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Developing Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer

Thomas E. Wiese

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Xavier University (XU) and the Tulane Cancer Center (TCC) will build a core of human talent that will address scientific problems such as drug resistance and the effect of environmental agents on breast cancer (BC) in the African-American community. A multi-part research and training program will generate data, develop new research programs and train new faculty and African-American students in BC research. The first component will fund two research projects. The Wang and Burow project will elucidate a previously unexplored cellular signaling mechanism that leads to drug resistance in breast carcinoma cells derived from African American women and women of other ethnicities. The Wiese and Hill project will identify and characterize the genes and gene products associated with BC cell proliferation induced by exposure to pesticide mixtures and is relevant to the African American community in Southern States where pesticide exposure is relatively high. The second part of the program aims to increase the number of faculty at XU involved in BC research by supporting two junior faculty members to develop BC research projects with a TCC mentor. The third objective will support research training of XU undergraduates and pharmacy students. The fourth objective will provide workshops, seminars and research opportunities in BC research for the XU community. This program will enhance the understanding of unique aspects of BC development and progression among African American women and will contribute to the elimination of the “mortality gap” between African-American BC patients and women of other ethnicities.

No subject terms provided.
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Introduction
African American women are at higher risk for breast cancer (BC) mortality compared with their white counterparts. Over the past decade BC mortality has decreased 1%-2% per year in white women, but not in African-American women. The resulting “mortality gap” is a serious national problem, and understanding the reasons for it and developing solutions must be a high priority. Thus, BC research must focus on developing breast cancer models that would aim to accurately predict the disease development and progression among African-American women. We are convinced that increasing the involvement of African American students in BC research will greatly contribute to increasing awareness of the disease in the African American community, which in turn will increase the likelihood of early detection of the disease. Furthermore, the focus on the unique aspects of BC in African American women will lead to better understanding of the disease, and to better treatment options for African American women. This will eventually minimize or eliminate the BC “mortality gap”. To this end we are developing a training program at Xavier University of Louisiana (XU) in collaboration with the Tulane University Cancer Center (TCC). More than 90% of Xavier’s student body is African American has active programs (MBRS, MARC, RISE, NSF/MIE) designed to increase the number of minority students pursuing careers in medical and biomedical research. Through this BC training program, African American students will have the opportunity to become involved in BC research. Tulane (TU) and Xavier have a long history of collaborations involving joint centers and programs and individual collaborations between Tulane and Xavier faculty and staff are common. This new initiative will provide funds for yet another collaboration offering a unique opportunity for XU researchers to establish a BC research program for the benefit of XU students and, eventually, the African-American community. The goals of the training program are to create an environment that fosters BC research, in which XU investigators will receive substantive training and to complete substantive research projects of high relevance to the eradication of BC. The program will enable XU investigators to publish their results in peer-reviewed literature and advance toward independently funded BC research programs. The program includes two full research projects that involve an XU researcher and a qualified TCC mentor. The program will identify two additional XU researchers who have expressed an interest in BC research but do not have prior funding in BC. Participating XU faculty will get the opportunity to network and learn about BC research through participation in the TCC weekly seminar program and the signal transduction workshop that will focus on breast and prostate cancer. The two additional XU faculty involved will develop a mini-proposal in Y1-2 and carry out pilot studies with the advisory of a mentor faculty from TCC in Y2-4. The results of all program research studies will be used as a basis for future proposals in the area of BC. Yearly symposia will be held to provide information to XU students and faculty as well as to enrich the experience of the participating members regarding research opportunities in BC. Multiple project group meetings will be held each year to discuss current data, manuscripts in preparation, funding opportunities and issues regarding project operations.

Year Two Progress
The most significant factor effecting progress in Y2 of this program was the effect of Hurricane Katrina. Even so, significant research progress was made before Katrina in 2005 (see below). The storm closed Xavier University from September through December 2005 and inflicted severe flood damage on the campus. While Xavier and Tulane did down size the faculty after the storm, all faculty involved in this program were retained and did select to return. Xavier University of Louisiana did an amazing recovery from the storm and after all buildings on campus damaged by flood waters, reopened in January 2006. While no lab facilities involved in this program were directly impacted by flooding, all perishable supplies were lost. I addition, faculty involved in the program were burdened with an increased teaching load after the evacuation and to make up for the lost time in the fall semester 2005, Xavier continued the fall in Jan-April 2005 and plans to run the Spring semester 2006 in the summer of 2006. Thus, time for research activities had been reduced in the programs Y2 and Y3. All teaching loads and schedules are expected to return to normal in Fall 2006. To make up for lost time and to save this program, we plan on asking the DOD for a one year funded extension in May of 2006.

During the Katrina evacuation, the Tulane Cancer Center and the Louisiana Cancer Research Consortium (LCRC), our partner in this DOD program offered $25,000 grants to re-supply cancer research labs impacted by
Katrina. This DOD Xavier program was allowed to compete for one of these Tulane/LCRC grants, the PI Dr. Wiese submitted a proposal while evacuated, it was awarded in November 2005 and these funds have been used since January 2006 to re-supply the labs of Dr. Wiese and Wang at Xavier so that this program can recover. See Appendix 1 for more information on this funded recovery proposal.

The period form end August 2005 through the end of this reporting period, April 2006, has been largely lost to evacuation and recovery activities. During the evacuation, all faculty and staff involved in the program moved around to various locations and then settled somewhere for a few months until the January return to Xavier. Dr. Wiese and his wife initially went to Memphis, then Detroit (family in the Detroit area) and then settled in Ann Arbor as a guest faculty in the Pharmacology Department at University of Michigan. Dr. Wang went to Arkansas and then to Philadelphia where he and his family lived with his brother. Research staff went to various locations and a significant effort was made to stay in contact and to work at some level on research related activities using computers, etc. When Xavier re-opened in January 2006, Dr. Wiese returned to New Orleans alone and moved into a FEMA trailer in the Xavier parking lot. Plans are for Dr. Wiese’s wife to return once a housing rental can be procured some time in 2006. Dr. Wang moved back with his family into a rental in up town New Orleans. Once Xavier reopened, all time has been spent on either teaching, cleaning out labs, ordering supplies and the slow re-establishment of research activities.

Body

Task 1
Complete two substantive research projects of high relevance to eradication of breast cancer

Project 1
Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses”

Guangdi Wang, Ph.D., Department of Chemistry, Xavier University of Louisiana PI (Trainee)
Mathew E. Burow, Ph.D., Department of Medicine, Tulane University School of Medicine (Mentor)

Year Two Progress
Aim 1: To demonstrate the requirement for and the role of the MEK5 pathway in survival signaling and suppression of apoptosis in MCF-7 breast carcinoma cells.

The activation status of the MEK5-Erk5 pathways can be monitored by Western blot analysis using phospho-state specific antibodies against phospho-Erk5. We initially wanted to determine the relevance of Erk5 activation in a number of human breast cancer tumors. To this end we developed immunohistochemical staining method to examine phospho-Erk5 (measure of MEK5-Erk5 activation) levels in breast carcinoma samples. Clinical correlation was obtained by studying expression of phospho-ERK5 in clinical samples of breast cancer tissue. Using TARP (Tissue Array Research Program) slides we found expression of phosphor ERK-5 in 30 of 39 (76.9%) samples (Figure 1). These findings underscore the importance of further study of this signaling pathway as a potential target in breast carcinoma.
Figure 1. Immunohistochemical analysis of phosphorylated ERK-5 in human breast and ovarian carcinoma using a semiquantitative scoring system. Staining was performed using Santa Cruz anti phospho ERK5 using TARP arrays of human breast and ovarian carcinomas. Phospho-Erk5 staining was blindly analyzed and scored. Staining intensity is indicated using a semiquantitative method (<5% = 0, 5%-30% = +1, >30% = +2). The numbers of breast or ovarian samples identified in each group are indicated in parenthesis. Representative images from each staining group are shown side-by-side with respective H&E stained samples.

These results indicated that distinct populations of breast carcinoma expressing activated Erk5 or not existed among clinical breast cancer samples. To determine if well established breast cancer cell lines also displayed difference in activation of the MEK5-Erk5 pathways we again used phospho-Erk5 measurements this time with Western blot analysis of a number of human breast carcinoma cell lines (Figure 2). In addition to MCF-7 cells, both T47D and ZR-75 cells are model of ER-positive breast carcinoma. SKBR3, MDA-MB-231 cells are well established ER-negative breast cancer models. MDA-MB-361 is utilized as a model of Her2/neu positive breast cancer (153-154). Variants of MDA-MB-361 exists and here we use MDA-MB-361(−) cells that are ER-negative. Using Western blot analysis against ERalpha, total Erk5 or phospho-Erk5 we can observe a clear enhanced activation of Erk5 (as measured by phospho-ERk5 levels) in SKBR3, MDA-MB-231 and MDA-MB-361 cells. Interestingly this activation of Erk5 is inversely correlated with ER expression. Lack of or loss of ER-alpha is indicative of a poor prognosis in breast cancer patients. Additionally the ER-negative cell lines used here exhibit aggressive in vivo growth, hormone-independence and enhanced apoptotic resistance. Our results suggest that phospho-ERK5 may be associated with a more aggressive phenotype.

