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14. ABSTRACT Although great strides have been made in breast cancer screening and treatment, it remains the second highest cause of cancer-related deaths for women in the United States. Current prevention focuses on oral administration of tamoxifen which decreases breast cancer incidence but increases the risk for secondary uterine cancer. In addition, tamoxifen may not be effective in preventing those lesions that are estrogen receptor (ER) negative based on its primary function of suppressing cell proliferation by blocking the estrogen receptor. We hypothesize that programmed cell death is dysregulated in premalignant and malignant breast cells which permits both ER-positive and ER-negative cells to avoid cell death. We intend to investigate whether treating premalignant breast cells with a molecular genetic-based agent may be effective alone or in concert with tamoxifen treatment to induce cell death in both ER-positive and ER-negative cells. Ultimately, we envision delivering genetic-based preventive agents and/or tamoxifen directly to the breast ductal lobe of these high risk individuals thus eliminating any potential for tamoxifen-induced uterine cancers.					
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TITLE:

Breast Cancer Prevention by Inducing Apoptosis in DCIS Using Breast Ductal Lavage

INTRODUCTION:

Although great strides have been made in breast cancer screening and treatment, it remains the second highest cause of cancer-related deaths for women in the United States. Current prevention has focused on oral administration of tamoxifen which appears to decrease breast cancer incidence but increases the risk for secondary uterine cancer. In addition, tamoxifen may not be effective in preventing those lesions that are estrogen receptor (ER) negative based on its primary function of suppressing cell proliferation by blocking the estrogen receptor. We hypothesize that programmed cell death (PCD) is dysregulated in premalignant and malignant breast cells which permits both ER-positive and ER-negative cells to avoid cell death. We intend to investigate whether treating premalignant breast cells with a molecular genetic-based agent (antisense bcl-2/bcl-xL oligonucleotide) may be effective alone or in concert with tamoxifen treatment to induce cell death in both ER-positive and ER-negative cells. Ultimately, we envision using the newly developed technique of breast ductal lavage to not only screen women for increased risk in developing breast cancer, which is currently being performed, but to also use this technique to delivery genetic-based preventive agents and/or tamoxifen directly to the breast ductal lobe of these high risk individuals thus eliminating any potential for tamoxifen-induced uterine cancers.

BODY:**Transfer of Funds from the University of Pittsburgh to Wake Forest University School of Medicine**

On May 27, 2005 the University of Pittsburgh formally notified Ms. Brenda Merson, Contract Specialist at the USAMRAA Grant Transfer Section, that the University was releasing the funds for this project. I was notified on June 8, 2005 that the initial funds had arrived at the Wake Forest University School of Medicine. Thus, progress was made regarding this issue since last year's annual summary report.

Approval for the Use of Human Anatomical Substances

Although I currently have approval from the Wake Forest University School of Medicine's IRB to obtain breast ductal lavage samples, I am still awaiting final approval from the DoD's Human Subject Protection Office. Ms. Patricia Dubill indicates she will need additional documentation for final approval. I am currently in the process of obtaining these documents: 1. local IRB approval of DoD recommended revisions – should have approval shortly; 2. continuing review approval documentation from Wake Forest IRB – currently have; 3. consent form used to obtain samples at Magee – Womens Research Institute for the exempt study – I have contacted Dr. Jean Latimer multiple times regarding documentation but have yet to receive; 4. IRB approval letter from Magee-Womens Research Institute to obtain samples for the exempt study – I have contacted Dr. Jean Latimer multiple times regarding documentation but have yet to

receive. Progress has been made on this subject since last year's annual summary report but final approval still awaits.

Statement of Work

Task 1. Determine expression pattern of the PCD regulatory genes bcl-2, bax, and bcl-xL in primary DCIS cultures (Months 1-6):

- a. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.

Task 2. Determine whether down-regulation by genetic manipulation of the anti-apoptotic genes bcl-2 and/or bcl-xL alone or in conjunction with physiological preventive doses of tamoxifen has the highest induction of PCD in primary DCIS cell cultures (Months 6-18):

- a. Treatment with antisense and control oligonucleotides and/or tamoxifen.
- b. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.
- c. Quantify mRNA for bcl-2 or bcl-xL using a PCR-based assay.
- d. Determine effect of treatment on programmed cell death markers using assays for DNA fragmentation and caspase activation.

Task 3. Determine expression pattern of the PCD regulatory genes bcl-2, bax, and bcl-xL in cells obtained by breast ductal lavage (Months 18-24):

- a. Protein analysis of Bcl-2, Bax, and Bcl-xL using immunofluorescent staining.

Task 4. Determine whether down-regulation by genetic manipulation of the anti-apoptotic genes bcl-2 and/or bcl-xL alone or in conjunction with physiological preventive doses of tamoxifen has the highest induction of PCD in cells from breast ductal lavages (Months 24-36).

