Personal Statement

My name is Matt Lordo and I am a biomedical science major at the Ohio State University. Since my first day walking into a human cancer genetics lab, I have known that I want research to be a part of my life. Being involved in research as an undergraduate has enriched my college experience as it offered me the connection between years of science classes and real-world applications that can ultimately save lives. The inherent sense of discovering the unknown greatly appeals to me, which is why I would like to pursue research and medicine as a profession. Having the financial means to conduct this research will expedite my scientific career and will hopefully allow me to conduct research in graduate school and in my professional life.

The field of research is an ever-growing field that continues to grow in complexity with each new discovery. Collaboration is an intrinsic part of any research project, so it is important for me to contribute my findings and knowledge to other researchers who may be able to draw connections to other fields of research. In this way, one finding can lead to many discoveries across all fields of research. Having financial support through the STEP program will help me conduct this research and could provide me with transportation expenses to travel to research forums across the country. Research forums provide an excellent opportunity to talk with other researchers in the field and these connections can lead to long term professional relationships.

Working in a research lab has taught me many important skills, both experimental and personal. Not only is learning techniques and assays such as Real-Time Polymerase Chain Reaction and Western blotting beneficial, but learning how to manage my time and developing a strong work ethic have proven equally important to me. Balancing three different experiments in the lab while simultaneously being enrolled as a full-time honors student has made me manage my time as efficiently as possible. Being involved in research has also increased my analytical abilities and has made my thought process about answering the unknown much more scientific. This has proven beneficial in many of my classes at Ohio State by allowing me to think critically about the material and the mechanisms that underlie it.

After my undergraduate career, I hope to pursue an MD/PhD with a focus on pathology and cancer biology, which will undoubtedly demand time management skills. I became interested in pursuing an MD/PhD after realizing that I enjoyed interacting with patients through volunteering at the medical center and then going to lab and working on research that will ultimately benefit the same patients. Physicians and researchers are not the result of a dichotomy in the medical field, rather they are collaborators working towards a similar goal; the physicians work on present patients, while the researchers work on treatments to prevent future patients from facing the same ailments.

Hopefully I have shown how dedicated I am to the field of research and how funding from the STEP program will help me be able to continue this important research by offering me financial stability for my project. By performing this important research, I will hopefully be able to present at forums that will also allow me to network with other cancer researches and provide a group of professionals who can mentor and support me throughout my professional career.
Fifty Word Summary

My STEP fellowship will consist of a research project that aims to elucidate the cause of breast cancer. Loss of tumor suppression in breast tissue increases tumor growth rates. The STEP stipend will be used to fund this project and I hope to present my findings in journals or conferences.
Project Proposal – Matthew Lordo

Project Title: Elucidating Tumor-Stroma Communications in Breast Cancer
Project Advisor: Dr. Sarmila Majumder
Goal: To identify novel targets leading to loss of stromal PTEN in cancerous epithelial cells. These novel targets may serve as therapeutic options for breast cancer patients.

I have chosen to pursue the undergraduate research experience for my STEP fellowship. This fellowship will help me carry out the following project to identify epithelial soluble factors that lead to loss of PTEN in fibroblasts, which has been shown to catalyze tumor growth. Also, I am currently taking a lab technique class as part of my major (BMS 2900H) and will be taking a class next semester (BMS 3891H) that focuses on writing and preparing for research presentations which will help me carry out the proposed project using the proper assays and analyses.

Methodology and Anticipated Results

Human mammary fibroblasts will be cultured in media that has been conditioned by cancerous mammary epithelial cells and will then be analyzed to determine changes in PTEN mRNA and protein levels. A panel of breast cancer cell lines was obtained from the ATCC and consists of: MCF10A (normal mammary epithelial cell line), MCF7, SK-BR-3, SUM159, MB231, MB468, T47D, BT474, and ZR5.1. Preliminary data and experiments have shown that media conditioned by SK-BR-3 cells downregulates PTEN protein levels in fibroblasts while conditioned media (CM) from MB468 cells had little effect. Furthermore, there was no significant change in PTEN mRNA levels for either treatment group. These data suggest that we will be able to segregate the cell lines into two groups after we collect data from additional cell lines: 1) cells that can knockdown PTEN in the stromal fibroblasts and 2) cells that have no effect on PTEN in the fibroblasts. Further analysis of CM from additional cell lines will help to categorize the cell lines into these groups.

