

Kentucky Lung Cancer Research Program

Cycle 6 Grant Abstracts

<u>Principal Investigator</u>		<u>Grant Research Title</u>
Paula Bates, Ph.D.	UL	An Innovative Approach for the Discovery of Novel Therapeutics and Molecular Targets in Lung Cancer
J. Scott Bryson, Ph.D.	UK	Allogenic cell therapy of lung cancer
Donald Cohen, Ph.D., Alan Kaplan, Ph.D.	UK	Hypoxia-driven macrophage-mediated immunotherapy of lung cancer
Edward Hirschowitz, M.D.	UK	Identification of novel lung tumor proteins using tumor infiltrating B cells
Robert Mitchell, Ph.D.	UL	Prevention of lung cancer with embryonic stem cell vaccine
Martha Peterson, Ph.D.	UK	Modifying lung cancer apoptosis through alternative RNA splicing
Jamie L. Studts, Ph.D.	UL	Attitudes and beliefs regarding nicotine vaccines as treatment and prophylaxis for nicotine dependence
John Trent, Ph.D.	UL	Development of antimetastatic CXCR4 inhibitors
Brian Wattenberg, Ph.D.	UL	Identification of novel inhibitors of the oncogenic enzyme sphingosine kinase as potential anti-cancer therapeutics
John Yannelli, Ph.D.	UK	Combined immunotherapy and nonmyeloablative chemotherapy in the treatment of non small cell lung cancer: A Pilot Study

Principal Investigator: **Paula Bates, Ph.D., University of Louisville**

Research Title: An Innovative Approach for the Discovery of Novel Therapeutics and Molecular Targets in Lung Cancer

Aptamers are DNA or RNA oligonucleotides that can bind to specific proteins via recognition of their three-dimensional structure. Thus, they are mechanistically similar to therapeutic monoclonal antibodies, but may have certain advantages over antibodies, such as stability, non-immunogenicity, ease of manufacture and efficient in vivo clearance. The Principal Investigator (PI) and her colleagues recently discovered and developed an aptamer named AGRO 100 (also called AS 1411), which became the first anticancer aptamer to ever be tested in clinical trials. This drug is currently in Phase I clinical trials and preliminary results indicate that it has promising clinical activity with no serious side effects. The mechanism of AGRO 100 was initially unknown, but the PI discovered that it works by binding to a protein called nucleolin, which she subsequently showed is a novel molecular target that is expressed selectively on the surface of cancer cells. Based on this previous experience, the rationale behind this proposal is that a directed approach to identify oligonucleotides that bind specifically to lung cancer cells will likely lead to the discovery of other potentially therapeutic aptamers and new molecular targets. Aptamers can be identified by in vitro evolution techniques, such as SELEX, which involves iterative selection and amplification of oligonucleotides from a starting pool containing billions of different sequences. Usually, this is applied to select for sequences that bind to a specific protein, but in this case, we propose to use it to select aptamers that can bind to lung cancer cells but not to non-malignant lung cells. Once we have identified the aptamers, we will characterize their properties, including structural characteristics and biodistribution in mice bearing lung cancer xenografts. Next, we plan to identify the molecular targets of the selected aptamers using a variety of molecular biology and mass spectrometry techniques, similar to those used to identify nucleolin as the target of AGRO100. The aptamers developed in this project could potentially be used to treat and/or detect lung cancer, whereas the discovery of new molecular targets may ultimately lead to novel small molecule therapeutics.

Principal Investigator: **J. Scott Bryson, Ph.D., University of Kentucky**

Research Title: Allogenic cell therapy of lung cancer

Therapeutic strategies for non-small cell lung carcinoma (NSCLC) include the use of surgery, chemotherapy and radiation. Many NSCLC patients present with advanced disease that is not treatable by surgery and does not respond to chemotherapy or radiation treatment. An alternative option would be the use of allogeneic stem cell therapy (ACT). Allogeneic stem cell therapy involves the transfer of donor stem cells and immune cells into an immunosuppressed patient. Patients are immunosuppressed to prevent rejection of the donor graft. Immune cells in the donor have the potential to respond against transplantation antigens on the patient's tumor cells. A major complication of this procedure is graft-versus-host disease (GVHD). It has been shown that a beneficial anti-tumor response is associated with the development of GVHD. Allogeneic stem cell therapy has been successfully utilized to treat a number of malignancies, including solid tumors. This procedure has been shown to mediate the regression of renal cell carcinoma lung metastases. Even more interesting is a recent report that has demonstrated the elimination of NSCLC following ACT. With this in mind, we will utilize an animal transplantation model to test the central hypothesis that the allogeneic immune response that develops following ACT will be effective in controlling the growth of lung cancer. Experiments are designed to determine the timing of the development of ACT complications, including GVHD and idiopathic pneumonia syndrome in the lung. We will determine the effectiveness of the GVT response that develops after ACT against murine lung tumors. Finally, studies will be conducted that will identify the immune cells that mediate tumor killing in the murine ACT model. These studies will provide the basis for future development of this procedure to enhance the GVT response and reduce regimen related toxicities.

