

# Kentucky Lung Cancer Research Program

## Cycle 7 Grant Abstracts

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Principal Investigator: **Susanne Arnold, M.D., University of Kentucky**

Research Title: Early detection of lung cancer in a high-risk population defined by pulmonary function testing, biomarker identification, and computerized tomography scanning

Computed tomography (CT) screening is a highly promising tool with which to detect lung cancer in its earliest, and potentially most curable, stages. Well recognized risk factors for lung cancer include tobacco exposure, age, and the development of chronic obstructive lung disease. By screening a population of participants from Appalachian Kentucky who meet stringent risk-based entry criteria, this project proposes to increase the early detection rate of lung cancer in the population at highest risk for its development, thus increasing the yield of the screening test. We propose to define this higher risk population by requiring subjects to: a) be 55 to 75 years of age, b) have with poor pulmonary function (FEV1/FVC<70%) and c) exhibit excess tobacco exposure (> 40 pack years). Our hypothesis is that lung cancer screening using low-dose CT scans at a community level, in a population at high risk for lung cancer is valid, feasible and efficacious for early detection of lung cancer.

We also intend to describe the relative incidence of benign and malignant nodules found on screening CT scans and prospectively collect biological specimens necessary to discover and develop biomarkers for the early detection. We will do this through a collaboration with rural hospitals within southeastern Kentucky, where lung cancer incidence is amongst the highest in the country. The Marty Driesler Cancer Project is already an ongoing, community-based research initiative, with ongoing projects in lung cancer, esophageal and liver cancers. This proposal will continue the important work begun in by the Marty Driesler Project and ensure the success of CT screening in rural Kentucky. This is exactly the type of research called for by the National Institutes of Health, as this project has developed collaboration between basic, translational, and clinical investigators, community clinicians and clinical practices to develop an innovative project with practical application in a rural community setting.

Principal Investigator: **E. Penni Black, Ph.D., University of Kentucky**

Research Title: Dusp4 activity in non-small cell lung cancers: an opportunity for intervention?

Lung cancer is the leading killer of all cancers and Kentucky has the highest rate of incidence and mortality from this dreaded disease. Although lung cancer is the most preventable cancer, those who do not choose to stop smoking will likely become the population that most needs the best available treatment for lung cancer. It is important to acknowledge that great strides have been made in treatment options that extend the lifespan of those afflicted. However, five-year survival rates are very low. We propose that development of new therapeutics targeted to specific molecules important in the biology of non-small cell lung cancer is as important focal point of research at this time. As such, we've developed new methods to interrogate large sets of genetic data to identify new molecules that may be important in lung cancer biology and thus, potential new 'druggable' targets. In this proposal we will describe dissection of the function of one such molecule, Dusp4, which is a potential tumor suppressor gene in non-small cell lung cancer.

Principal Investigator: **Jason Chesney, M.D., University of Louisville**

Research Title: Targeting Choline Kinase in Lung Cancer

Metabolomic analyses of non-small cell lung adenocarcinomas have identified a marked increase in the metabolite phosphorylcholine relative to adjacent normal lung tissues. Choline kinase is activated in lung adenocarcinoma cells to catalyze the phosphorylation of choline into phosphorylcholine which is then converted via the Kennedy pathway to phosphatidylcholine, a metabolic reservoir for lipid second messengers required for survival and growth. In preliminary studies, we demonstrate that the introduction of oncogenic *ras* into immortalized bronchial epithelial cells causes a marked increase in choline kinase expression and the concentration of phosphorylcholine. Silencing of choline kinase using siRNA species attenuates this increase in phosphorylcholine which in turn reduces the lipid messenger phosphatidic acid and attenuates the anchorage-independent growth of *ras*-transformed bronchial epithelial cells in both soft agar and athymic mice. Taken together, these preliminary observations suggest that choline kinase may prove useful as a lung cancer-specific molecular target for the development of anti-neoplastic agents. *Our hypothesis is that high choline kinase activity is selectively required by lung cancer cells to provide a metabolic reservoir of lipid second messengers for ras signaling and tumorigenic growth.* We propose to test this hypothesis by pursuing the following two specific aims:

1. To examine the effects of stable shRNA silencing of choline kinase on the intracellular phosphorylcholine concentration, lipid second messengers, *ras*-dependent signaling, viability and anchorage-independent growth of normal, immortalized and *ras*-transformed bronchial epithelial cells.
2. To determine the effect of cre-induced genomic deletion of choline kinase on the growth and viability of established *ras*-dependent lung adenocarcinomas in mice.