Figure 2. Erk5 activation in breast cancer cells. Six human breast cancer cells were grown in 5%CS-DMEM and harvested for immunoblot analysis of phospho-Erk5, total-Erk5, or ER-alpha. Lanes:
1) SKBR3,
2) MDA-MB-231,
3) MDA-MB-361(−)
Aim 1: (b). Implicate apoptotic suppression as a mechanism for MEK5-mediated survival and drug resistance (Months 12-24).

We next wished to establish a model system to allow us to examine the effects of MEK5-Erk5 signaling on breast cancer progression and tumor growth. To investigate the role for direct activation of MEK5 in the regulation of breast cancer cell phenotype, MCF-7 cells (our N variant (SENSITIVE) from above) were used to establish a stable constitutively active MEK5 (CA-MEK5) overexpressing cell line. The use of a CA-MEK5 allows activation of the downstream Erk5 in the absence of any growth factor or survival signal. MCF-7 cells were transfected and stable clones were selected with empty vector (MCF7-VEC) or CA-MEK5 (MCF7-CA-MEK5) (Figure 3A). Using these stables cell systems we next set out to determine if loss of clonogenic survival induced by TNF, tamoxifen or the pure antiestrogen fulvestrant (ICI 182,780), was affected by overexpression constitutive active MEK5(CA-MEK5). Using a clonogenicity assay (126,127), we demonstrate that expression of CA-MEK5 in a sensitive cell type (MCF-7) can shift the cell survival/death signaling balance leading to a resistant phenotype (Figure 3B).

![Figure 3](image-url)

**Figure 3.** Generation of MEK stables and CA-MEK5-mediated resistance to loss of clonogenic survival. (A), MCF7 cells expressing either constitutively active MEK5 (MCF7-MEK5) or vector (MCF7-VEC) were examined by western blot analysis for expression of MEK5 (upper panel) or Erk5 (bottom panel). (B), MCF7-MEK5 and MCF7-Vec cells were plated for clonogenic survival assay and treated with TNFα (10 ng/ml), 1 µM ICI 182, 780 (ICI), or 1 µM 4hydroxy-tamoxifen (OHT) for 18 hr. Following this, cells were cultured in a fresh media and observed daily for 1-2 weeks. Colonies were fixed, stained with crystal violet and counted. Data is displayed as percent clonogenic survival from untreated control cells (100%) ± S.E.M. (n=3).

Using a SCID model of in vivo tumorigenesis we demonstrate that MEK5 expressing cells possess an early tumor onset and greater tumor growth than VEC cells (Figure 4A). Of significant interest was the observation that the MEK5 cells were capable of tumor formation in the absence of exogenous estrogen while the ER-positive VEC parent MCF-7 cells were unable to form tumors without estrogen as far out as 50 days (Figure 4B), demonstrating MEK5’s role in progression to a more malignant hormone-independent phenotype. Consistent with this data was the observation that MEK5 cells will continue to proliferate in the absence of estrogen in vitro as well (not shown). The ability of MEK5 to lead to both an anti-estrogen resistant phenotype in vitro and a hormone-independent phenotype in vivo in many ways parallels what is observed in advanced/recurrent ER+ breast carcinoma’s progression to therapeutic resistance.
Figure 4. MEK5 activation enhances in vivo tumor growth and leads to hormone-independence. (A), MCF7 cells expressing either constitutively active MEK5 (MCF7-CA-MEK5) or vector were injected (s.c.) into the flanks of NOD-SCID mice in the presence of slow release estradiol pellets (1.5 mg, 60 day release) (n=5/group). (B), MEK5 (MCF7-CA-MEK5) or vector were injected (s.c.) into the flanks of NOD-SCID mice. Tumor growth was monitored biweekly after palpable tumor formation and was represented as tumor volume (mm3) ± S.E.M. (n=5).

Aim 2: To characterize differences in protein expression between MCF-7N (APOP-Sensitive), MCF-7M (APOP-Resistant) and ZR-75-30 breast carcinoma cells and identify anti-apoptotic proteins, such as Survivin, within MEK5-expressing cell lines.

(a) Prepare samples for 2D gel separation. (Months 18-24).
(b) Separate proteins on 2D gel electrophoresis, compare differences in protein expression, and perform in-gel tryptic digestion of excised protein products. (Months 24-36).
(c) Sequences obtained from tryptic digests will be used to characterize and identified protein expression differences between drug resistant ZR-75-30 and MCF-7 breast carcinoma cells with a focus on known anti-apoptotic proteins or novel apoptotic domain containing proteins (BC1-2 homology (BH), baculovirus IAP repeat (BIR0, caspase activation recruitment domain (CARD), etc.). (Months 24-36).

Continued Collaboration between Dr. Wang (Xavier), Dr. Burow (Tulane Cancer Center), and Dr. Cai (Proteomics Lab at the Children’s Hospital)

Although all research activities were abruptly interrupted by Hurricane Katrina for an extended period of time (from August 29 through January 17), collaboration between the researchers resumed as soon as all of our facilities were back up and running in early January. Dr. Wang’s lab at Xavier University became functional in early February; Dr. Burow maintained his lab at Baylor Medical School in Texas while trying to restore full operation at Tulane’s Cancer Center; Dr. Cai returned to New Orleans in late October to help restore the Proteomic Facilities Lab at Children’s Hospital which was mostly functional by December of 2005.

Dr. Burow’s lab has provided one batch of cell samples to Dr. Wang in March of 2006. Due to insufficient protein quantities in both cell lines, 2D gel separation resulted in protein spots that were too faint to perform any proteomic characterization. To improve productivity during this important stage of method development, we have decided to culture the MCF-7 cell lines in Dr. Thomas Wiese’s laboratory at Xavier University where cell culture facilities and expertise are available. Dr. Wiese has successfully cultured and harvested MCF-7Mek5 and MCF-7N cells for initial comparative study of proteomics of the breast cancer cell lines. Preliminary results are summarized below.

**Preliminary Data:** Using the newly installed two-dimensional gel electrophoresis system, we have developed and optimized working protocols for i) preparation of protein homogenates from cultured breast carcinoma cells; ii) isoelectric focusing of the protein homogenates in an immobilized pH-gradient gel for the first dimension separation, iii) SDS-PAGE second dimension electrophoresis, and iv) gel imaging (see Figure 5 for example 2D gels). Work is underway to identify protein spots that can yield meaningful information on
differential protein expression (both qualitative and quantitative) between the MCF-7MEK5 and MCF-7N cell lines.

**Figure 5a.** 2D gel electropherogram of protein extracts from MCF-7Mek5 cells using pH 3~10 IPG strips and 11 cm 8~16% linear gradient SDS PAGE gel, and coomassie stain.

**Figure 5b.** 2D gel electropherogram of protein extracts from MCF-7N cells using pH 3~10 IPG strips and 11 cm 8~16% linear gradient SDS PAGE gel, and coomassie stain.

**Image Analysis:** Coomassie-stained gels were scanned with a Gel Doc-XR image system (Bio-Rad) using PDQuest software (Bio-Rad) and the proteins of interest were marked for excision. Image analysis of the 2-gels of the two breast cancer cells (Figure 2) revealed very similar protein expression with more than 2100 spots
detected on each gel. For preliminary examination, the spots which appear to have sufficient amounts of proteins for LC/MS analysis were excised. Of the 153 spots excised, C2, C5, C7, C11, D9, E11, F2 and H8 were found only in MCF-7Mek5 cells and D4, F7, F8, G1, G3 and G4 were detected only in the MCF-7N cell line. A complete list of excised gel spots is given in Table 1.

Table 1. List of Excised Gel Spots for MCF-7NvU and MCF-7Mek5

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Some other spots appearing different in the two breast cancer cell lines were of low concentrations and were barely detectable by coomassie staining. At the present expression level these spots could not be identified by LC/MS, and it will be necessary to run preparative gels on which more proteins can be loaded. Some of the identified spots are listed in Table 2 (for the MCF-7N cells) and Table 3 (MCF-7MEK5 cells) below. The mass spectrometric identification of all excised gel spots is being carried out using the linear trap tandem mass spectrometer at Children’s Hospital in New Orleans.

Most of the identified proteins are the same in the two breast cancer cell lines. For example, B01, B02, D02, E04, E05, F04 spots from MCF-7N are identical to B03, B04, D01, E02, E06, F01, F03 spots from MCF-7Mek5, respectively. Two differing spots have been identified. In the drug sensitive MCF-7N cell line, gel spots E03 and F09 (Table 2) represent different proteins than the corresponding E01 and F06 spots (Table 3) from the drug resistant MCF-7Mek5 cell line, respectively. Further experiments are underway to confirm the preliminary findings and to gain insight into the mechanism of drug resistance at the proteomic level.

