PROTOCOL TITLE: A study to identify the potential effects of extracorporeal shockwave therapy alone and in combination with Avastin.

I. NON-TECHNICAL SYNOPSIS:

Breast cancer is characterized by rapid aberrant growth of tissue in the mammary glands. The speed of cancer growth is enabled by the rapid development of a robust vasculature to provide nutrients to the tumors. One relatively recent approach to mitigating cancer growth is to inhibit the development of the vasculature supplying blood to the tumor. One drug that has shown substantial success in this is bevacizumab (Avastin, Genentech). Avastin targets a growth factor that signals for vascular development. By interfering with this signaling pathway this drug slows the growth of tumors, and has been shown to enhance the therapeutic effects of other chemotherapy drugs. The FDA has approved Avastin in combination with Paclitaxel (Bristol-Myers Squibb) for the treatment of breast cancer. Paclitaxel inhibits mitotic division, slowing the growth of rapidly dividing cells. Nab-Paclitaxel (Abraxane, Abraxis BioScience, LLC.) is and new form of Paclitaxel with the advantages of linear pharmacokinetics, high tumor retention, improved antitumor efficacy, and reduced toxicity. Shockwave therapies have also been used in combination with anticancer drugs to increase their effectiveness. The mechanical disruption of cellular structures may temporarily increase the permeability of the tissue to these drugs, thereby increasing their concentration within cancerous tissues. To date, only focused and high-energy shockwave devices have been used toward this research. This study aims to find the effects of un-focused extracorporeal shockwave therapy (ESWT, DermaGold™ shockwave technology, Tissue Regeneration Technologies) on breast cancer morphology alone and in combination with the known anticancer agent Avastin and Nab-Paclitaxel.

II. BACKGROUND:

II.1.1. Avastin and Paclitaxel for the Treatment of Cancer:

Breast cancer is a common and growing problem for the civilian as well as military population. According to a report written for the American Cancer Society, breast cancer is the number one cause of death for women ages 20-59. The U.S. active-duty military population differs from the U.S. general population in its exposure to cancer risk factors and access to medical care (Zhu et al, 2009) with a higher incidence of breast cancer in the military population. Even with the overall decrease trend in death rate due to cancer, the American Cancer Society estimates that 192,379 women will develop breast cancer in 2009.

Avastin and Nab-Paclitaxel are two therapeutic drugs quickly becoming a part of the standard of care for late stage metastatic breast cancer. Both drugs target the rapid development of vasculature and aberrant tissue growth characteristic of cancer.

Bevacizumab (Avastin, Genentech) is a humanized monoclonal antibody with specific affinity for vascular endothelial growth factor (VEGF). Administration of Avastin inhibits the signaling effects of VEGF, thereby reducing the body's ability to produce new vasculature. This has clear therapeutic advantages in oncology as tumors are characterized by the rapid development of a robust vasculature to support the aberrant growth. Genentech states that "Avastin is indicated for the treatment of patients who have not received chemotherapy for metastatic HER2-negative breast cancer in combination with paclitaxel. The effectiveness of Avastin in metastatic breast cancer is based on an improvement in progression free survival. There are no data demonstrating an improvement in disease-related symptoms or increased survival with Avastin. Avastin is not indicated for patients with breast cancer that has progressed following anthracycline and taxane chemotherapy administered for metastatic disease."

Paclitaxel (Bristol-Myers Squibb) is a mitotic inhibitor that acts therapeutically by preventing the breakdown of microtubules during cell division. The FDA has approved the use of Paclitaxel in combination with Avastin specifically as this combination was found to prolong the progression of metastatic breast cancer in a phase III clinical trial. Nab-Paclitaxel (Abraxis BioScience, LLC.) is a new albumin-bound formulation of the drug which is Cremophor-free. Cremophor is an additive used to stabilize the emulsion in injextable paclitaxel, but is itself toxic. The main advantages of Nab-Paclitaxel include linear pharmacokinetics, high tumor retention, improved antitumor efficacy, and reduced toxicity because of the elimination of Cremophor. However, the tumor response even to the improved formulation of paclitaxel is typically only 30% to 35%.

The chemotoxicity of paclitaxel results in compensatory upregulation of VEGF-A in tumor cells. The excess VEGF-A helps the tumor cells to survive during chemotherapy to the extent that they will continue to grow once therapy is stopped. This is the purpose of combining this treatment with bevacizumab. Bevacizumab is a monoclonal antibody to VEGF. When bevacizumab bind VEGF it can no longer signal tumor vasculature to grow. This results in a drastically reduced blood supply to the tumor, and increases the chances of remission.

As Avastin and Nab-Paclitaxel are quickly becoming a part of the standard of care for late stage metastatic breast cancer, experimental treatment groups with this combination will serve as an important correlate by which to evaluate the effects of ESWT.

II.1.2. <u>Shockwave Therapy for the Treatment of Cancer:</u>

A number of technological advances have permitted the application of mechanical energy in order to produce a therapeutic clinically relevant biological effect in this manner. A promising technology is extra-corporeal shockwave therapy (ESWT; DermaGold™ shock wave technology, Tissue Regeneration Technologies (TRT), Woodstock, GA). ESWT generates an acoustic pressure wave that penetrates tissue and produces a favorable biological response in the target tissue by an incompletely

understood mechanism. The positive effects of ESWT are shown in promoting wound healing include accelerated closure of difficult-to-heal chronic wounds, an anti-microbial effect, and enhanced healing of bone fractures. Previous results published by the CWI team showed effects of ESWT on angiogenesis.