We anticipate that shRNA silencing or genomic deletion of choline kinase will attenuate the neoplastic potential of *ras*-transformed bronchial epithelial cells without causing significant toxicities to normal bronchial epithelial cells. The tertiary structure of the human choline kinase protein was recently solved and in future studies we intend to identify small molecule inhibitors that interrupt the substrate-binding domain of choline kinase using computational screening.

Principal Investigator: **Anita Fernander, Ph.D., University of Kentucky**

Research Title: Examination of the Breathe Free for Women stop smoking program among African-American women: A Pilot Study

Lung cancer is the third most common cancer and the leading cause of cancer death among African American (AA) women and rates of lung cancer morbidity and mortality are continuing to rise within this population. Smoking is the primary avoidable cause of illness and death related to lung cancer and AA women bear a disproportionate burden. Abstaining from smoking is the single most important preventive health behavior AA women can engage in to reduce their chances of illness and death related to lung cancer. Standard smoking cessation programs designed for the general population have failed to show a significant impact on reducing smoking rates among AA women. Clinical practice guidelines suggest that smoking cessation programs are multimodal, intensive and relevant to the population being treated. The Breathe Free for Women (BFFW) smoking cessation program fulfills this criteria but has not been examined for its effectiveness among AA women. The short-term objective of this developmental pilot study is to examine the effectiveness of the BFFW program among AA women. The long-term objective of this study is to improve smoking cessation intervention efficacy and decrease smoking among AA women. Identifying whether this standard intervention is beneficial for AA women has both theoretical and applied implications for researchers and practitioners addressing the critical public health issue of reducing smoking among AA women and subsequent disparate rates of lung cancer.

Principal Investigator: **Ramesh Gupta, Ph.D., University of Louisville**

Research Title: Effect of estrogen on polycyclic aromatic hydrocarbon (PAH)-mediated lung cancer

Lung cancer is the leading cause of cancer-related deaths in the United States. Though cigarette smoking is considered the predominant cause of lung cancer, only one in 12 women develop lung cancer based on latest account. These data suggest that some endogenous factors play a vital role in one's susceptibility to this disease. Epidemiological data indicate that female lung cancer related mortality is on the rise, partly due to increase in smoking and partly due to as yet undefined factors. Estrogen has been suspected to contribute to lung cancer in females, but there is no clear evidence (Berge et al., 2004). Exposure to cigarette smoke induces cytochrome P450s (CYPs), primarily, CYP1A1 and 1B1, which are key isozymes in metabolism of the main female estrogen, 17 $\beta$ estradiol in extrahepatic tissues, including lung. We hypothesize that overexpression of CYPs by exposure to carcinogenic dibenzo[a,l]pyrene (DBP) present in the human environment, including cigarette smoke will elevate redox-active estrogen metabolites, particularly potentially 4-hydroxy metabolites, in the target (lung) tissue, leading to increased oxidative DNA damage and promote DBP-mediated lung cancer. An established DBP-mediated A/J mouse lung tumorigenesis model will be used to test our working hypothesis. The hypothesis is supported from our preliminary data in which many known metabolites of 17 $\beta$ -estradiol were detected in the lung tissue of untreated female A/J mice, and that cigarette smoke exposure which induced CYP1A1 and 1B1, resulted in the accumulation of 4-hydroxy metabolites. The following two specific aims will be pursued to meet our objectives: Aim 1. To determine effects of 17 $\beta$ -estradiol, DBP and their combination on DBP-induced DNA adducts, estrogen metabolites and estrogenmetabolizing enzymes in the lung tissue of A/J mice. Aim 2. To determine if tissue DNA adducts and estrogen metabolites associated with co-treatment with 17 $\beta$ -estradiol and DBP can be diminished by inhibition of P450s. Tissue DNA adducts, estrogen metabolites and selected P450s will be measured by established <sup>32</sup>P-postlabeling, GC-MS, and real-time PCR/immunohistochemistry, respectively. Data from this pilot project will reveal the effect of tissue metabolites of estrogen metabolites in environmental PAH-mediated lung cancer model. Preliminary data generated in this project will set ground to apply for a major NCI grant to determine effect of estrogen on PAH-mediated lung cancer and its prevention.