Table 2. Identified proteins of selected gel spots from the drug sensitive MCF-7N cell line (in progress)

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Table 3. Identified proteins of selected gel spots from the drug resistant MCF-7Mek5 cell line (in progress)
Plan to Purchase an HPLC-Tandem Mass Spectrometer
Dr. Wang has secured approximately $200,000 in external funds toward purchasing an HPLC-MS/MS system for dedicated proteomic analysis. Because the instrument costs over $340,000, he is seeking to rebudget most of the year two carryover fund of the DoD BC grant and some of the year three funds for the instrument purchase. Upon approval, Dr. Wang will be able to acquire a complete mass spectrometry system equipped with a two dimensional HPLC for proteomic characterization. The availability of a proteomic mass spectrometer will significantly increase Dr. Wang’s productivity on the breast cancer research project and will serve as an important analytical platform for Xavier faculty to conduct cancer-related research.

Aim 3: In this task we will use RNA interference strategies to validate a role for the Erk5 pathway in downstream gene expression and in suppression of chemotherapeutic drug-induced apoptosis. Our preliminary analysis revealed survivin expression was increased in drug-resistance and MEK5 expressing breast carcinoma cells. Subsequently we will characterize the role of these downstream targets such as Survivin, in suppression of apoptosis and drug-resistance.

Aim 3: (a) Optimize pSUPER base RNA interference (RNAi) suppression of ERK5 expression in breast carcinoma cells (Month 15-18).

Aim 3: (b) Confirm a role for Erk5 signaling in MCF-7N-CA-MEK5, and MCF-7M-(RESIST) cell survival using pSUPER-Erk5-RNAi. (Months 18-28).

We next sought to confirm a role for Erk5 signaling in the observed phenotype of MEk5 overexpressing MCF-7 cells. RNA interference (RNAi) is a novel method which through either transient transfection of siRNA or through the use of Pol III driven shRNA expression constructs stably ablates/suppresses expression of specific genes. In our lab, we have used siRNA and shRNA-expression constructs to selectively ablate/suppress target genes. In Figure 6 we demonstrate the use of shRNA expression constructs to generate MCF7-MEK5 cells stably targeting Erk5. Here we show that MCF7-MEK5 cells stably expressing Erk5-shRNA demonstrate a downregulation of Erk5 mRNA (Fig 6A) and protein levels (Fig 6B) as compared to empty-shRNA vectors. The ability of MEK5 expression to enhance tumor formation in immunocompromised mice is suppressed by RNAi-mediated ablation of Erk5 expression (Figure 5C).

![Figure 6. Erk5-RNA interference partially suppresses MCF7-MEK5 tumor growth](image)

Figure 6. Erk5-RNA interference partially suppresses MCF7-MEK5 tumor growth. MCF7-MEK5 cells were transfected with pCMV-hygro along with either empty pSUPER-GFP (pSUPER-VEC) or pSUPER-GFP containing Erk5 shRNA sequences (pSUPER-Erk5-RNAi) and stable clones were selected in hygromycin. Decreased expression of Erk5 was confirmed with PCR (A), and western blot analysis (B). (C), MCF7-MEK5-(VEC) or MCF7N-MEK5-(Erk5-RNAi) cells (5 X 10^5) were injected (s.c.) into the flanks of SCID-Beige mice in the presence of slow release estradiol pellets (0.72 mg, 60 day release) (n=5 /group). Tumor growth was monitored daily after palpable tumor formation and was represented as tumor volume (mm3) ± S.E.M. (n=5).
Aim 3: (c) Develop/validate RNAi strategies for Survivin suppression using pSUPER method as above.

Use RNAi to implicate Survivin expression in drug resistance and apoptotic signaling of MCF-7 and ZR-75 breast carcinoma cells (Months 24-36).

While focusing effort on the RNAi strategies for ERK5 knock-down we have been exploring the mechanisms and targets of Survivin regulation by MEK5-Erk5.

Regulation of biological responses to MAPK signaling involves stimulation of transcription factor mediated gene expression. The ability of MEK5 to mediate cell survival may in part occur through activation of transcriptional regulation of survival gene expression. We and others have previously shown an important role for NF-κB activation in the determination of cell survival decisions (21, 25). Using MCF-7 breast carcinoma cells, we demonstrate above that transfection of CA-MEK5 slightly increasing basal activation in the absence of stimuli as well as potentiates the ability of TNF and PMA to induce NF-κB activity (Figure 7B). We have confirmed that stable MCF-7-MEK5 cells exhibit a higher basal and TNF-inducible NF-κB luciferase activity as well (not shown). A number of signaling pathways converge at different steps of the NF-κB cascade to stimulate activity. While MEK5 may directly activate the signaling cascade (IKK-IκB-NFκB), potentiation of TNF induced NF-κB activity suggests that MEK5 may coordinately integrate with TNF to enhance NF-κB, resulting in a shift from death receptor signaling towards one of survival. Additionally, STAT3 is critical in regulation of cytokine and growth factor mediated cell survival and its constitutive activation in certain cancers has been demonstrated to suppress apoptosis. Along with NF-κB, STAT3 has also been demonstrated to be targeted by MAPK mediated signaling. Using a similar reporter gene assay for STAT3-mediated transcription we show that CA-MEK alone is capable of potently stimulating STAT3 activation (Figure 7A). To investigate the mechanism by which MEK5 signaling may increase NF-κB activity we examined basal expression of NFκB-p50, NFκB-p65 and IκB-α in MCF-7-VEC versus MCF-7-MEK5 cells. Western blot analysis revealed a higher basal expression of p65 in the MCF-7-MEK5 cells similarly basal levels of IκB-α were lower in MCF-7-MEK5 cells. This suggested that MEK5 may function by activating IKK-IκB-p65 pathways leading to NF-κB transcription activation. These results do not rule out the possibility that MEK5 may have direct effects on P65 or IκB expression (Figure 8A). gene expression profiling (Affymetrix analysis not shown)
of MCF-7-CA-MEK5 cells versus MCF-7-VEC cells revealed increased expression of the key anti-apoptotic gene Survivin. The results suggest that the suppression of cell death by MEK5 may in part occur through upregulation of Survivin. Western blot analysis confirmed a higher expression of Survivin in MCF-7-MEK5 cells (Figure 8A). To determine if the effects of MEK5 on Survivin expression required NF-kB, MCF-7-MEK5 cells were treated with inhibitors of NF-kB, SN50 (128) or Bay11-7082 (21). RT-PCR analysis revealed that NF-kB inhibition partially suppressed MEK5 induced Survivin expression (Figure 8B). This was further confirmed by examining the full length Survivin-promoter linked to luciferase(129-132) (Figure 8C).

(d) Develop, validate and use RNAi strategies for novel targets identified from proteomic analysis of drug resistant breast carcinoma cells from Aim 2. (Months 36-48).

The data from above suggests that higher levels of Erk5 activation are observed in ER-negative breast cancer cell lines. Additionally our data in the ER-positive MCF-7 line suggests that overexpression of CA-MEK5 drives these cells to an ER-independent phenotype. We have focused on ER signaling as a target regulated by MEK5-Erk5 while continuing our proteomic studies to identify novel markers of drug resistance. We hypothesized that at some level the estrogen receptor pathways are affected by or by-pass MEK5-Erk5 signaling. To determine the effects of MEK5 on ER signaling, an ERE-luciferase reporter assay was used in both transient (Figure 9A) and stable (Figure 9B) transfected MCF-7-(CA-MEK5) cells. The data demonstrate a partial suppression of estrogen mediated transcription in transients and complete suppression of ERE-activity in stable MCF7-CA-MEK5 cells.
FIGURE 9: Suppression of estrogen receptor function by transient and stable CA-MEK5 expression. (A), MCF-7 cells were transiently transfected with ERE-luc (200ng/well) along with 300 ng/well of either vector or CA-MEK5 and treated 6 hours later with vehicle (DMSO) or 17β-estradiol (1 nM). Cells were harvested the next day for ERE-luciferase assay. Data is displayed as relative light units normalized to vehicle treated vector transfected cells (100) from a representative experiment. (B), Stable-MCF-7-(CA-MEK5) or MCF-7-VEC cells were transfected with ERE-Luc (200 ng/well) and treated 6 hours later with vehicle or 17β-estradiol (1 nM). Data is represented as normalized RLUs from three independent experiments.

To determine if activation of the MEK5-Erk5-Pathways affects ER expression levels we examined by PCR the expression of ERalpha in The MCF7-VEC, MCF7-MEK5-(shRNA-empty) and MCF7-MEK5-Erk5-shRNA cells (Figure 10). These data show that in the MEK5 overexpressing cells, ERalpha is downregulated at the mRNA and protein levels. Blockade of the MEK5-Erk5 pathways by shRNA-Erk5 restores ERalpha expression at both the protein (Figure 10A) and mRNA levels (Figure 10B).

