety
 Fax: (650) 225-4682 or (650) 225-4683

(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

AND:

PI: Rita Nanda, M.D.
University of Chicago
Fax: (773) 834-3834

AND:

QA Coordinator
University of Chicago
Clinical Trials Support Office
Fax: (773)-702-8855

MedWatch 3500a Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500a form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500a form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that bevacizumab caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

11.1.3 Forms

The UC IRB Adverse Event form is available on-line at:
<http://ors.bsd.uchicago.edu/HS/newirbforms>. The MedWatch form is available at <http://www.fda.gov/medwatch/safety/3500a.pdf>.

11.2 Registration Guidelines

All patients must be registered with the University of Chicago Registrar Bernadette Libao (phone 773-834-1758) prior to the commencement of treatment. Confirm all selection criteria listed in Section 3.0, then call the Registrar with the following information:

- Provider of information
- Study # and Institution
- Treating Physician
- Patient name and hospital ID number
- Patient's zip code of residence
- Date of signed informed consent
- Race, gender, date of birth of patient
- Diagnosis and date of initial diagnosis

11.2.2 Data Submission

Institutional data collection will be conducted through a secure, password protected database that will be maintained by Dr. Nanda and her research staff.

On-study	Submit specific registration packet and source documentation prior to registration.
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Weekly	Fax weekly flow sheets and toxicity and chemotherapy summary forms by noon on Friday of each week, for review at the weekly Breast Cancer Clinical Research Conference.
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Evaluations	At each evaluation as specified in the protocol, complete the extent of disease form, specify response (CR, PR, SD, PD) on the flow sheet and submit source documentation of the response (CT, x-ray, physical exam).
Off-study	Submit a flow sheet documenting date of treatment completion.
Follow-up	Submit follow-up and notification of death form every 3 months, documenting disease progression, second line therapy, last date known alive or date of death.

11.3 **Data and Safety Monitoring**

Data Safety and Monitoring will occur at the weekly University of Chicago Breast Oncology Clinical Research Conference, which are lead by senior level medical oncologists. At each meeting, all active breast cancer studies will be reviewed for safety and progress toward completion. Toxicities and adverse events will be reviewed at each meeting and a Data Safety and Monitoring form will be filled out for each protocol and signed by either the principal investigator or another physician in the Breast Oncology Program.

12. STATISTICAL CONSIDERATIONS

12.1 **Study Design/Endpoints**

The trial is a single-arm phase II study with progression free survival (PFS) as the primary endpoint. In an open-label, randomized, phase III study conducted in women with untreated metastatic breast cancer, the median PFS was 6.1 months in patients treated with weekly paclitaxel (Miller et al. 2005). The median PFS was prolonged to 11.0 months in the group receiving weekly paclitaxel plus bevacizumab. In the proposed trial, we anticipate the similar size of effect will be observed for the combination of carboplatin and bevacizumab in patients with metastatic ER, PR and HER2/neu negative breast cancer. We will test the null hypothesis that the median PFS is 6 months against the alternative hypothesis that it is 11 months or greater. Specifically, Kaplan-Meier curve will be constructed and the lower bound of the Brookmeyer-Crowley 90% confidence interval of the median will be calculated and compared with 6 months (Brookmeyer & Crowley 1982). Assuming a uniform accrual (2 patients per month) over time, additional 6 months after the last patient is registered, no loss of follow-up within 6 months, and an exponential distribution of PFS event time, 36 patients are required to enroll in order to achieve an approximate 80% power with one-sided alpha of 0.10. The powers under other alternative hypotheses are listed in the Table below.

Median PFS under	Power	Expected
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alternative hypothesis		number of event
9 months	53%	24
10 months	68%	22
11 months	79%	21
11.1 months	80%	21
12 months	86%	20
13 months	91%	19

12.2 Sample Size/Accrual Rate

The planned sample size is 36 patients total. With an expected accrual rate of 2 patients per month, the total accrual time will be approximately 18 months. They will be followed for additional 6 months after the last patient is enrolled.