Principal Investigator: **Claudia Hopenhayn, Ph.D., University of Kentucky**

Research Title: Lung cancer survival in Kentucky: A multifactorial approach

More people are at risk of developing or dying from lung cancer in Kentucky than any other state in the country. This disease is especially deadly, with very lung cancer patients surviving only two years after diagnosis. Although survival is largely determined by how early the disease is diagnosed, there are other factors that can also influence how long a patient survives, such as the risky behaviors and treatments related to a patient's lung cancer. Since modern medicine is getting better at detecting lung cancer early, these other factors could become even more important in trying to improve lung cancer survival. In order to determine which factors play an important role in surviving early stage lung cancer, we will review 5000 cancer cases from the Kentucky Cancer Registry (KCR). KCR data from 1998-2003 will provide data on early stage lung cancer patients for a study of previous cancer cases, their clinical profile, and their treatments. In addition, with the help of the Kentucky Lung Cancer Research Program's Clinical Trials Network (CTN), we will survey 200 newly diagnosed cancer patients in the early stages of disease. Lung cancer patients will be recruited at seven or more CTN-affiliated hospitals, representing multiple regions of Kentucky, and will be surveyed with respect to their smoking history and habits, family history of lung cancer, job history, and whether they may have come into contact with substances known to cause lung cancer. The responses will then be linked to KCR data for these patients to create a more comprehensive database than the KCR alone can provide. Finally, this comprehensive database will be used to determine which causes, treatments, and other factors most influence survival of lung cancer.

Principal Investigator: **Carolyn Klinge, Ph.D., University of Louisville**

Research Title: Mechanisms for Gender Differences in Lung Adenocarcinoma

The higher frequency of lung cancer in women than in men smokers implicates gender-dependent factors lung cancer etiology. Estrogens are the female sex hormones that play a critical role in breast and endometrial cancers, but little is known about estrogen action in lung cancer. Indeed, up until 1997, lung was not considered to be a target of estrogen. Estrogens work by binding to a protein called estrogen receptor (ER). In turn, ER binds to DNA and stimulates production of messenger molecules that make cells divide. There are two types of ERs in cells: ER $\alpha$  and ER $\beta$ . Although a few people have examined ER $\alpha$  and ER $\beta$  expression in human lung cancer, but the results are mixed. We took a different approach and studied not only ER expression, but if estrogen would stimulate human lung adenocarcinoma cells to divide. Our results were that estrogen caused lung cancer cells from women, but not men, to grow and that the drug tamoxifen, which is used clinically to treat breast cancer, blocked estrogen-stimulated lung cancer cell growth! The present set of experiments is a follow-up study to determine how estrogens cause lung adenocarcinoma cells to grow and how antiestrogens block this growth. We have 4 overall Specific Aims which are goals of the proposed experiments: 1) Determine if chimeric estrogen receptors (ER) ER $\alpha$ -VP16 and ER $\beta$ -VP16 activate estrogen-response element (ERE)-driven luciferase activity in transfected lung adenocarcinoma cells. 2) Identify estrogen-regulated genes in lung adenocarcinoma cells from females and males. 3) Determine the efficacy of E2- and ICI 182,780- Protac (Proteolysis Targeting Chimeric) molecules in inhibiting lung adenocarcinoma cell proliferation and estrogen target gene expression. 4) Determine if E2, sodium arsenite (NaAsO<sub>2</sub>), and cadmium chloride (CdCl<sub>2</sub>) rapidly activate non-genomic/membraneinitiated ER activation of MAPK activity in lung adenocarcinoma cells. Other investigators have demonstrated that cadmium and arsenite act like estrogens in female reproductive tissues, but no one has evaluated if they have estrogenic activity in lung. If they do, this could be a reason why women, smokers or non-smokers have twice the lung adenocarcinoma compared to male smokers. It is our hope that the successful completion of these experiments will define the molecular mechanisms of estrogen, cadmium, and arsenite action in lung adenocarcinoma and could provide rationale for clinical trials for antiestrogens to treat patients with lung adenocarcinoma.