Figure 10. Stable MEK5-Erk5 activation suppresses ERalpha expression. MCF7-VEC, MCF-7(CA-MEK5)-(shRNA-empty) and MCF-7(CA-MEK5)-(shRNA-Erk5) cells were grown in 5%CS-serum containing media to remove any endogenous estrogens from the experiment and harvested after 72 hours in estrogen free media. (A), Western blot analysis of ERalpha expression with beta-actin
measured as a control. (B) RT-PCR analysis of ERalpha expression, Erk5 expression, or GADPH (control) expression. Data shown is representative analysis of three independent experiments.

Project 2
Interactions of estrogen and progestin active environmental chemicals on BC cell proliferation, survival and gene expression

Thomas E. Wiese, Xavier University College of Pharmacy PI (Trainee)
Steven R. Hill, Tulane University School of Medicine (Mentor).

Aim 1: Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival.
(a). Develop treatment mixture matrix and plan for proliferation experiments (Months 1–2).
(b). Perform cell proliferation studies with binary mixtures of pesticides (Months 1–18)
(c). Identify mixtures with novel effects on cell proliferation (Months 6–20).

Year Two Progress
Research Assistant
This project started with Ms. Suzanne Nehls as research assistant and then primary research assistant duties transitioned to Ms. Hanh Nguyen (see Y1 progress report). After 5 months of training (Jan 05–May 05), Ms. Hanh Nguyen informed Dr. Wiese that she would be leaving in June 05 to enter dental school. This was a surprise since Ms. Nguyen had indicated at the beginning that she was looking for a position for at least 2 years. Dr. Wiese advertised for a new assistant and was able to hire Mr. H. Chris Segar, a student that did his MS research in Dr. Wiese’s lab in 2002. Mr. Segar started on the project in June 2005 and immediately went through training. Mr. Segar brings more educational background to the position (BS biochemistry and MS in molecular toxicology), but lacked significant hands on research experience. His MS project involved entirely computer modeling simulations. Mr. Segar learned fast and was working out well when the lab operations were interrupted by Hurricane Katrina in August 2005. With the help of the DOD program staff, Dr. Wiese was able to keep paying Mr. Segar during the Katrina Evacuation (Sep-Dec 2005) and he returned to work when Xavier reopened in January 2006. Over all, the loss of Ms. Nehls in 2004 and the training of Ms. Nguyen and Mr. Segar has cost the project at least 8 months lost time.

Collaboration between Dr. Wiese at Tulane Cancer Center and Dr. Hill
Dr. Wiese has been in close contact with Dr. Hill since the start of this project through phone, email or meetings. While the project was designed to take place entirely in the Wiese lab, Dr. Hill continues to provide input on experimental design and data interpretation. The main contribution of Dr. Hill to this project has been discussions relating to the use of microarray technology to identify specific genes or classes of genes that may be related to the observed mixture effects (see Y2 progress of Aim 2 below). Dr. Hill has also provided insight regarding the management of the overall training program (see tasks 2 and 3 below).

Preliminary and Y1 results summary
The series of pesticides included in this study included isomers and metabolites of DDT and methoxychlor. Each are known to have weak estrogen, androgen and/or progesterone activity. A series of MCF-7 proliferation studies were conducted to identify novel interaction effects of binary mixtures of these compounds. The initial studies were designed to include one pesticide at the lowest observed effect level (LOEL) and the other at the highest dose possible (10−5 M). Experiments were also conducted to determine if mixing the pesticide (high dose) with sub-optimal concentrations of estradiol-17β (E2) enhanced estrogen induced proliferation. This series of experiments did not identify mixture combinations with more than the additive cell proliferation activity expected from the compounds alone at the same concentrations. These same
mixtures were examined in the MVLN estrogen responsive reporter gene assay where similar additive effects were also observed. See Y1 progress report for more information. At this point, Dr. Wiese decided to examine mixtures that contained one of the organochlorine pesticides along with one of three organophosphate pesticides. We have observed a positive sensitizing or potentiation effect of organophosphate pesticides on the weak estrogen dependant proliferation activity of organochlorine pesticides (increased potency). This action can be eliminated by antiestrogen and is likely estrogen receptor (ER) dependant. The observation that this sensitizing effect was not observed in the reporter gene system suggests that the mechanisms involved are more complex than a simple stimulation of classical ER transactivation activity. Finally, the observation of this sensitizing effect suggests a hypothesis that exposure to low levels of weakly estrogenic pesticides in combination with an organophosphate pesticide might result in more breast cancer cell proliferation than would be expected by the organochlorine alone. The organophosphate compounds in this study are known to have antiandrogen activity. Considering that androgen agonists are known to inhibit estrogen regulated processes in some cells, it is reasonable that treatment with antiandrogens may relieve such suppression, resulting in a relative increase in organochlorine induced estrogen activity. The organochlorine compounds in the study are considered persistent contaminants with long elimination half lives. Thus, chronic exposure to low concentrations may have more estrogenic activity than would be expected if cells are sensitized or stimulated by periodic exposure to organophosphate pesticides. Contamination from older pesticides that are no longer used might be more significant if one is exposed to current use pesticides. See Y1 progress report for more information.

Year 2 activities related to Aim 1 included completing the matrix of pesticide combinations in the proliferation and MVLN reporter gene assays. Mixture combinations that produced the most dramatic sensitization effect in the breast cancer cells were selected for microarray analysis in Aim 2. These are: Parathion and opDDT, Fenitrothion and opDDT, and HPTE and opDDT.

Aim 2: Conduct cDNA microarrays to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones. Preparations from cells grown and exposed to mixtures of hormone active pesticides in the Wiese Lab will be evaluated for differential expression of genes in the Tulane Center for Gene Therapy.
(a). Identify target genes related to breast cancer cell proliferation from literature searches that will be used in gene array studies (Months 1–12).
(b) Prepare cells for gene array analysis after exposure to mixtures of pesticides. (Months 9–24).
(c) Run gene array analysis on cell preparations and analyze data (Months 12–36).

Year Two Progress
In Year 1, we decided to use focused microarrays such as SuperArray rather than the Affymetrix arrays available in the Tulane Cancer Center. This will reduce the cost of the microarray experiments and allow us to focus more on nuclear receptor mechanisms and cancer related genes. In Y2, Dr. Wiese and Mr. Segar in the Wiese lab did background research on the SuperArray system and ordered some arrays to test at Katrina disrupted activities. Plans are to set up the SuperArray system in the lab once the lab is fully operational and we can repeat the novel mixture results identified in Y1 and refined in pre-Katrina Y2.

Aim 3: Confirm the expression pattern of genes identified by microarray through analysis of gene products (mRNA or protein).
(a) Select 6–10 genes that have been shown by differential display to have novel expression patterns as a result of pesticide mixture treatment (Months 14–36).
(b) Obtain probes for Northern blot analysis of selected genes (Months 14–36).
(c) Perform Northern blots to confirm expression observed in micro array studies (Months 24–48).
(d) Obtain antibodies for Western blot analysis of selected genes (Months 14–36).
(e) Perform Western blots to confirm expression observed in micro array studies (Months 24–48).

**Year One Progress**
No progress on this aim in year 2.

**Deliverables/measurable outcomes:**
Drs. Wang and Wiese will prepare or oversee the following:

1. **Semiannual reports will be submitted to the PI.**

**Year Two Progress**
These reports were submitted and have been used to make this progress report.

2. **Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36, 48).**

**Year Two Progress**
In Y2, one Xavier undergraduate student was involved in research activities supporting the breast cancer project in the Wiese lab. Ashley White, a chemistry major and Xavier RISE scholar joined the Wiese lab in May 2004 and worked on this project through project Y1 and into Y2. Ashley has very good lab skills, was well trained in the lab and after graduation, she planned to continue working on this project for the next few years while she was in pharmacy school at Xavier (started August 2005). However, Ashley did not return to Xavier after Hurricane Katrina. Another student will be selected to work on this project in the second half of 2006, after the lab is fully operational and after Xavier students are caught up with 2005-2006 school year courses running through the summer of 2006.

Dr. Wang will identify an undergraduate student to work on his project in the second half of 2006 or in early 2007. The method development required for his project in Y1 was not appropriate for student training. At the same time, the lab recovery process undertaking in first half of 2006 is also difficult for student training because the students is not able to generate a product.

Both Dr. Wang and Wiese feel that the most effective student training only occurs when the full time staff members of the lab have sufficient expertise in the methodologies used in the lab. Thus, Y1 in both labs was dedicated to building expertise so that students in Y2 and beyond will have sold faculty and staff mentors from which to learn lab skills.

Drs. Wang and Wiese also have experience mentoring Xavier students in the MARC and RISE programs at Xavier. Once these programs are reestablished on campus in late 2006, both of Drs. Wang and Wiese will be bringing in at least one additional student into their labs.