High-energy and focused lithotripsy shockwave therapies have been shown to increase cytocidal effects of known chemotherapy agents. Prior work includes the combination of Paclitaxel and high-energy shockwave therapy; however, no work has been found combining the monoclonal antibody Avastin and shockwave, nor the combination of Paclitaxel, Avastin, and shockwave therapy. Tissues treated with focused and high-energy lithotripsy differ from the proposed study in that these methods subject the tissues to much greater mechanical disturbances than un-focused extracorporeal shockwave therapy.

The purpose of this protocol/pilot study is to determine if the use of this technology is appropriate in the treatment of breast cancer.

II.1.3. Innoculation Procedures:

Two different inoculation procedures will be used. The primary procedure will involve a simple inoculation of a cell suspension into the mammary fatpad. The secondary procedure will involve locating the fatpad initially by incision, followed by inoculation with the cell suspension. The secondary procedure described above has been used in prior studies, and is the standard to ensure the fatpad is accuractly located prior to inoculation. However, since the treatments in this study affect wound healing, it will be better for the study to avoid wounding the animal prior to chemo and shockwave therapy. The secondary method will be used for comparison, to determine if inoculation without locating the fatpad surgically produces similar tumor growth characteristics. If so, these results will be used as a basis for refinement of future studies.

II.2. Literature Search for Duplication:

II.2.1 Literature Source(s) Searched: ***(BRD Search is mandatory, as well as a FEDRIP or a CRISP search; Medline and other databases are optional but recommended):*** The following databases were searched: PubMed, Web of Science

II.2.2 Date of Search: Aug 4, 2009

II.2.3 Period of Search: 1998-present

II.2.4 Key Words of Search: extracorporeal shockwave therapy, orthotopic breast cancer, Avastin, Paclitaxel, murine breast cancer model.

II.2.5 Results of Searches: No duplications found.

III. OBJECTIVE\HYPOTHESIS:

The objective of this study is to treat murine breast cancer with ESWT alone and in combination with Bevacizumab and Nab-Paclitaxel to determine the potential effects of un-focused shockwave on cancer growth and metastases. The major hypothesis of this study is that the temporary increase in cell permeability due to shockwave therapy will increase the effectiveness of the drugs in treating breast cancer. Shockwave therapy alone is also expected to have some therapeutic effects. The primary measurement will be using immunohistochemistry for histological analysis of the morphological changes of the tumors in the breast tissue.

IV. Experimental Design and General Procedures:

The proposed study will be a randomized, controlled, small animal study. Breast tumors will be induced by injection of a tumor cell suspension into the mammary fatpad, and by incision over the fatpad followed by injection into the fatpad. One human cell line and one murine cell line will be used. These lines are well characterized highly metastatic cell lines. The human cell line will be SUM1315MO2, The murine cell line will be 4T1.

Experiment: Murine Orthotopic Breast Cancer

Groups of **10** mice will be assigned per experimental group and will receive assigned treatments with an additional 20% increase in the number animals to account for unforeseen complications.

Groups		Treatment
A)	*Murine cell line w/ Primary inoculation method*	
1	Tumor injection	no treatment
2	Tumor injection	ESWT
3	Tumor injection	Bevacizumab + Nab-Paclitaxel
4	Tumor injection	Bevacizumab + Nab-Paclitaxel + ESWT
B)	*Human cell line w/ Primary inoculation method*	
1	Tumor injection	no treatment
2	Tumor injection	ESWT
3	Tumor injection	Bevacizumab + Nab-Paclitaxel
4	Tumor injection	Bevacizumab + Nab-Paclitaxel + ESWT
C)	*Human cell line w/ Secondary innoculation method*	
1	Tumor injection	no treatment
2	Tumor injection	ESWT
3	Tumor injection	Bevacizumab + Nab-Paclitaxel
4	Tumor injection	Bevacizumab + Nab-Paclitaxel + ESWT

Total number of animals needed: 12 groups x (10+2) mice per group = 144

Histological Evaluation

Animals will be sacrificed at day 28. Blood, breast tissue, bone, liver and lung will be

collected for further analysis. Blood cells will be examined by Fluorescence Activated Cell Sorting analysis to count the number of GFP-labeled tumor cells in circulation. Fresh tissues of breast, bone, liver and lung will be sliced to 1-5 mm thick slices placed between glass slides for direct examination under the fluorescent microscope for the presence of GFP-expressing tumor foci. The number of GFP-expressing tumor foci per field of examination from 10 random sites of five different
slides for each organ will be calculated and compared among different groups of animals.

Other Analysis:
Flow cytometry will be used as the metric for measuring apoptosis using blood samples. The degree of apoptosis seen is an indicator of how effective the treatment is.

Morphometric analysis will be done using Pictzar to determine the effects of the treatment on wound healing in group **B**. Since this group is undergoing a procedure that involves an incision, and since the drugs administered may have adverse effects on wound healing, this analysis is necessary to quantify the effects of the drugs.

V. Study Endpoint: All mice will be euthanized 28 days after the first treatment is given. In case of an adverse event requiring early euthanasia (i.e. unalleviated pain, severe dehydration, unresolved injury or infection, loss of locomotion, severe weight loss, unresolved inappetence), premature death or other reasons judged by the PI and the attending veterinarian as incompatible with the animal's survival, specimens are to be stored in a refrigerator for the investigator. Animals that are euthanized or die prior to the study endpoint will be considered for analysis as long as death was a direct result of the procedures in the study. Prior to premature euthanasia, all attempts should be made to contact the PI or PI staff. If investigative staff is unavailable, DLAMs attending veterinarian should direct the proper course of action. Upon approval from the PI, animal carcasses are to be disposed of in accordance with DLAM SOPs.

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