12.3 Analysis of Secondary Endpoints

This trial will also calculate response rate and duration of response and correlate to tumor *BRCA1* promoter hypermethylation. Possible risk factors for progression free survival (e.g. *BRCA1* methylation status, tumor VEGF expression) will be examined using the log-rank test., The proportional hazards Cox model will be applied in the multivariate analysis. Hazard ratio along with 95% confidence interval will be reported.

12.4 Reporting and Exclusions

Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with carboplatin and bevacizumab.

Evaluation of response. All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. 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). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.] All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) 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

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

The UNIVERSITY OF CHICAGO
The Division of the Biological Sciences • The University of Chicago Hospitals

CONSENT BY SUBJECT FOR PARTICIPATION IN A RESEARCH PROTOCOL

Protocol Number: _____ Name of Subject: _____
Medical History Number: _____

STUDY TITLE: A Phase II Study of Carboplatin and Bevacizumab (Avastin®)
Combination Therapy for Basal-like Metastatic Breast Cancer

Doctors Directing Research:	<u>Rita Nanda, M.D.</u>	<u>Funmi Olopade, M.D.</u>
Address:	5841 S. Maryland Ave. Mail Code 2115 Chicago, IL 60637	5841 S. Maryland Ave. Mail Code 2115 Chicago, IL 60637
Telephone Number:	(773) 834-2756	(773) 702-1632

You are being asked to participate in a research study. A member of the research team will explain what is involved in this study and how it will affect you. This consent form describes the study procedures, the risks and benefits of participation, as well as how your confidentiality will be maintained. Please take your time to ask questions and feel comfortable making a decision whether to participate or not. This process is called informed consent. If you decide to participate in this study, you will be asked to sign this form.

You are being asked to take part in this study because you have breast cancer that cannot be cured by surgery and/or radiation alone. Your breast cancer is estrogen receptor negative, which means that it will not shrink by blocking the effects of estrogen (a type of hormone) in your body. When tumors are estrogen negative, they usually are faster growing and harder to shrink with the drugs we have now.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to find out what effects (good and bad) the combination of carboplatin (a chemotherapy drug) and bevacizumab (also call Avastin, a therapy which targets a protein that is commonly positive in estrogen receptor negative breast tumors) has on your breast cancer and you. This research is being done because there are currently no treatments to cure recurrent or metastatic (spread of cancer cells from one area of the body to another) breast cancer.

The purpose of this study is to find out how well the combination of carboplatin and bevacizumab can shrink tumors, and how serious the side effects are.

Both carboplatin and bevacizumab are FDA-approved drugs, but they are not approved for use in breast cancer, therefore the use of this combination in breast cancer is considered experimental.

Each drug on its own has not been as good at shrinking tumors in women with breast cancer as we would have liked, but the reason we are doing this study is because we think that the combination of the two will work well in people who have estrogen receptor negative tumors.

A second purpose of the study is to find out if certain changes in cancer affect the response to these chemotherapy drugs. Some breast cancers turn off a gene that repairs damage to cancer cells. We will examine your tumor (that was removed at the time of your diagnosis) to see if it has this change or not, and see if that matters for how well the chemotherapy shrinks the cancer.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 36 people will take part in this study at the University of Chicago.

WHAT IS INVOLVED IN THE STUDY?

If you agree to take part in this study, you will need to have the following tests and procedures to determine if you meet the trial requirements.

- Physical exam, including measurements of your height, weight, vital signs (blood pressure, temperature, and heart rate), and performance status (your ability to perform daily tasks)
- You will be asked about any medications you are taking
- Blood and urine tests (about half a tablespoon of blood will be taken)
- If you are a woman who can have children, a pregnancy test (1 teaspoon of blood) will be done.
- CT scan (a special type of x-ray that creates 3D pictures of your body) or MRI (uses magnets to create pictures of your body) and possibly a chest x-ray will be performed to measure the amount and size of tumors (cancer) in your body.

Study Procedures

While you are receiving the study drugs you will need to repeat many of these tests and procedures. All these procedures are considered part of standard care, that is, they would be done if you were receiving chemotherapy even if it were not part of a clinical trial.