3. **One competitive grant application will be submitted by the end of the funding period.**

**Year Two Progress**
**Submission of a major equipment proposal to DoD for the acquisition of a tandem mass spectrometer.**
In an effort to build up Xavier’s capability to conduct independent proteomic research, Dr. Wang submitted a major equipment proposal to DoD’s to the Army Research Office for consideration under ARO Broad Agency Announcement W911NF-05-R-0001 in 2004. The proposal was entitled “High Performance Liquid Chromatography-Tandem Mass Spectrometry for Enhancement of Teaching and Research at Xavier University” and asked for $196,392 for the purchase of an HPLC-MS/MS system. One of the major justifications for the proposal is the ongoing breast cancer research project for which the availability of such an instrument is essential. This proposal was not funded.
After returning to Xavier in January 2006 from the Katrina Evacuation, Drs. Wiese and Wang identified funds from 3 grants that could be rebudgeted to purchase the core components of the HPLC and Mass Spectrometry equipment requested in the above proposal. After, obtaining approval from funding agencies, rebudgeting was done and the equipment was purchased in the first quarter of 2006. Thus, the core instrumentation equipment required to do proteomic analysis will be set up in Dr. Wang’s lab in 2006.

**Submission of a P20 planning grant to the NCI**

A P20 planning grant was developed and submitted to the NCI in Y1. More details are provided in the Y1 progress report of Task 3 below.

4. **Papers will be submitted to peer reviewed journals through the funding period.**

**Year Two Progress**

No manuscripts were submitted in Y2.

**Training deliverables:**

1. The Tulane Cancer Center in conjunction with the Section of Hematology and Medical Oncology and The Cell Signaling group will be directly involved in providing breast cancer research training for Xavier Investigators.

**Year Two Progress**

The support provided from the TCC to each project is described within the progress reports of each project above. In addition, TCC support for the program as a whole is detailed in Task 3 below.

2. **Toward the end of the project period, Drs. Wang and Wiese will be Co–PIs in writing an R01 grant in collaboration with Drs. Burow and Hill.**

**Year Two Progress**

No R01 collaborative grants are in preparation at this time. We expect that during Y3, the planning for at least one collaborative grant will be started.

**Task 2**

Assist two Xavier junior faculty to become more competitive in breast cancer research

a. **Identify two Junior Faculty with interest in breast cancer research (Month 1).**

**Year Two Progress**

In the summer of 2004, Dr. Wiese and Dr. Klassen (coPI of the XU Prostate Training Grant) began identification of XU faculty that were interested in cancer research. This process resulted in one XU chemistry faculty, Dr. David Wolfgang, and one XU biology faculty, Dr. Mary Carmichael, submitting interest statements and CVs. Dr. Wiese then met with Dr. Hill to discuss potential mentors. Dr. Carmichael has been matched with Dr. Asim B. Abdel-Mageed from TCC on a Prostate Cancer project and is now developing a project proposal. Dr. Wolfgang was matched with Dr. Charles Miller at the Tulane Cancer Center in Spring 2005 and they were well along on developing a project when Katrina hit.

Efforts to get Xavier College of Pharmacy clinical faculty involved in developing a research project have not produced a viable team.

b. **Establish participation of the selected Junior Faculty in Tulane Cancer Center seminars and the weekly signal transduction workshop focused on breast and prostate cancer (Month 2).**
Year Two Progress
A regular group of XU faculty involved in the DOD Breast and Prostate cancer projects are attending the TCC and LSU CC seminars held most Thursdays at noon. Attendance has reduced after the Katrina evacuation since faculty are involved in additional responsibilities and lab recovery efforts. The LCRC signal transduction workshops have not yet started meeting. Dr. Wiese has discussed this situation with Dr. Hill who is in the process of reorganizing these workshops after the Katrina recovery. Xavier faculty involved in the DOD BC and PC programs have meet and decided to form a regular work in progress seminar meeting at Xavier starting in Fall 2006.

c. Determine Tulane Cancer Center mentors for the Junior Faculty and submit a two–page mini proposal for review of the PI and alternate PI (Month 6).

Year Two Progress
Dr. Wolfgang has been matched with Dr. Miller and they have developed the following idea for a mini-proposal:

The protein p23 is up regulated in cancer cells. It is a co-chaperone of heat shock protein 90 (Hsp90). Hsp90 is involved in the maturation of signaling proteins such as estrogen receptor. Activation of the estrogen receptor is important in the formation and progression of breast cancer. p23 stimulates Hsp90 to release its substrates. Dr. Miller has mouse fibroblast cells in which he has knocked-out the p23 gene. He also has stably transfected some of those cells with the p23 gene. This provides two cell lines with the only difference being the expression of p23. We will conduct experiments with various drugs such as geldanamycin, novobiocin, and herbimycin. The molecule 17-allylamino geldanamycin is already in clinical trials. These drugs function to inhibit Hsp90 activity. Normal cells and cancerous cells both have the same amount of Hsp90 but cancer cells have more of the Hsp90 in its active dimmer form. p23 helps keep Hsp90 in the active form. We will be looking for differential toxicity between the cells with p23 and the cells without p23. The goal is to find drugs with toxicity toward the p23 cells.

Initially the work will be done at Tulane. Since I have limited experience with cell culture the work will initially be carried out at Tulane. Currently my Tuesdays are free to go to Tulane. Once I’m comfortable working with the cells I can move the work to Xavier. Dr. Tom Weise has a functioning cell culture incubator and hood. The Chemistry Department also has an incubator and hood that are currently not in use. I currently have one Chemistry senior student working on another project but I have three Biology sophomore students who I would like to get involved with p23 project.

See Appendix 2 and 3 for CVs of Drs. Wolfgang and Miller.

d. Junior Faculty collect preliminary data (Months 7–36).

Year Two Progress
See Task 2 c for current status of the Wolfgang-Miller project.

e. Junior Faculty develop grant proposal (Months 36–48).

Year Two Progress
No Progress in this area in Y1.

Task 3
Establish infrastructure that will create an environment that fosters breast cancer research, in which
Xavier faculty will receive substantive training and become more competitive for DoD funding

**Background and Year Two Progress**

When Xavier was awarded the DOD Breast Cancer grant in April 2004, Dr. Rosenzweig, the project PI, announced that she would leave Xavier in May 2004. A plan was formulated where Dr. Wiese, PI of one of the research projects in the Breast Cancer training program would take over program PI responsibilities along with his research project. He would be provided release time for both tasks and be assisted by a part time administrative assistant that would be hired. Dr Wiese served 5 years as a joint faculty between Tulane and Xavier before moving full time to Xavier in 2003. While at Tulane, he became a member of the Tulane Cancer Center and developed a good working relationship with Dr. Steven Hill, Tulane coPI of this project. Dr. Wiese also had also developed a good working relationship with Dr. Klassen, Xavier Chemistry Department, coPI of the XU-YU DOD Prostate Cancer training program, when Dr. Klassen utilized the cell culture facilities in the Wiese lab in 2003-2004.

Unfortunately, Dr. Klassen elected not to return to Xavier after Katrina. Dr. Wiese, the PI of the Xavier DOD BC program has been asked by the Xavier administration to replace Dr. Klassen as PI of the Xavier DOD Prostate program effective Feb 2006.

The Xavier DOD BC and PC programs continue to operate in parallel with meetings, seminars and discussion sessions involving both groups. In Y1, we established an email list serve for all Xavier and Tulane faculty involved in both XU DOD cancer training projects and this mechanism has been very helpful for rapid communication of cancer center events, project meetings and organizing car pools to LCRC seminars.

It should be noted that the Tulane Cancer Center is part of the Louisiana Cancer Research Consortium (LCRC) that includes the LSU Cancer Center. The LCRC was devised in 2002, involves significant funding from the state of Louisiana and will eventually be housed in a new building between the Tulane and LSU medical centers in New Orleans. The LCRC is co-directed by Dr. Roy Weiner (Director of Tulane Cancer Center) and Dr. Oliver Sartor (Director of the LSU Cancer Center). Drs. Klassen and Wiese were invited to the first annual LCRC retreat in January 05. The planning process and meetings that took place at this retreat clearly stated that all Xavier faculty interested in or doing cancer research were welcome to participate in the LCRC through adjunct appointments in Tulane or LSU departments. In addition, Dr. Roy Weiner has keep in close contact with Dr. Wiese regarding the Xavier DOD Breast Cancer training program and has made it clear that he is personally committed to helping Xavier faculty develop cancer research projects and programs. He has opened up all the resources of the Tulane Cancer Center core facilities to Xavier researchers and has invited Xavier faculty to be involved in the Tulane Cancer Centers cancer research symposia held each fall. This Mauvernay Research Excellence Award program includes seminars and posters related to cancer research and concludes with a dinner where TCC faculty meet the invited speakers. Several of the XU faculty involved in the DOD cancer training programs attended the Mauvernay Research Excellence Award program in fall 2004 and Drs. Hill and Weiner made a special effort to introduce the XU faculty to TCC faculty and to the invited speakers. Dr. Weiner also has included clinical faculty from the Xavier College of Pharmacy in ongoing initiatives at the Tulane Cancer Center.