- a. Treatment with antisense and control oligonucleotides and/or tamoxifen.
- b. Protein analysis of Bcl-2, Bax, and Bcl-xL using Western blotting and immunofluorescent staining.
- c. Quantify mRNA for bcl-2 or bcl-xL using a PCR-based assay.
- d. Determine effect of treatment on programmed cell death markers using assays for DNA fragmentation and caspase activation.

Unfortunately *Tasks 1* and *2* are, at this time, completely reliant on the collaboration with Jean Latimer, PhD from the Magee-Womens Research Institute. At the time this Award was submitted and granted, Dr. Latimer had, and still has, several primary explant cell lines established from DCIS and normal breast tissues. Dr. Latimer had agreed from the beginning and more recently had been added as a paid consultant to this award to supply these cell lines for *Tasks 1* and *2*. Despite countless requests on my part and many promises from Dr. Latimer to supply these cell lines, I have yet to receive any cells. Therefore, until I receive these cell lines from Dr. Latimer or find another source for similar cell lines, *Tasks 1* and *2* will remain incomplete.

As stated in my last annual summary report, I have embarked on collecting breast ductal lavage samples to accomplish *Task 3* using very limited internal funding. I have been able to obtain informed consent from twenty patients recruited through the Wake Forest School of Medicine. Breast ductal lavage samples were obtained from eighteen of these patients. However, the vast majority of these lavage samples had a very limited number of cells to evaluate. I have been in consultation with the surgeons performing the ductal lavage procedure and the company (Cytoc) who manufactures the devices used in the lavage procedure. We are hopeful to rectify this problem shortly and to be able to successfully obtain sufficient cell numbers for *Task 3* and *4*. We have been evaluating a limited number of ductal lavage samples using the protocols designed to accomplish *Task 3* in the above "Statement of Work".

Regarding the Concerns and Comments of the Reviewer of Last Year's Annual Summary Report

I fully appreciate the Reviewer's concerns and comments regarding the ethics of starting *Task 3* before starting/completing *Tasks 1* and *2* and the Reviewer's concern regarding violating federal regulations. I would like to further clarify and hopefully alleviate the Reviewer's concerns.

Reviewer Comments: "There appears to be a serious, fundamental disjoint here. The PI is commencing *Task 3* without having provided any evidence of the proof of principle that would have been tested in the outcome of *Tasks 1* and *2*, which employ primary cultures of excised tissue from DCIS. *Task 1* asks whether there is indeed any altered regulation of the antiapoptotic proteins. *Task 2* tests antisense suppression in vitro. *Task 3* parallels *Task 1* in evaluating antiapoptotic protein expression in fluid obtained from volunteers subjected to ductal lavage.", "Most important, it is clear that the PI is currently conducting, with "internal funding," the more invasive clinical component of the research, that of lavage as opposed to that of the testing of routinely excised tissue. Three of the four recruited patients have been lavaged even before the PI has obtained the DCIS material with which to conduct *Tasks 1* & *2*." and "It is unclear that anyone would allow *Task 3* to proceed without favorable outcomes in *Tasks 1* and *2*." **Response:** Ideally, I would have preferred to accomplish *Task 1* and *2* prior to beginning *Task 3*. However, I have not yet been able to obtain the DCIS and normal breast primary cell lines from Dr. Latimer for *Task 1* and *2* as explained above. Furthermore, although it is anticipated that the protein expression pattern identified in DCIS primary explant cultures will also be representative of protein expression patterns identified in cells obtained from breast ductal lavage samples, this may or may not be the case. Therefore, it is still necessary to identify the protein expression pattern proposed in *Task 3* using ductal lavage samples regardless of what may be identified in *Task 1*. Thus, *Task 3* stands alone and can be performed prior to *Task 1* and *2*.

Reviewer Comment: "This reviewer recommends that the human subjects issues for this grant be subject to careful scrutiny. Given that the "horse has already left the barn," so to speak, it is reasonable to be concerned that, when Army funding resumes, the PI might continue with the recruitment of women for lavage, in the absence of any demonstration of proof of principle on routinely excised tissue. This reviewer recommends that *Tasks 1* and *2* be successfully completed before any work can commence on *Task 3*." **Response:** I have explained the rationale for proceeding with *Task 3* prior to completing *Task 1* and *2* in the above response. If the U.S. Army Medical Research and Materiel Command still

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agrees with the Reviewer's recommendation or needs further clarification of the rationale, please notify me at the earliest possible moment.

Reviewer Comments: "The PI has commenced with the last phase using non-Army funds. The information provided in the report does not necessarily violate any federal regulations, but it raises concerns." and "Also, the PI should make certain that the funds used are not at all derived from the U.S. Army or he may be in violation of Army regulations." **Response:** No U.S. Army funds have been used in the obtainment or evaluation of the breast ductal lavage samples.

KEY RESEARCH ACCOMPLISHMENTS: N/A

REPORTABLE OUTCOMES: N/A

CONCLUSIONS: N/A

REFERENCES: N/A

APPENDICES: N/A