You will see your doctor every 3 weeks. Each visit will consist of your doctor asking questions about you and any side effects you may be having. Your doctor will also perform a physical examination including measurements of your weight, vital signs and performance status. At each visit you will have your blood drawn as described below.

Blood tests will be done every three weeks while taking carboplatin chemotherapy to be sure you are not experiencing any side effects. The total amount of blood taken each time is about half a tablespoon.

Urine tests will be done about every six weeks while you are taking the study drugs.

You will have a CT scan or MRI every six weeks while you are receiving the study drugs. This will allow you and your doctors to know whether or not the cancer is shrinking.

Study Drug Administration

The study drugs will be given on an outpatient basis. The drugs will be given intravenously (through a tube in a vein).

You may be given medicines before you receive the study drugs to help prevent some side effects.

Every three weeks, the carboplatin will be given by vein over 30 minutes and the bevacizumab will also be given by vein. The first dose of bevacizumab will take 90 minutes. If you tolerate that well (do not experience any serious side effects), the later doses will take 60 minutes.

Every time you get a dose of carboplatin and bevacizumab you start a new "cycle." Because you will be given a dose of these drugs every three weeks, a new cycle starts every three weeks.

You can continue to take the study drugs as long as your cancer does not get worse and as long as you do not experience side effects that are too bad.

If you have to stop taking either the carboplatin or bevacizumab because of bad side effects, your study doctor may decide that you can continue to take the other study drug alone as long as your cancer is not getting worse.

If your cancer goes away completely while you are on this study, you will stop taking the carboplatin and your study doctor may decide that you should continue to receive bevacizumab until your cancer comes back or you experience serious side effects.

End of Study

After you have stopped taking the study drugs you will be asked to return to the clinic for a final visit. During this visit a physical exam including measurements of your weight, vital signs, and performance status will be done. Blood and urine tests (about half a tablespoon of blood) will be done. You will be asked about any side effects you may still be experiencing. A CT or MRI scan may be done to measure your tumors.

Special Research Tests

As part of this study, the researchers would like to use a piece of your tumor for special research tests. You will be asked to sign a separate consent form which explains these research tests.

During this study, Dr. Nanda and her research team will collect information about you for the purposes of this research. This will include name, initials, contact information (address, telephone numbers, and/or email), social security number (for follow-up purposes), medical record number, demographic information (gender, birth date, race/ethnic back-ground), medical history (including medications), cancer history, dates (of consent, drug administration, side effects and other medical procedures), results of physical exam, weight, height, vital signs, performance status, side effects, results of blood and urine tests, pregnancy test (if applicable), chest x-rays and/or reports, CT and/or MRI scans and/or reports, including tumor measurements, tissue samples and reports, and the results of any other tests to determine your disease status.

HOW LONG WILL I BE IN THE STUDY?

You may remain on study as long as your cancer is not getting worse and your side effects are not too bad. The decision about how long to continue if your disease is not getting worse is up to you and your doctor.

Dr. Nanda may decide to take you off of the study without your consent if:

- Your cancer gets worse even though you are receiving the study drugs;
- Side effects of the study drugs are too hard on you or too dangerous for you;
- You develop another illness or condition which would not allow you to continue;
- You do not comply with study procedures;
- New information about the drug combination becomes available and this information suggests the drugs will be ineffective or unsafe for you;
- The study drug are no longer available;
- The study is stopped.

You can also choose to stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study doctor and your regular doctor first.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for experiencing side effects including those listed below. You should discuss these with the study doctor and/or your regular doctor. There also may be other side effects that we cannot predict. Other drugs will be given to make side effects less serious and uncomfortable. Many side effects go away shortly after carboplatin and bevacizumab are stopped, but in some cases side effects can be serious or long-lasting, **permanent**, or even **fatal**.