One result of this close relationship between Drs. Weiner and Hill of the TCC and Xavier University is the submission of a P20 planning grant to the NCI in February of 2005 (see Abstract in Y1 Progress Report Appendix). This grant is specifically designed to plan long term collaborations between cancer centers and minority serving institutions. Through a series of meetings starting in October 2004, a P20 grant was developed between the Tulane Cancer Center and Xavier University with Dr. Weiner as the Tulane PI and Dr. Kathleen Kennedy, Associate Dean, Xavier College of Pharmacy as the Xavier PI. At the same time, the PI and co-PI of the Xavier DOD Breast Cancer Training Program, Drs. Wiese and Hill became the P20 grant program managers for each respective institution. Drs Wiese and Hill also took responsibility for the majority of the organization, planning and preparation of this panning grant over a 5 month period leading up to submission in February.
2005. This NCI P20 program grant was awarded in August 2005 with a start date of October 1, 2005 (during the Katrina evacuation). The good working relationship of Dr. Wiese and Hill, developed largely from the DOD Breast Cancer Training Program and other prior activities, was critical to working out the complex details of this P20 proposal that involved two very different universities. We feel that the DOD cancer training programs between Tulane and Xavier provided the critical mass required to put together this P20 grant and that the combination of these programs will contribute significantly to the development of self sustaining cancer research programs at Xavier in the future.

The review of the Xavier DOD Breast Cancer Training program requested that an administrative assistant be hired to assist the PI in grant management tasks as well as in planning meetings and coordinating communication between all those involved at XU and TU. In August 2004, Mr. Sergio Alcantera was hired as a part time program manager for this project. See Y1 progress report for more details. Mr. Alcantera moved his family to California after Katrina leaving this position open.

With the award of the NCI P20 training grant in 2005, Xavier now had two program grants that had openings a program assistant. With the help of Dr. Roy Weiner at the Tulane Cancer Center and a search process at Xavier, a suitable candidate was identified in early 2006. Ms. Stephanie Colbert was hired by Xavier in February 2006 to support both the DOD BC program and the NCI P20 grant working under the supervision of Dr. Wiese, PI of the DOD BC program and manager of the NCI P20 program. For Ms. Colbert’s CV, see Appendix 4.

a. Grant membership in the Tulane Cancer Center to Xavier researchers. Drs. Wang and Wiese will be granted a status of contributing members and the junior faculty will be granted a status of associate members. Please see attached TCC publication for the definitions (Month 1).

**Year One Progress**

At this time, only Dr. Wiese has formal membership in the Tulane Cancer Center because he is an adjunct faculty in the Biochemistry Department of the Tulane school of Medicine. Dr. Wang will be granted membership in the cancer center once he is approved as an adjunct in a Tulane department. Dr. Burow (mentor for Dr. Wang) is currently exploring the potential for an adjunct appointment in his home department of Internal Medicine. Cancer center membership for Dr. Wolfgang will occur once he has been matched with a research mentor and receives an adjunct appointment at Tulane.

b. Include Xavier researchers in Tulane Breast Cancer focus group and Journal Club (Months 2).

**Year One Progress**

The Tulane Cancer Center Focus Groups are now combined with the LSU Cancer center under the LCRC. At the LCRC retreat in January 2005, the following focal groups were established: Molecular Genetics, Molecular Signaling, Immunology, Epidemiology and Clinical Research. To date, while membership in these groups as been established, regular meetings of these groups has not occurred. We plan to hold some of these focal group meetings at Xavier in Y2.

c. Grant access to core research facilities at Tulane Cancer Center (Month 1).

**Year One Progress**

Access to TCC and LCRC core facilities has been granted to Xavier faculty. These cores include: Genomics, Proteomics, Biostatistics/Bioinformatics, Immunology, and Tissue Acquisition.

d. Include a student in each research project (Month 2 for Drs. Wang and Wiese and Month 8 for the junior faculty).
Year One Progress
See Task 1 above.

e. Establish a monthly brown–bag lunch meeting to bring up research related issues, review proposals and papers, or brainstorm on new directions to improve the cancer program (Month 1).

Year One Progress
Due to the busy schedules of both TCC and XU faculty involved, monthly meetings have proven to be difficult to organize. We have held a number of group and sub group meetings to discuss aspects of individual projects. Many meetings were also held to develop the P20 planning grant. In Y2, we plan to establish a set date for monthly meetings that will coincide with the weekly seminars at the TCC that many faculty from both institutions are going to anyway. We also hope to have mini-program meetings before or after the monthly/bimonthly LCRC focal group meetings.

e. Hold an annual workshop, open to all in the Xavier and Tulane communities and Xavier student body, for all BC participants to present results of the preceding year. Faculty, students, and staff will attend and at least one person from each group will present a talk; students will present posters (Months 12, 24, 36, 48).

A. First workshop titled "Molecular Signaling in Breast Cancer" (Month 12).
B. Second workshop titled "Breast Cancer and the African American Community" (Month 24).
C. Third workshop titled "Funding Opportunities in Breast Cancer Research" (Month 36).
D. Forth workshop titled "Drug Design and Delivery in Breast Cancer" (Month 48).

Year One Progress
Efforts to hold a joint Breast and Prostate symposia in the spring semester 2005 were thwarted by scheduling conflicts within the university. Dr. Wiese and Klassen are now planning on holding the Y1 symposia in the first or second week of classes of fall semester 2005. We feel that this timing will allow for good student and faculty attendance and also serve as an advertisement for the Breast and Prostate training programs to the whole Xavier community before they get bogged down in the semester. This first symposia will bring in speakers that can directly impact the research projects underway in both training programs and will also include posters from each project. Then, in the spring of 2006, we will hold the Y2 symposia at a time that does not conflict with the various spring activities at Xavier. We will work with the Xavier Festival of Scholars Program in the Fall of 2005 so we can hold our cancer symposia along with the annual Festival of Scholars at Xavier. This way, students presenting at the festival can also participate in the cancer symposia and learn of cancer research opportunities on campus as well as cancer research careers.

f. Subscribe to breast cancer related journals (Month 1).

Year One Progress
After a survey of the cancer research journals available to the Xavier and Tulane communities, we have purchased a subscription to the online journal Breast Cancer Research. In Y2, we will subscribe to the journal Proteomics and the XU-TU Prostate Cancer program will purchase another cancer related journal subscription. Access to Tulane library resources is still limited for XU faculty. Only faculty with adjunct appointments have off campus online access. In Y2 we must establish XU faculty as adjuncts at Tulane to resolve this problem.

Key Research Accomplishments

- We have shown that over expression of MEK5 increases breast cancer tumor volume independent of estrogen.
• We have shown that the combination of organophosphate and organochlorine pesticides can interact to enhance the estrogen activity of the organochlorine.

**Reportable Outcomes**

1. MCF-7 MEK5 cells that stably express MEK5.

**Conclusions**

In Y1 of this training program, we have established two collaborative breast cancer research projects and have identified faculty that can develop additional projects in Y2. Most importantly, we have built a framework of activities for XU faculty to utilize for interaction with the TCC to develop cancer research initiatives involving Xavier undergraduate and pharmacy students.

**Year Two Synergy and Opportunities**

In Y2, we plan to build on established interactions with the XU-TU DOD Prostate Cancer program. Examples of the resulting synergy will be the cancer research symposia, group training activities such as attendance to seminars and TCC focal group meetings, research collaborations and perhaps even sharing TCC mentors that support both the breast and prostate training programs. We also expect to start interaction between XU students involved in both cancer training programs, will encourage student training between the two programs and will include student presentations in the symposia. In Y2, we will also explore the potential for interacting with other HBCUs that have DOD cancer grants to develop a program where students from HBCUs doing cancer research spend a summer at another HBCU institution learning techniques and gaining experience.

**Year Two Challenges**

The top program priority of Y2 is to establish Dr. Wolfgang and an XU Pharmacy clinical faculty with a TCC mentor to develop a project. The challenge in this process is two fold. Finding the right TCC faculty that is not already overloaded with research and academic responsibilities and then finding the time in the heavily loaded schedule of the clinical faculty so they can develop a project. In Y2 we expect to increase the number of XU students working on the cancer projects. Drs. Wang, Wolfgang and Wiese will pick up MARC and RISE students in the summer and fall semester. Dr. Wolfgang presents a summer program at XU for RISE students where they learn basic molecular techniques. Through this process, he will gain good rapport with the RISE students which should result in an easy transition for the students into research projects. We hope to get XU pharmacy students involved in this program through clinical faculty who develop projects. The two primary research projects of this program must continue to develop capacity for their projects. Dr. Wang will develop a closer interaction with his mentor Dr. Burow through more frequent meetings and focal group research presentations so he can learn more cancer biology and Dr. Burow can learn more about mass spectrometer capabilities. Dr. Wiese must continue training his new research assistant so that his project will stay on schedule. Drs Wiese and Klassen will work closer with the TCC and LCRC in an effort to get the cancer research focal groups and works shops running on a continual basis. We feel this is critical to the success of the training aspects of the program. Release time for research is a problematic issue for XU faculty in the College of Pharmacy. While XU College of Arts and Sciences faculty have a well defined formula where percent effort on a research project translates into reduced teaching load or summer salary (with no teaching), faculty in the college of pharmacy maintain their same teaching load (<50% that of Arts and Sciences faculty) regardless of research funding. Thus, the load for Dr. Wiese was the same in the 2004-2005 academic year as it was in 2003-
2004 even though in 2004-2005 he was supported 40% by the DOD Breast Cancer grant for PI and research responsibilities (dedicated 40% of his time). In a normal year, this would not be a problem. In Y1 of this program, Dr. Wiese lost an experienced research assistant and had to search for and train a new person in the lab. This situation with faculty load and release time in the XU College of Pharmacy is also a hinderance for recruiting clinical faculty to develop a cancer research project. The administration of the XU College of Pharmacy is planning to develop a faculty load policy in the near future.