Principal Investigator: **Charles Lutz, M.D., Ph.D., University of Kentucky**

Research Title: Differential Expression of NKG2D Ligands in Airway SCC

Cigarette smoking induces cancers in the lung and upper airways. Among lung and upper airway cancers, squamous cell carcinomas (SCC) are very prevalent and have a grim prognosis, especially after metastasis. Our long-term goal is to design treatments that stimulate killer lymphocytes to attack these cancers. SCC are recognized by the powerful killer lymphocyte receptor, NKG2D. Target proteins for human NKG2D, such as MICB and ULBP1, can activate killer lymphocytes. However, NKG2D target proteins also may play complicated roles in regulating the immune system. We have discovered that metastatic SCC resist human killer lymphocyte attack, but paradoxically make more of the MICB protein. Airway SCC selectively suppress the ULBP1 protein, but suppression is reversed by a new cancer treatment drug that inhibits an important cellular protein, the proteasome. We propose that MICB helps SCC to metastasize, whereas ULBP1 inhibits SCC growth. To test this idea, we will measure MICB and ULBP1 RNA in airway primary SCC tumors and lymph node metastatic tumors from the same patient, test whether MICB or ULBP1 changes SCC stimulation of killer lymphocytes, and test whether MICB or ULBP1 changes human SCC growth and metastasis in experimental mice. Based on strong preliminary evidence, we also propose that SCC specifically suppress ULBP1 and that drug-induced ULBP1 upregulation cannot be explained by current theories of immunity. To test this idea, we will identify how the ULBP1 gene is turned on and what genetic control regions are important. In our translational research, results with human biopsy specimens will lead to new ideas that will be tested in the test tube and in experimental animals. Our results will be highly significant, because they will help us understand how SCC escape immune destruction and metastasize to distant sites. Our research may lead to new drugs that make airway SCC more susceptible to immune attack.

Principal Investigator: **Haval Shirwan, Ph.D., University of Louisville**

Research Title: Modulation of Tumor Stroma for Immunotherapy

Therapeutic vaccines represent an attractive treatment modality for the management of cancer, primarily due to their specificity and ability to induce long lasting immunological memory that may prevent recurrences. However, the therapeutic potential of cancer vaccines remains to be realized partially due to the complex nature of interactions taking place between the immune system and cancer cells in the course of tumor progression. These interactions are primarily regulated by two opposing forces; immune mechanisms that target the tumor for destruction and tumor-mediated counter-mechanisms that enable the tumor to evade the immune system. The most significant immune evasion mechanisms are immune regulation imposed by tumor stroma serving as a physical and immunological barrier and tumor cell modulation of immunological signals critical to the generation of an effective immune response. However, tumor stromal cells also express various immunological receptors, such as HVEM for LIGHT, that can be targeted for modulation for the development of effective therapeutic cancer vaccines. The main objective of this proposal is to convert tumor cells into professional immune stimulatory cells and tumor environments into “pseudo” secondary lymphoid structures for the generation of effective anti-tumor immunity. This will be accomplished by engineering tumor cells with a novel and practical approach, designated as ProtEx™ that we recently developed, to display on their surface two immune stimulatory proteins, LIGHT and 4-1BBL, that are implicated in the regulation of lymphoid structures required for an effective immune response, recruitment of immune cells into these structures, and the activation and differentiation of these immune cells into effectors cells that not only destroy the tumor, but also establish a long-term immunological memory that may safe guard against recurrences. Proof-of-principle in a lung cancer model will facilitate the application of this approach to the clinic for the treatment of cancer.

Principal Investigator: **Peter Spielmann, Ph.D., University of Kentucky**

Research Title: Development of prenyl function inhibitors as therapies for lung cancer

Lung cancer is the number one cause of cancer-related death for both men and women in the United States and will kill approximately 4,450 Kentucky citizens this year. Substantial evidence points to the central role of proteins normally modified by a prenyl group in lung cancer progression. Mutated forms of the prenylated protein K-Ras are found in 20-30% of all non-small cell lung cancer (NSCLC). There are two types of prenyl groups, the farnesyl group attached to proteins by the enzyme FTase and the geranylgeranyl group attached by the related enzyme GGTase I. Prenylation is essential for proper function of the modified proteins and K-Ras is normally farnesylated. Farnesylation of Ras and other proteins is inhibited by a new class of anti-cancer drugs called farnesyltransferase inhibitors (FTI) which reduce the growth of lung tumor cells in cell culture and animal models. However, FTIs have shown little efficacy as single agents in human lung cancer clinical trials. Proteins critical for cell survival, including K-Ras, evade the action of FTIs by becoming geranylgeranylated by GGTase I. Geranylgeranylated K-Ras is fully functional and GGTase I is not inhibited by FTIs. We propose to develop a novel class of K-Ras function inhibitors that would bypass the evasion of the FTIs and lead to new treatments for lung cancer. We seek to modify K-Ras in an FTase dependent fashion with unnatural prenyl groups that prevent K-Ras function. We have prepared and tested a variety of unique, drug-like molecules that hold great promise as lung cancer therapies.