Risks Associated with Carboplatin

Likely:

- Low white blood cells (which leads to an increased risk of infection)
- Low platelet counts (which can lead to an increased risk of bleeding)
- Decreased number of red blood cells (may make you feel tired)
- Nausea
- Vomiting
- Pain
- Weakness

Less Likely:

- Numbness or the feeling of “pins and needles” in hands and feet
- High levels of some blood tests of liver and/or kidney function (usually with no symptoms)
- Low levels of sodium, potassium, calcium and/or magnesium in the blood (may cause muscle cramps, irregular heart beats, low blood pressure, nausea, vomiting)

Rare but Serious:

- Allergic reactions may include rash, itching, hives, swelling, difficulty breathing, and/or high or low blood pressure

Risks Associated with Bevacizumab:

Likely:

- Low white blood cells (which leads to an increased risk of infection)
- Infection with decreased white blood cell count or fever
- Weakness
- Pain
- Abdominal pain
- Headache
- High blood pressure
- Diarrhea
- Nausea
- Vomiting
- Loss of appetite
- Stomatitis
- Constipation
- Upper respiratory infection
- Nose bleeds
- Shortness of breath
- Too much protein in urine
- Sores in the mouth, throat, stomach, or intestines
- Rash

Less Likely:

- Blood clots, which may lead to heart attack or stroke which can be **life-threatening** or **fatal** (these events are more likely to occur in subjects over 65 years)
- High blood pressure
- Kidney damage, which may include blood and protein in the urine or swelling

Rare but Serious:

- Hole in the digestive tract (including stomach or intestines) can be **life-threatening** or **fatal**
- Delay in wound healing
- Bleeding (which can be **life-threatening** or **fatal**)
- **Life-threatening** bleeding in the brain (can cause confusion, blindness or vision changes, seizure, and other symptoms). This is known as Reversible Posterior Leukoencephalopathy Syndrome (RPLS) and this condition is usually reversible, but in rare cases, it is potentially **life-threatening** and may have long-term effects on brain function.
- Heart Failure
- Severe allergic reaction while receiving the infusion, may include high blood pressure, wheezing, chills, chest pain, headache, sweating

In addition, there is a risk that the combination of the drugs might have worse side effects than any one of the drugs alone.

Reproductive Risks: Because the effect of the study drugs on an unborn baby are not certain, you should not become pregnant while on this study. You should not breast feed your baby while on this study. If you are able to have children, you must use birth control (condom, diaphragm, or not having sex) while on this study. Ask about counseling and more information about preventing pregnancy.

Blood drawing risks: There may be bruising, bleeding or inflammation at the sites where blood samples are taken. Care will be provided to avoid these complications.

MRI risks: Risks from the MRI test may include an allergic reaction to the contrast dye and a feeling of claustrophobia (fear of closed-in spaces) that can make you feel anxious and nervous. You will be required to rest still in a dark and closed space during the time of testing. If you have a fear of closed spaces (claustrophobia) you may not be able to undergo this test or you may require additional medications to prepare you for taking the MRI test. The radiologist (a doctor specializing in the field of radiology) who will conduct the MRI test will explain the procedure to you in detail.

CT risk: Risks from the CT scans may include an allergic reaction to the contrast dye or kidney problems from the dye. Also, you might have problems with claustrophobia, which may

make it hard to get the test or require you to have additional medicines before you have the test. The doctor who does the test will explain everything to you in detail.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct benefit to you. The benefit of carboplatin and bevacizumab at this dose given to subjects with breast cancer is not known. However, the information gained from this study could benefit other individuals with this type of disease in the future.

WHAT OTHER OPTIONS ARE THERE?

Instead of being in this study, you have these options:

- Standard chemotherapy (chemotherapy with approved drugs).
- Other experimental drugs.
- Comfort care only, where treatments are directed only at reducing symptoms, relieving suffering and maximizing comfort, dignity, and control. In comfort care only, treatment is not directed at curing, slowing, or reversing your disease.

Please talk to your doctor about these and other options.

The decision whether or not you wish to participate in this study will not affect your care at the University of Chicago Hospitals.

WHAT ARE THE COSTS?

Clinical services provided during a clinical trial are either research-related or related to usual medical care. Research-related services are done to complete the research and the costs are not the responsibility of you or your insurance.

The bevacizumab (Avastin®) will be provided to you free of cost by Genentech, the pharmaceutical company that makes the drug.

The costs that are considered research-related for this study include the pregnancy test (if needed).