Principal Investigator: Donald Cohen, Ph.D., Alan Kaplan, Ph.D., University of Kentucky

Research Title: Hypoxia-driven macrophage-mediated immunotherapy of lung cancer

Lung tumors contain not only the tumor cells, but also a variety of normal cells whose function is to help the lung tumor grow. One of these normal cells is an immune cell, known as the macrophage. An important function of macrophages (M0) is to kill tumor cells, but within lung tumors, this critical function is blocked due to inhibitory factors released within the tumor. One of these inhibitory factors, IL-10, blocks the ability of macrophages to attack tumor cells and inhibits generation of immune responses that can effectively attack the tumor. Recent studies have shown that M0s in tumors of mice can be modified so that they can attack tumor cells and generate other anti-tumor immune responses. Importantly, these studies showed that a large percentage of the mice were cured of their tumors and continued to live tumor-free. Based on these observations, we propose to test a therapeutic model in mice with lung tumors to determine if this form of therapy can be adapted to the treatment of lung cancer. We will use bone marrow transplantation in which several genes are first inserted into the marrow cells before transplantation. The bone marrow cells will be induced to become M0s and then transplanted into mice with lung tumors, where they will travel to the lung tumor. The inserted genes will be turned on within the tumor where they will attack the tumor cells and help generate other immune responses that will also be able to kill the tumor cells. To be certain that these immune responses will work effectively inside of the tumor, we will also treat the mice by injection of a drug to block the activity of the inhibitory factor, IL-10. We will evaluate the effectiveness of this novel therapy by determining 1.) how efficiently the modified M0s travel to the tumor and activate their inserted genes; 2.) how effectively the immune system was able to generate immune responses against the tumor; 3.) whether mice can be cured of their lung tumors. The results of these studies will establish proof of principle for the potential of this novel form of lung cancer therapy to allow macrophages and other immune cells to attack lung tumor cells in tumor-bearing mice and to "cure" mice of their growing tumors.

Principal Investigator: **Edward Hirschowitz, M.D, University of Kentucky**

Research Title: Identification of novel lung tumor proteins using tumor infiltrating B cells

A multiple marker approach is a logical strategy to compensate for genotypic and phenotypic heterogeneity of lung cancer. Novel proteomic techniques are continually being applied to identify circulating proteins and expand the range of available lung cancer markers. We believe the antibody response to tumor antigens is a highly sensitive and specific indicator of the plasma proteome and provides a rational alternative to marker discovery. B cells that hone to the site of corresponding antigen (tumor-infiltrating B cells), are a self-selected tumor-specific subset of the patient's antibody repertoire. B cells isolated from fresh tumor and immortalized by Epstein-Barr Virus (EBV-TIB) are thus a concentrated and renewable source of tumor specific antibodies. Preliminary data shows how we have already identified four unique tumor antigens from limited screening of a phased-displayed tumor cell cDNA library with antibodies from cultured autologous EBV- TIB. This proposal describes our approach to library screening, protein identification, monoclonal EBV:TIB isolation, antibody / validation and finally the use of antigen-specific human monoclonal antibodies to test for circulating proteins present in NSCLC patient, but not normal, plasma samples. In context, we hypothesize an assortment of antigen-specific human monoclonal antibodies to previously unknown tumor proteins can identify and dramatically expand the number of available cancer biomarkers measurable in the peripheral circulation. Commercially available multiplex assay platforms such as Luminex, designed for multiple biomarker measurement, will allow rapid translation from discovery to diagnostic stages of project development. Data from this innovative discovery-phase proposal will confirm proof of principle and be incorporated into a more comprehensive NIH proposal.