Principal Investigator: **Jill Suttles, Ph.D., University of Louisville**

Research Title: Targeting TRAFs as an Anti-tumor Strategy

CD40 is a protein present on the surface of many types of normal cell types, including certain classes of white blood cells, as well as cells of the blood vessel wall and skin. CD40 acts as a receptor and transmits signals to the cell when it interacts with another protein known as CD154. The signals CD40 transmits often result in cell division or the production of inflammatory proteins. The cell surface expression of CD40 has also shown to be common to a number of tumor types, including those of lung, breast and ovary. CD40 expression on lung carcinomas has been shown to be correlated with metastatic spread and is considered an indicator of poor prognosis. Co-expression of both CD40 and CD154 on tumor cells allows for the continuous activation of CD40 signaling pathways that can promote tumor growth and metastasis. Thus, we hypothesize that blockade of CD40 signaling in tumors may promote tumor regression. CD40 cannot transmit signals to the cell on its own and relies on the use of “adapter proteins” known as TRAFs (TNF Receptor-Associated Factors). We have found that blockade of the TRAF-CD40 interaction with use of cell-permeable TRAF binding peptides (TRAFBPs) inhibits CD40 signaling events and reduces the cell division (proliferation) of a number of both human and murine carcinomas. Using mouse lung tumor cells that express both CD40 and CD154, we have shown that treatment of these cells (grown in tissue culture dishes) with TRAFBPs inhibits their proliferation as well as the production of proteins favorable for tumor growth and metastasis. The aims of this proposal are 1) to determine the efficacy of TRAFBPs in the prevention of tumor growth and metastasis in animals using the mouse lung carcinoma model, and 2) elucidate the precise impact of TRAFBPs on the intracellular signaling pathways coordinating cell proliferation and tumor promoting protein production in both the murine model as well as in human lung carcinomas.

Principal Investigator: **Douglas Taylor, Ph.D., University of Louisville**

Research Title: Exosomal microRNA in the Detection and Diagnosis of Lung Cancer

Poor early detection coupled with ineffective treatments for advanced disease is responsible for the low 5-year survival rates of lung cancer patients. Development of new diagnostic/prognostic markers would significantly enhance its early detection and improve survival. Emerging evidence has implicated altered microRNA (miRNA, small noncoding RNAs that regulate gene expression) regulation in lung cancer pathogenesis. miRNA profiling of tumors has identified signatures associated with the patient's clinicopathologic characteristics. While miRNA profiling has shown promise, its current application is limited to tissue biopsies. To have utility in diagnosis/screening, tumor-associated miRNA needs to be identified prior to clinical symptoms or a demonstrable mass. Our proposal to utilize circulating tumor exosomal miRNA as diagnostic markers is based on (1) the production and release of exosomes by tumor cells and (2) the presence of specific oncogenic miRNA associated with circulating tumor-derived exosomes (our preliminary data shows hsa-miR-21 and hsa-miR-155 association with circulating tumor exosomes). Our hypothesis is that identification of specific miRNAs associated with circulating tumor-derived exosomes are markers for diagnosis, staging, prognosis and response to treatment. To address this hypothesis, we will isolate and quantitate circulating exosomes from lung cancer patients at various stages and grades and compare with similar material isolated from patients diagnosed with benign lung masses. The miRNA associated with circulating tumor-derived exosomes from a small set of patients (n=10/group) will be profiled to define all miRNAs associated with circulating exosomes. The presence of specific miRNAs will be correlated with the presence of malignant disease. After identifying the specific miRNAs associated with exosomes, a larger set of patient specimens, representing different stages and outcomes, will be analyzed for specific miRNA by real-time PCR to determine the presence and level of these miRNAs and to correlate the specific exosomal miRNAs with clinicopathologic characteristics.

Principal Investigator: **Sucheta Telang, M.D., University of Louisville**

Research Title: Targeting 6-phosphofructo-2-kinase/fructose2,7-bisphosphate-4 in Lung Cancer