Usual medical care costs include any and all services that are considered medically necessary for your disease. This will include the cost of the carboplatin, any medications given before receiving the study drugs, administration of the study drugs, blood and urine tests, x-rays, CT and/or MRI scans, clinic visits, and physical exams. The cost of this usual, ongoing medical care will be the responsibility of you or your insurance, and may include deductibles and co-

payments. Similarly, this care will be subject to all the same requirements and restrictions of your insurance.

In the event of physical injury resulting from this research, if emergency care is needed the University of Chicago Hospitals will provide it to you free of charge. If non-emergency care is necessary, the University of Chicago Hospitals will provide it to you at your cost.

WILL I BE PAID FOR MY PARTICIPATION?

You will receive no payment for taking part in this study.

WHAT ABOUT CONFIDENTIALITY?

Study records that identify you will be kept confidential. Your information is being collected and maintained on paper copies and computer databases. This information will be kept indefinitely. These records will be kept in locked offices. Access to these records is limited to research personnel (including the study doctors, nurses, and data managers).

The data collected in this study will be used for the purpose described in the form. By signing this form, you are allowing the research team access to your medical records, which include Protected Health Information. Protected Health Information (PHI) consists of any health information that is collected about you, which could include your medical history and new information collected as a result of this study. The research team includes the individuals listed on this consent form and other personnel involved in this study at the University of Chicago.

As part of the study, Dr. Nanda and her research team will report the results of your study-related procedures and tests explained above to Genentech (the study sponsor). This will include initials, demographic information (gender, birth date, race/ethnic background), medical history (including medications), cancer history, dates (of consent, drug administration, side effects and other medical procedures), results of physical exam, weight, height, vital signs, performance status, side effects, results of blood and urine tests, pregnancy test (if applicable), chest x-rays and/or reports, CT and/or MRI scans and/or reports, including tumor measurements, tissue samples and reports, and the results of any other tests to determine your disease status. This information is being sent because these reviews are done to assure the quality of the study conduct and the study data. Additionally, this information will be reported for data analysis, to confirm study results, publish study findings, and report side effects.

The study sponsor or their representatives, including monitoring agencies, may also review your medical record. Please note that these individuals may share your health information with someone else. If they do, the same laws that the University of Chicago must obey may not protect your health information.

Your records may be reviewed by federal agencies whose responsibility is to protect human subjects in research including the Food and Drug Administration (FDA) and Office of Human Research Protections (OHRP). In addition, representatives of the University of Chicago, including the Institutional Review Board, a committee that oversees the research at the University of Chicago, may also view the records of the research. If your research record is reviewed by any of these groups, they may also need to review your entire medical record.

The results from tests and/or procedures performed as part of this study may become part of your medical record.

During your participation in this study, you will have access to your medical record. Dr. Nanda is not required to release to you research information that is not part of your medical record.

This consent form will be kept by the research team for at least six years. The study results will be kept in your research record and be used by the research team indefinitely. At the time of study completion, either the research information not already in your medical record will be destroyed or information identifying you will be removed from study results. Any research information in your medical record will be kept indefinitely.

Data from this study may be used in medical publications or presentations. Your name and other identifying information will be removed before this data is used. If we wish to use identifying information in publications, we will ask for your approval at that time.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. If you choose not to participate in this study, your care at the University of Chicago/University of Chicago Hospitals will not be affected. You may choose not to participate at any time during the study. Leaving the study will not affect your care at the University of Chicago/University of Chicago Hospitals.

If you choose to no longer be in the study and you do not want any of your future health information to be used, you must inform Dr. Nanda in writing at the address on the first page. Dr. Nanda may still use your information that was collected prior to your written notice.

Data Safety and Monitoring, which is when doctors, nurses and other research personnel review the data from the study to ensure that it is safe to continue, will occur on a weekly basis for this study. We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay on this study.

You will be given a signed copy of this document. This consent form document does not have an expiration date.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

You have talked to _____ about this study and you had the opportunity to ask questions concerning any and all aspects of the research. If you have further questions about the study, you may call Dr. Nanda at (773) 834-2756.