Principal Investigator: **Robert Mitchell, Ph.D., University of Louisville**

Research Title: Prevention of lung cancer with embryonic stem cell vaccine

Our work is focused on prevention rather than therapy of lung cancer. It takes advantage of the well known antigenic similarity between tumor cells and early embryonic cells. Given this antigenic similarity, we reasoned that vaccination of mice with allogeneic embryonic stem cells (ESC) might confer cross-immunity against lung cancers. In support, we find that such vaccination, especially with GM-CSF as an adjuvant, is 80-100% effective in preventing the outgrowth of an aggressive lung cancer (Lewis lung carcinoma). This immunity is dependent on cytotoxic T lymphocytes because (1) splenocytes from vaccinated animals are very effective in killing cultured Lewis lung carcinoma cells and (2) in vivo depletion of CD8+ T lymphocytes completely abrogates the anti-tumor effects of vaccination. ESC vaccination also prevents lung cancer development in a murine model of carcinogen + inflammation-induced lung cancer. To advance these observations we propose to: (1) Evaluate the effectiveness of alternative ESC vaccination modalities (microspheres which deliver GM-CSF instead of STO fibroblasts producing the cytokine; irradiated vs. live ESC) on prevention of the outgrowth of a transplantable cancer. (2) Using improvements from aim 1, determine the effectiveness of ESC vaccination against an established model of spontaneous, carcinogen-mediated mouse pulmonary carcinoma. (3) Determine the effectiveness of ESC vaccination against transgene-mediated mouse lung adenocarcinoma. (4) Identify ESC vaccine-induced immuno-reactive lung tumor cell antigens. The results may bring us closer to applying this concept of pre-emptive vaccination against lung cancer to humans at high risk.

Principal Investigator: **Martha Peterson, Ph.D., University of Kentucky**

Research Title: Modifying lung cancer apoptosis through alternative RNA splicing

Lung cancer currently causes the most cancer-related deaths nationally and is a particularly heavy burden for Kentucky. More effective treatment strategies are essential to better control this disease. Defects in apoptosis pathways contribute both to the formation of tumors and the frequent failure of anti-cancer treatments. While most tumor cells retain the molecular machinery required for apoptosis, they have an altered balance between pro-apoptotic and anti-apoptotic factors and thus survive signals that should induce death. Many apoptosis factor genes are alternatively spliced to encode gene products with opposite functions. Thus, changes in alternative RNA splicing regulation, an important mechanism of gene regulation known to be altered in cancer cells, is likely to be a contributing factor in altering cellular apoptotic responses. Previous work with the Bcl-x gene has shown that an oligonucleotide that enhances pro-apoptotic Bcl-xS and decreases anti-apoptotic Bcl-xL mRNA levels makes the cells more sensitive to chemotherapy agents and radiation treatment. This provides a proof-of-principle that targeting alternative splicing of apoptosis genes alters the sensitivity of cells to death induced by anti-cancer treatments. I propose that modifying the splice patterns of multiple apoptosis factors to enhance expression of their pro-apoptotic forms in lung cancer cells will sensitize the cells to anti-cancer treatments; a combination of effective oligonucleotides, used in conjunction with standard treatments for lung cancer, will enhance treatment of drug-resistant tumors and lower the effective treatment dose to be less toxic to the patient. To test this hypothesis, this grant will characterize the splice patterns and expression levels of a set of apoptosis genes in a panel of lung tumor cells and normal lung, design and test oligonucleotides to redirect splice patterns of target apoptosis genes, and test oligonucleotide-transfected cells for increased sensitivity to drugs and radiation. At the end of two years I plan to have identified a set of genes whose processing and/or expression can be altered with antisense oligonucleotides to sensitize lung cancer cells to anti-cancer treatments. This should generate sufficient preliminary data to support an ROI grant proposal to expand this work to include appropriate pre-clinical animal models and to move toward translation applications.

Principal Investigator: **Jamie L. Studts, Ph.D., University of Louisville**

Research Title: Attitudes and beliefs regarding nicotine vaccines as treatment and prophylaxis for nicotine dependence

Tobacco use is the most preventable cause of illness in Kentucky and has been linked with health conditions, ranging from cardiovascular disease to cancer. Despite the known risks, rates of tobacco use remain above 25% in Kentucky and 20% nationwide. Several tobacco cessation approaches have shown efficacy in helping motivated tobacco users quit smoking, including nicotine replacement, pharmacotherapy, and behavioral approaches. Yet less than 1/3 of participants in even the most rigorous tobacco cessation programs achieve long term abstinence, and there remains ample room for improvement in developing effective tobacco cessation interventions. Recent research has led to the development of nicotine vaccines designed for use in tobacco cessation and prevention by reducing or eliminating the reward value of nicotine. Investigators have reported tremendous success with nicotine vaccines in animal models, and preliminary reports of phase I/II clinical trial data have been encouraging. While nicotine vaccines present a novel approach to tobacco cessation, researchers and clinicians have also suggested that nicotine vaccines could be administered to children and adolescents to prevent the development of nicotine dependence. However, there are many significant clinical, psychosocial, and ethical factors that warrant consideration regarding the utility of these vaccines. The purpose of this proposal is to explore attitudes and beliefs regarding nicotine vaccines as treatment and prophylaxis for nicotine dependence. The first specific aim is to explore attitudes and beliefs of current tobacco users regarding the use of nicotine vaccines as treatment for nicotine dependence. The second specific aim is to explore parental attitudes and beliefs regarding the use of nicotine vaccines with children as prophylactic agents against the development of nicotine dependence. This proposed project includes focus groups to identify the important parameters relevant to the potential uptake of nicotine for treatment and preventive purposes. Based on focus group data, the project will develop and administer survey questionnaires and apply conjoint analysis procedures to identify the crucial factors that influence decision-making regarding vaccine utilization. This data will be used to educate clinicians regarding the crucial factors in counseling patients regarding this novel approach to tobacco control and insure informed treatment decision-making.