Two million epithelial cells will be seeded onto a 100 mm cell culture plate in 8 mL of complete DMEM with 1% fetal bovine serum (FBS) and will be incubated for two days. The media will then be filtered using a 0.45 µm filter and diluted 1:1 with DMEM containing 1% FBS (Conditioned media, CM). Approximately 50,000 “Normal” fibroblasts will be seeded per well in a 6 well plate, and there will be two wells per fibroblast cell line to assess RNA and protein expression. As very little is known about the heterogeneity of stromal fibroblasts from patients with different subtypes of breast cancer, the experiment will be carried out in duplicate using at least 10 different fibroblast cell lines from patients with different breast cancer subtypes (2 from TNBC patients, 2 from ER+/PR+ patients, 2 from ER+/PR- patients, 2 from ER+/PR+/Her2+ patients and 2 from ER-/PR-/Her2+ patients) to see if they respond similarly to conditioned media from breast cancer cell lines. If there is a variable response between fibroblasts of the same subtype, additional fibroblast cell lines of that subtype will be tested to determine if there is a correlation between that subtype and the effects seen on PTEN after CM treatment or if it is variability among the fibroblasts themselves that leads to the different results.

Fibroblasts will be cultured in the CM for seven days. Each cell line will also have a control sample cultured in DMEM with 1% FBS for seven days. One well of each cell line will undergo RNA isolation while the other well will be used to make whole cell extract (WCE) for protein analysis. RNA will be isolated using TRIzol® reagent and reverse transcribed into cDNA.
using the Invitrogen SuperScript® III Reverse Transcriptase kit. The WCE will be made using Radio-Immunoprecipitation Assay (RIPA) buffer containing protease and phosphatase inhibitors. PTEN protein levels will be determined by western blot analysis using PTEN antibody and normalized to a GAPDH loading control. If a reduction in PTEN protein is observed in the CM treated fibroblasts compared to the control fibroblasts (1% FBS-treated), the corresponding cDNA will be tested for PTEN expression and normalized to GAPDH expression using RT-qPCR to see whether there was a reduction in PTEN mRNA. If a reduction in PTEN mRNA is observed, it will be concluded that the CM is affecting PTEN at the transcriptional level. If there is a decrease in protein but not at the mRNA level, it will be concluded that factors in the CM are destabilizing PTEN at the protein level.

Following analysis of the conditioned media samples, the epithelial cells will be grouped together based on whether PTEN was knocked down or not. A sample of conditioned media from each epithelial cell line will be submitted for mass spectrometry analysis. The two groups will then be compared and soluble factors common to both groups will be eliminated from further study. Only factors unique or altered in the epithelial group that knocked down PTEN will be considered further. We will first focus on factors that are altered in the maximum number of epithelial cell lines. However, any factors that are unique in TNBC cell lines or Her2+ cell lines will also be investigated, as TNBC patients with or without HER2 positivity have a poor outcome and less treatment options compared to ER/PR positive patients. Furthermore, loss of stromal PTEN in TNBC patients predicts poor survival.

Once the tentative gene set that could potentially destabilize PTEN is determined, each gene will be systematically knocked down using the respective shRNAs in the epithelial cell lines that downregulated PTEN protein level in fibroblasts. The knocked down cells will then be used to generate conditioned media lacking the soluble factor. The fibroblasts expressing moderate levels of endogenous PTEN will then be cultured in the conditioned media for seven days. The fibroblasts will then be harvested for RNA and WCE as before. It is expected that PTEN levels in the fibroblasts will remain unaltered after the conditioned media from knocked down cells were used to treat the cells, because the factors responsible for PTEN loss are depleted from the CM. These data can also be used to determine which genes play the most significant role in knocking down PTEN. It is likely that not all of the genes in the gene set will play an overtly important role in regulating PTEN in fibroblasts, where we might still observe PTEN downregulation after candidate gene knockdown in the cancer cells. However, for conditions where no PTEN mRNA or protein depletion is seen, it can be concluded that the knocked down gene plays a major role in stromal PTEN expression and stability—especially if the same results are seen across multiple fibroblast cell lines.

To confirm the role of the identified gene set in reducing PTEN levels in fibroblasts, these genes will be ectopically expressed in epithelial cells whose secretome does not contain the soluble factor of interest. We will use cDNA expression vectors to overexpress the protein. The cancer cells will be transfected with the expression vector for ectopic expression. It would be expected that CM from cancer cell lines where the candidate gene is upregulated, would reduce PTEN level, whereas there was no change seen before when the conditioned media lacked this factor. If knockdown of the gene shows stabilization of PTEN levels and upregulation of the same gene shows a loss of PTEN, it will be concluded that the soluble factor affects PTEN stability in stromal fibroblasts and will be further studied as a possible avenue to prevent tumorigenesis. This information will be important for focusing future studies.