If you have a research related injury, you should immediately contact Dr. Nanda at (773) 834-2756 or the hematology/oncology clinic answering service at 773-702-4400 and they will page your doctor or covering physician.

If you have any questions concerning your rights in this research study you may contact the Institutional Review Board, which is concerned with the protection of subjects in research projects. You may reach the Committee office between 8:30 am and 5:00 pm, Monday through Friday, by calling (773) 702-6505 or by writing: Institutional Review Board, University of Chicago, McGiffert Hall, 2nd Floor, 5751 S. Woodlawn Avenue, Chicago, Illinois 60637.

WHERE CAN I GET MORE INFORMATION?

You can contact the study chair, Rita Nanda, M.D. at (773) 834-2756.

You may call the **Cancer Information Service** at:
1-800-4-CANCER (1-800-422-6237) or **TTY: 1-800-332-8615**

Visit the NCI's Web Sites.

cancerTrials: comprehensive clinical trials information
http://www.cancer.gov/clinical_trials/

CancerNet™: accurate cancer information including PDQ
http://www.cancer.gov/cancer_information/.

CONSENT

SUBJECT

The research project and the procedures associated with it have been explained to me. The experimental procedures have been identified and no guarantee has been given about the possible results. I will receive a signed copy of this consent form for my records.

I agree to participate in this study. My participation is voluntary and I do not have to sign this form if I do not want to be part of this research study.

Signature of Subject: _____

Date: _____ Time: _____ AM/PM (Circle)

PERSON OBTAINING CONSENT

I have explained to _____ the nature and purpose of the study and the risks involved. I have answered and will answer all questions to the best of my ability. I will give a signed copy of the consent form to the subject.

Signature of Person Obtaining Consent: _____

Date: _____ Time: _____ AM/PM (Circle)

INVESTIGATOR/PHYSICIAN:

Signature of Investigator/Physician _____

Date: _____ Time: _____ AM/PM (Circle)

Version History: 05/07/07, IRB pending (initial approval)

The UNIVERSITY OF CHICAGO
The Division of the Biological Sciences • The University of Chicago Hospitals

CONSENT BY SUBJECT FOR PARTICIPATION IN A RESEARCH PROTOCOL

Protocol Number: _____ Name of Subject: _____
Medical History Number: _____

STUDY TITLE: A Phase II Study of Carboplatin and Bevacizumab (Avastin®)
Combination Therapy for Basal-like Metastatic Breast Cancer

Doctors Directing Research:	<u>Rita Nanda, M.D.</u>	<u>Funmi Olopade, M.D.</u>
Address:	5841 S. Maryland Ave. Mail Code 2115 Chicago, IL 60637	5841 S. Maryland Ave. Mail Code 2115 Chicago, IL 60637
Telephone Number:	(773) 834-2756	(773) 702-1632

You are being asked to participate in a research study. A member of the research team will explain what is involved in this study and how it will affect you. This consent form describes the study procedures, the risks and benefits of participation, as well as how your confidentiality will be maintained. Please take your time to ask questions and feel comfortable making a decision whether to participate or not. This process is called informed consent. If you decide to participate in this study, you will be asked to sign this form.

WHY IS THIS STUDY BEING DONE?

Tissue will be tested for an alteration called “*BRCA1* promoter methylation.” This is a change occurring in some cancer cells that may affect the cancer cell’s ability to repair damage. We will try to determine whether or not this change affects how your cancer responds to chemotherapy.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 36 people will take part in this study at the University of Chicago.

WHAT IS INVOLVED IN THE STUDY?

Samples of your cancer tissue (from a surgery or biopsy which has already been done and from which a piece of the cancer was saved—no new biopsy will be performed for this study) will be sent to the laboratory of Dr Rita Nanda at the University of Chicago.

We will use your tissue to do tests on your genes. Genes are the material that is passed from parents to children that determine the make-up of the body. The results of these tests will not be shared with you. The doctors directing this study will receive the results of these tests and will use this information for the purpose of this research study.

The cancer tissue will not be shared with anybody else or used for any other tests. Unused tissue will be returned to the pathology department it came from.