Principal Investigator: **John Trent, Ph.D., University of Louisville**

Research Title: Development of antimetastatic CXCR4 inhibitors

There is a severe lack of cancer drugs that work in late stage lung cancer, or for that matter late stage cancer as a whole. This is primarily due to the fact we can only treat the resulting symptoms not the cause. Unfortunately, by the time lung cancer is diagnosed 84% of it is in the later stages. There is a drastic need of drugs that inhibit metastasis, the actual mechanism for the spread of lung cancer. The overall goal of this proposal is to develop antimetastatic drugs based on our promising preliminary data. We have found lead compounds via structure-based drug design that inhibit chemotaxis and selectively bind to our target receptor, CXCR4. The hypothesis is that we can take our existing lead template compound and increase its anti metastatic ability via an interdisciplinary approach using structure-based drug design, chemical synthesis and biologically testing. We have found that it is the activated form of CXCR4, when targeted by in silico virtual screening, provided compounds that have experimental anti metastatic ability. Specific Aims. 1. To develop a full quantitative structure-activity relationship of our lead compounds and synthesize new derivatives for optimization of activity. We have discovered a series of compounds that inhibit the CXCR4-SDF-1 axis in in vitro chemotaxis assays. We will develop a full structure activity relationship to identify regions of the lead template that will be chemically modified to enhance the biological anti metastatic (chemotaxis) activity and selectivity for CXCR4. 2. To discover new antimetastatic lead compounds by targeting the CXCR4:Gai interface. We have successfully generated a structural model of the GPCR CXCR4 with its partner trimeric G proteins, Gai, and Gf3y. We will target the critical recognition site of Gai on CXCR4 with in silico virtual screening to identify compounds to be biologically tested. 3. To biologically test new compounds for antimetastatic potential and antagonist activity.

Principal Investigator: **Brian Wattenberg, Ph.D., University of Louisville**

Research Title: Identification of novel inhibitors of the oncogenic enzyme sphingosine kinase as potential anti-cancer therapeutics

Principal Investigator: **John Yannelli, Ph.D., University of Kentucky**

Research Title: Combined immunotherapy and nonmyeloablative chemotherapy in the treatment of non small cell lung cancer: A Pilot Study

The immunotherapy of cancer provides an alternative or adjuvant therapy for the treatment of cancer. Immunotherapy relies on the generation of a specific anti-tumor immune response. This response is generally mediated by either tumor specific CD4 helper or CD8 cytotoxic T cells (CTL) which home to the tumor bed and destroy disease. In addition, through cytokine release, the immune response can be enhanced and memory developed. Our laboratory has been utilizing a dendritic cell (DC) vaccine for the treatment of patients with all stages of non small cell lung cancer (NSCLC). Since 2003 we have delivered both prime and boost doses of autologous DCs pulsed with apoptotic bodies derived from an allogeneic lung cancer cell lines to 30 patients with the disease. All patients received both vaccine doses and immune assessment studies performed to date, have revealed that 14 of 21 patients evaluated possess lymphocytes which react to the vaccine as determined by gamma interferon ELISPOT analysis. The current proposal will extend this study to include the use of *in vitro* generated anti-tumor lymphocytes infused following a period of patient conditioning using the nonmyeloablative chemotherapy fludarabine and dexamethasone. The rationale is to increase specific T cell precursors using the DC vaccine. Following an *in vitro* step of expansion of T cells, patients are pre-treated with the chemotherapy to remove regulatory T cells (Tregs). Following chemotherapy the expanded T cell population will be infused with maintenance doses of IL-2 to insure T cell survival. We hypothesize that in the absence of Tregs, expanded T cell populations will infiltrate tumor nodules and cause tumor destruction. The protocol is written as a pilot study, 3 patients a year for 2 years. We anticipate developing the protocol and obtaining important clinical and laboratory data to provide preliminary results for an NIH submission in the fall of 2006 or spring of 2007. The PI has gathered a strong clinical research team which together with the strong existing laboratory team would predict a high likelihood of success.