References
NA

Appendices
Xavier LCRC Recover Grant p. 19
Hanh Nguyen CV p. 22
Sergio r. Alcantara CV p. 24
P20 Planning Grant p. 29
Title: Louisiana Cancer Research Consortium – Immediate Response Program
Sponsor: Louisiana Cancer Research Consortium (LCRC)

PART I: Investigator Information

Name: Thomas E. Wiese

Applicant Organization: Xavier University of Louisiana

Position Title: Assistant Professor of Biochemistry

Department/Division: Division of Basic Pharmaceutical Sciences, College of Pharmacy

Cancer Program: Molecular Signalling

Applicant Eligibility: (please indicate)

X LCRC member with existing extramural funding

☐ LCRC member with funding that LCRC has granted, e.g., awarded bridge or seed funds

☐ A new recruit to whom LCRC has pledged support for an initial period

Title and Funding Source of Project(s) Relief/Assistance is Sought:

Developing a Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer; DOD W81XWH-04-1-0557

Specify Any Overlap With Other Funding Requests:

No Overlap with other funding requests.
PART II: Program Leader Review & Comment

Cancer Program: Molecular Signalling

Program Leader: Steven Hill

Score (1-10): (10 being the highest): ____________________________________________

Comments on Merit & Program Relevance:
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
PART III: Narrative, Budget & Budget Justification (2 page max.)

Introduction
The DOD funded project “Developing a Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer” awarded in April 2004 is part of a concerted effort to develop cancer research capacity at Xavier. This initiative also includes DOD Prostate Cancer (awarded February 2004) as well as a DOD Ovarian Cancer (under review) training programs that involve mentors from the LCRC. All three of these programs are linked to the LCRC where established cancer researchers from the Tulane Cancer Center are serving as research mentors for Xavier faculty. The Breast and Prostate programs at Xavier each include two research projects and the development of two new projects through the funding period. Thus, these programs have the potential for establishing eight to ten cancer research projects at Xavier that would each be part of the LCRC community. This proposal addresses replacement supplies needed to restart Year 2 of the Xavier-Tulane DOD Breast Cancer program.

Tasks and Aims
- **Task 1** Complete two substantive research projects of high relevance to eradication of breast cancer
- **Task 2** Assist two Xavier junior faculty to become more competitive in breast cancer research
- **Task 3** Establish infrastructure that will create an environment that fosters breast cancer research

The support requested in this proposal addresses only the research projects underway in Task 1. Research projects under Task 2 are in development and require no supplemental funds.

Research Project 1
“Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses”, Guangdi Wang, Ph.D., Department of Chemistry, Xavier University of Louisiana PI (Trainee); Mathew E. Burow, Ph.D., Department of Medicine, Tulane University School of Medicine (Mentor)

**Aim 1:** To demonstrate the requirement for and the role of the MEK5 pathway in survival signaling and suppression of apoptosis in MCF-7 breast carcinoma cells (Burow Lab).

**Aim 2:** To characterize differences in protein expression between MCF-7N (APOP-Sensitive), MCF-7M (APOP-Resistant) and ZR-75-30 breast carcinoma cells and identify anti-apoptotic proteins, such as Survivin, within MEK5-expressing cell lines (Wang Lab).

**Aim 3:** In this task we will use RNA interference strategies to validate a role for the Erk5 pathway in downstream gene expression and in suppression of chemotherapeutic drug-induced apoptosis (Burow and Wang Labs).

We have established that expression/activation of the MEK5-Erk5 pathways promotes enhanced tumor growth/formation and progression to hormone-independence of in vivo breast tumors in immunocompromised mice. MEK5 cells were capable of tumor formation in the absence of exogenous estrogen while the ER-positive VEC parent MCF-7 cells were unable to form tumors without estrogen, demonstrating MEK5’s role in progression to a more malignant hormone-independent phenotype. The ability of MEK5 to lead to both an anti-estrogen resistant phenotype in vitro and a hormone-independent phenotype in vivo in many ways parallels what is observed in advanced/recurrent ER+ breast carcinoma’s progression to therapeutic resistance. We have developed and optimized working protocols for i) preparation of protein homogenates from cultured breast carcinoma cells; ii) isoelectric focusing of the protein homogenates in an immobilized pH-gradient gel for the first dimension separation, iii) SDS-PAGE second dimension electrophoresis, and iv) gel imaging. Work is underway to identify protein spots that can yield meaningful information on differential protein expression (both qualitative and quantitative) between the MCF-MEK5 and MCF-VEC cell lines as well as the development of methods for spot cutting, trypsin digestion, and peptide separation and identification by HPLC-MS/MS. We have further established that shRNA targeting of Erk5 abrogates the ability of MEK5 to enhance tumor growth through the use of RNA interference (RNAi) through either transient transfection of siRNA or through the use of PolII driven RNAi expression constructs to stably ablate/suppress expression of specific genes. The ability of MEK5 expression to enhance tumor formation in immunocompromised mice is suppressed by RNAi-mediated ablation of Erk5 expression.

Project 2
“Interactions of estrogen and progestin active environmental chemicals on BC cell proliferation, survival and gene expression”, Thomas E. Wiese, Xavier University College of Pharmacy PI (Trainee); Steven R. Hill, Tulane University School of Medicine (Mentor).

**Aim 1:** Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival (Wiese Lab).

**Aim 2:** Conduct cDNA microarrays to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones. Preparations from cells grown and exposed to mixtures of hormone active pesticides in the Wiese Lab will be evaluated for differential expression of genes in the Tulane Center for Gene Therapy (Wiese Lab and LCRC Cores).

**Aim 3:** Confirm the expression of gene pattern identified by microarray through analysis of gene products (mRNA or protein) (Wiese Lab).
We have observed a positive sensitizing or potentiation effect of organophosphate pesticides on the weak estrogen dependant proliferation activity of organochlorine pesticides (increased potency). The organochlorine compounds in the study are considered persistent contaminants with long elimination half lives. Thus, chronic exposure to low concentrations may have more estrogenic activity than would be expected if cells are sensitized or stimulated by periodic exposure to organophosphate pesticides. Contamination from older pesticides that are no longer used might be more significant if one is also exposed to current use pesticides. Year 2 activities will include completing the matrix of pesticide combinations in the proliferation and estrogen regulated reporter gene assays to include all combinations of concentrations. In addition, ER binding assays will be used to determine if mixtures have effects on compounds binding to receptor. Mixture combinations that produce the most dramatic sensitization effect in the breast cancer cells will be selected for microarray analysis in Aim 2.

**Losses to Projects 1 and 2 from Hurricane Katrina**

Storm related events such as wind and floods shut off power to the Wiese and Wang labs at Xavier and the Burow lab at the Tulane Medical Center. While none of these labs were structurally damaged by the storm (no broken windows or flooding), each lab suffered the total loss of refrigerators and freezers. All perishable lab supplies, kits and samples were lost along with many chemicals that are not stable long term at room temperature or higher. Cell cultures in the Wiese and Burow labs were stored in liquid nitrogen cryo tanks. Each set of tanks from both labs was removed to a safe location in September and all are currently being maintained. Thus, this request for support addresses only perishable supplies and kits to support cell culture experiments and 2D gel analysis and does include the purchase or procurement of cell lines.

**Budget**

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<th>Item</th>
<th>Cost</th>
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<tr>
<td>General Chemicals, Reagents and Molec Bio Kits</td>
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<tr>
<td>Tissue Culture Reagents and Supplies</td>
<td>$11,000</td>
</tr>
<tr>
<td>2D Gel Reagents and Supplies</td>
<td>$5,000</td>
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<tr>
<td><strong>Total Budget</strong></td>
<td><strong>$25,000</strong></td>
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**Budget Justification**

A sum of $9,000 is requested for **General Chemicals, Reagents and Molec Bio Kits** to restart this projects research activities in the Wiese, Burow and Wang labs. In the Burow lab, $3,000 is requested for obtaining new MEK5 antibodies as well as Erk5 siRNA. In the Wang Lab, $2,000 is requested to replace protein assay kits and standards as well as buffers, ampholytes and RNAse and DNAse. In the Wiese lab, $4,000 is requested to replace all steroid and pesticide standards, obtain new recombinant ER kits used in receptor binding assays obtain new luciferase assay reagents used for reporter gene assays.