Your sample and medical information will be labeled with a code number, not your name. The researchers performing the special tests will not be able to link your sample to your name or personal information.

In addition to the information collected on the main study, Dr. Nanda and her research team will collect your name, date of consent, tumor sample, medical history, including response to the study drugs, and any side effects while taking carboplatin and bevacizumab (Avastin).

HOW LONG WILL I BE IN THE STUDY?

You do not have to do anything to participate in this additional research.

WHAT ARE THE RISKS OF THE STUDY?

There is a slight risk of loss of confidentiality. Care will be taken to protect your personal information.

ARE THERE ANY BENEFITS TO TAKING PART IN THE STUDY?

There will be no direct benefit to you by taking part in this research.

You may help scientists understand why people react to or handle the combination of carboplatin and bevacizumab (Avastin) differently. This may help identify who is more likely to respond to carboplatin and bevacizumab (Avastin) and who may experience side effects.

WHAT OTHER OPTIONS ARE THERE?

You have the option not to participate in the study.

You must agree to allow your tumor sample to be used to participate in the drug administration study.

The decision whether or not you wish to participate in this study will not affect your care at the University of Chicago Hospitals.

WHAT ARE THE COSTS?

There will be no cost to you for these special research studies.

WILL I BE PAID FOR MY PARTICIPATION?

You will receive no payment for taking part in this study.

WHAT ABOUT CONFIDENTIALITY?

This information is in addition to confidentiality discussed in the CONSENT FORM for the main study.

As part of the study, Dr. Nanda and her research team will report the results of your study-related procedures and tests explained above to Genentech (the study sponsor) and the National Cancer Institute. This will include the results of the special research tests and your medical history including your response to carboplatin and bevacizumab (Avastin)

Your records may be reviewed by federal agencies whose responsibility is to protect human subjects in research including the Food and Drug Administration (FDA) and Office of Human Research Protections (OHRP). In addition, representatives of the University of Chicago, including the Institutional Review Board, a committee that oversees the research at the University of Chicago, may also view the records of the research. If your research record is reviewed by any of these groups, they may also need to review your entire medical record.

This consent form will be kept by the research team for at least six years.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. If you choose not to participate in this study, your care at the University of Chicago/University of Chicago Hospitals will not be affected.

You may choose not to participate at any time during the study. Leaving the study will not affect your care at the University of Chicago/University of Chicago Hospitals.

If you choose to no longer be in the study and you do not want your sample to continue to be used, you must inform Dr. Nanda in writing at the address on the first page. Dr. Nanda may still use your information that was collected prior to your written notice.

You will be given a signed copy of this document. This consent form document does not have an expiration date.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

You have talked to _____ about this study and you had the opportunity to ask questions concerning any and all aspects of the research. If you have further questions about the study, you may call Dr. Nanda at (773) 834-2756.

If you have any questions concerning your rights in this research study you may contact the Institutional Review Board, which is concerned with the protection of subjects in research projects. You may reach the Committee office between 8:30 am and 5:00 pm, Monday through

Friday, by calling (773) 702-6505 or by writing: Institutional Review Board, University of Chicago, 5841 S. Maryland Avenue, MC 1108, Chicago, Illinois 60637.

CONSENT

SUBJECT

The research project and the procedures associated with it have been explained to me. The experimental procedures have been identified and no guarantee has been given about the possible results. I will receive a signed copy of this consent form for my records.

I agree to participate in this study. My participation is voluntary and I do not have to sign this form if I do not want to be part of this research study.

Signature of Subject: _____

Date: _____ Time: _____ AM/PM (Circle)

PERSON OBTAINING CONSENT

I have explained to _____ the nature and purpose of the study and the risks involved. I have answered and will answer all questions to the best of my ability. I will give a signed copy of the consent form to the subject.

Signature of Person Obtaining Consent: _____

Date: _____ Time: _____ AM/PM (Circle)

INVESTIGATOR/PHYSICIAN:

Signature of Investigator/Physician _____

Date: _____ Time: _____ AM/PM (Circle)

Version History: 03/26/07, IRB pending (initial approval)