Lung cancer cells are found to need a sugar called glucose to grow more rapidly than normal healthy cells. This characteristic of lung cancer cells is used in a highly sensitive test called a PET scan which can detect and monitor lung tumors in the body. The increased use of glucose in the body is allowed partly by a group of enzymes named PFKFB 1-4. Until recently, one member of this family of enzymes (called PFKFB3) was believed to be the only enzyme responsible for the high use of glucose by many cancer cells. We have however surprisingly found that another enzyme from this group called PFKFB4 is over-produced by lung cancer cells and not the PFKFB3 as was previously thought. ***Our hypothesis is that PFKFB4 is required by lung cancers to use high amounts of glucose and therefore grow more rapidly than normal lung tissues.*** We want to know if PFKFB4 is necessary for lung cancer cells to use glucose since we can then develop drugs that will inhibit this enzyme and possibly the growth of lung cancer cells while leaving normal cells (that do not depend on glucose) unharmed. We plan to both over-produce and delete the PFKFB4 enzyme in lung cancer cells and then examine the effects on the ability of the cells to use glucose and grow as a cancer in a plate. We also intend to delete the PFKFB4 gene in mice and examine the effect on lung tumor growth. We predict that without PFKFB4 the lung cancer cells will not be able to use glucose and therefore not be able to grow as tumors. We hope that these experiments will help to explain the function of this enzyme in the utilization of glucose. Our ultimate goal is to use this knowledge to develop new drugs that target the special ability of lung cancer cells to use high amounts of glucose and therefore help to stop the growth of lung cancer in patients.

Principal Investigator: **Zhigang Wang, Ph.D., University of Kentucky**

Research Title: A transgenic mouse model for understanding Rev 1 and lung cancer

Cancer causing agents are called carcinogens. Many carcinogens can attack our genetic material called DNA. A result of such chemical attacks is the alteration of the normal DNA structure, called DNA damage. DNA damage can cause mutations. Some mutations can transform normal cells into tumor cells and eventually into cancers. Therefore, mutations form the foundation on which cancers are being built. In cells, the conversion of DNA damage into mutations requires several proteins including Rev1 and Rev3. Our laboratory has isolated the human REV1 gene, a process called gene cloning. Furthermore, several other groups and our group have demonstrated that the Rev1 protein plays a critical role in making mutations following exposure to carcinogens. Accordingly, we believe that Rev1 protein is a key player in lung cancer development. Furthermore, we believe that, if we can inhibit Rev1 function in cells, we might be able to prevent cancer to form in the first place. This is a novel idea of cancer prevention by inhibiting the Rev1 protein. That is, we would like to attack the foundation of cancer to make it impossible to build up. These are our long-term goals for research and development, which would require considerable financial support from the Federal government such as the National Institutes of Health (NIH). To be competitive for obtaining a major NIH funding, we will need to establish an animal model that is suitable for these research and development. Therefore, we propose to establish such an animal model through the support by the Kentucky Lung Cancer Research Program. The mouse model that we will produce utilizes a state-of-the-art biotechnology called conditional knockout transgenic mouse. Through this technology, we can mimic Rev1 inhibition by inactivating the mouse Rev1 gene. With this model, we can then ask whether Rev1 indeed plays a key role in lung cancer development and whether it is feasible to prevent cancer by inhibiting the Rev1 protein.

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

Research Title: Generation of cytotoxic T lymphocytes (CTL) against the cisplatin resistant phenotype displayed by non small cell lung cancer

Lung cancer is the leading killer due to cancer of men and women in the United States. Conventional therapies are limited in their ability to impact the disease. Patients treated with the chemotherapeutic drug Cisplatin, often re-occur even following what appear to be significant durable responses. Cisplatin binds to DNA and forms adducts, thus preventing DNA synthesis, transcription and eventual translation of critical cellular proteins associated with the neoplastic state. Resistant tumor cells, however, evolve mechanisms to: 1) prevent drug uptake into the cytoplasm, 2) increase drug efflux from the cytoplasm out of the cell, 3) immobilize drug in the cytoplasm, and 4) repair damage done by DNA adducts formed as a result of drug binding to the DNA. The current proposal suggests that the proteins that the tumor cells express to maintain the chemo-resistant phenotype can serve as targets for the immune system, specifically cytotoxic T lymphocytes (CTL). The PI has developed a unique model system of NSCLC tumor cell lines and associated clones which express both Cisplatin sensitive and resistant phenotype. The proposal will utilize the Cisplatin resistant tumor cell clones to generate CTL which can recognize and kill resistant tumor cells. The PI suggests a future immunotherapy strategy where-by patients are immunized using DC vaccines which express proteins associated with the chemo-resistant state. Following chemotherapy with the specific agent, the patients will be boosted with DC vaccines, thus increasing the CTL precursor frequency against those tumor cells which will eventually develop Cisplatin resistance. The current study is for 2 years and will provide the PI with critical "Proof of Principal" data that the hypothesis is well founded and feasible. The data generated will be used to submit an RO-1 and eventually utilized to develop new and innovative immunotherapies for patients with this deadly disease.