A sum of $11,000 is requested for **Tissues culture Reagents and Supplies** required to restart cell culture activities in the Wiese and Burow labs. Funds are requested to purchase fetal bovine sera and dextran coated charcoal treated fetal bovine sera as well as to obtain DMEM media, HBSS and DPBS buffers, trypsin, antibiotic solution, nonessential amino acids, l-glutamine, sodium pyruvate, insulin, etc.

A sum of $5,000 is requested for **2D gel Reagents and Supplies** used in the proteomic characterizations done in the Wang lab. Items that must be replaced include: IEF and SDSPAGE standards, Silver Stain kits, IEF ready strips and PAGE gels.
Scientific Executive Committee Review Proceedings & Outcomes

Committee Comment:

Final Disposition:
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
David E. Wolfgang

POSITION TITLE
Assistant Professor of Chemistry

eRA COMMONS USER NAME

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<tr>
<td>The Pennsylvania State University, State College, PA</td>
<td>B.S.</td>
<td>1987-90</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>Cornell University, Ithaca, NY</td>
<td>Ph.D.</td>
<td>1993-98</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>State University of New York at Stonybrook, Stonybrook, NY</td>
<td>Post. Doc.</td>
<td>1999-2002</td>
<td>Chemistry</td>
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NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions
2002-Present  Assistant Professor, Chemistry Department, Xavier University of Louisiana, New Orleans, LA.
1999-2002     Postdoctoral Research Fellow, Chemistry Department, State University of New York at Stonybrook, Stonybrook, NY
1993-1998     Graduate Student/Assistant, Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY
1990-1993     Laboratory Technician, Johns Hopkins School of Medicine, Baltimore, MD

Honors
Member: American Chemical Society

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.


C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

Ongoing Research Support

Active

None

Pending

None

Planned

None
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Charles A. Miller III

POSITION TITLE
Associate Professor

EDUCATION/TRAINING

<table>
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<th>INSTITUTION AND LOCATION</th>
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<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<tbody>
<tr>
<td>Univ. Alabama in Birmingham, Alabama</td>
<td>B.S.</td>
<td>1976-81</td>
<td>Biology</td>
</tr>
<tr>
<td>New York Univ., NYC, NY</td>
<td>Ph.D.</td>
<td>1985-90</td>
<td>Environ. Oncology</td>
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Employment / Experience

1979-1981: Laboratory Technician with Dr. Roy Mundy (anti-malarial drug toxicity) and Dr. Awni Sarrif (polycyclic aromatic hydrocarbon toxicity), Pharmacology Dept., University of Alabama in Birmingham, AL
1981-1982: Research Technician with Dr. Charles Balch and Dr. Toru Abo (function and origin of natural killer cells), Wallace Cancer Center, University of Alabama in Birmingham, AL
1983-4: Research Technician with Dr. Michael Brattain (colon cancer cell biology and biochemistry), Pharmacology Dept., Baylor College of Medicine, Houston, TX
1985-90: Graduate student with Dr. Max Costa (genotoxicity of metals), Environmental Medicine Dept., New York University, NY
1990-4: Postdoctoral fellow with Dr. David Kowalski (DNA replication), Molecular and Cellular Biology Dept., Roswell Park Cancer Inst., Buffalo, NY
1994-2000, Assistant Professor (Ah receptor signaling), Environmental Health Sciences Dept., Tulane Univ. School of Public Health and Tropical Medicine, New Orleans, LA
2000-present, Associate Professor (role of chaperones in Ah receptor signaling), Environmental Health Sciences Dept., Tulane Univ. School of Public Health and Tropical Medicine, New Orleans, LA

Awards and Honors

Shell Oil graduate fellowship recipient, 1988-90
Delta Omega, Public Health Honor Society, elected 1999 and member to date.
Member of Society of Toxicology’s Committe on Diversity Initiatives

Relevant Publications


32. A. Alnafisi, J. Hughes, G. Wang, and C.A. Miller III, Evaluating polycyclic aromatic hydrocarbons and environmental samples for human aryl hydrocarbon receptor activation using a yeast bioassay. in revision, Env. Toxicol. Chem. 2006


**Current Research Support**

NIH/NCI Project number: 1R33CA101622-01A2, C.A. Miller, P.I., Project period 9/1/04 to 8/31/07. Title: “Evaluating toxicity in humanized yeast.” (50% effort)

NIH/NCI COBRE grant, P. Deininger, P.I., with C.A. Miller as co-investigator, Project period 9/30/04 to 6/30/09, Title: “Mentoring a Cancer Genetics Program.” (10% effort)

LA State Board of Regents, C.A. Miller, Project period 2/1/06-1/31/07 “Phenotype of a p23 null mouse” ($10,000 for supplies, 0% effort).

**Recent Support**

Cancer Association of Greater New Orleans, C.A. Miller III, P.I. Dates of Project 4/1/03-5/31/04 Title: Generation of a p23 null mouse. Major goal of this project is to breed chimeric mice and establish a p23 null mouse strain.

NIH Project Number: R29ES09055, C.A. Miller III, P.I., Dates of project: 7/98-6/03 (no cost extension through 6/04) Title: Aryl hydrocarbon receptor structure and interactions. Major goals of this project are to elucidate the effects and interactions of chaperones on Ah receptor signaling. Project Number: NIH S11ES09996, R. Blake, P.I./ with T. Wiese and C.A. Miller as co-Investigators, Dates of project: 8/99-7/02, Title: General method to clone and characterize steroid receptors from...
wildlife species. Major goals of this project are to clone estrogen receptors from fish and characterize their binding and activation by xenobiotics.
Stephanie M. Colbert

2915 Laurel Street • New Orleans, LA 70115 • Home: 895-5590 • Work: 520-5326
scolbert@xula.edu

EDUCATION

University of Minnesota, Minneapolis, MN
1992, Bachelor of Arts, History

Additional Education:
1987 Fall Semester, University College Dublin, Dublin, Ireland
Courses in Irish Studies

2000-2002, Tulane University College, New Orleans, LA
Web Site Development

EXPERIENCE

February 2006 – present Program Assistant
Xavier University of LA, New Orleans, LA 70125
National Cancer Institute P20 Cancer Research and Education Planning Grant
and Department Of Defense Cancer Training Partnership Grants

Grant Management for NCI grant and both DOD grants (breast cancer and
prostate cancer), which includes budget management and procurement of
program supplies and equipment (using Xavier’s Banner system);
preparing minutes of committee meetings; maintaining all program
records; and organizing and scheduling all program related events
including meetings, workshops, and retreats.

March 2001-February 2006 Senior Program Coordinator
Tulane University Health Sciences Center, New Orleans, LA
Clinical Research Curriculum Award Program

I was responsible for initiating, managing and coordinating the CRCA
program. Part of my duties included coordinating and maintaining course
curriculum in conjunction with the School of Public Health to assure
compliance with accreditation guidelines for the MPH in Clinical Research.
Additionally, I supervise the recruitment process from initial inquiry through
acceptance and graduation, and serve as academic advisor to all students. I
am involved in marketing the Program through the use of advertisements,
brochures and web site maintenance. Utilizing the Tulane TAMS system, I
manage the accounts and budgets, order supplies, and initiate payroll
paperwork. I also schedule and coordinate the yearly Program Retreat as well
as monthly research seminars.
December 1994- March 2001

**Administrative Secretary**
Tulane University Health Sciences Center, New Orleans, LA
Department of Medicine, Section of Gastroenterology and Hepatology

In this position, I was responsible for the management of the departmental office, consisting of the Section Chief, seven faculty members, eight fellows, a section accountant and two study coordinators. A primary duty was coordinating the Section Chief’s calendar, balancing the time needed for the Chief’s clinical and administrative responsibilities. I also arranged the monthly On-Call and Attending schedules for both faculty and fellows, and coordinated the Junior Core Lectures, Pathophysiology and Case Conferences, and Journal Club, all of which were held monthly. I managed the Fellowship Program, which included monthly evaluations and rotation schedules, as well as ensuring compliance with ACGME regulations to maintain accreditation. I scheduled interview visits for potential faculty and fellows, including all travel arrangements and faculty meetings. I supervised students workers as needed.

January 1994- August 1994

**Principal Secretary**
University of Minnesota Medical School, Minneapolis, MN
Cardiovascular Division, Section of Electrophysiology

The scope of my duties included organizing and initiating all current drug and device studies, including the necessary paperwork and forms to ensure compliance with Food and Drug Administration requirements as well as Internal Review Board requirements. I maintained dated flow sheets for all regulatory documents, submissions and protocol amendments. I was responsible for the storage and inventory of the drug supply. This position included extensive word processing tasks for up to 15 simultaneous studies, as well as the needs of five faculty members.

REFERENCES AVAILABLE UPON REQUEST