

Kentucky Lung Cancer Research Program

Cycle 1 Grant Abstracts

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Principal Investigator: **Mansoor M. Ahmed, Ph.D., University of Kentucky**

Research Title: TGF-beta signaling and radiation response in lung carcinoma

Lung carcinoma is the fourth leading cause of cancer mortality in the US, with more than 157,400 estimated deaths in 2001. The state of Kentucky has the highest incidence of lung cancer with highest estimated deaths of all cancers in 2001. Surgery, radiation therapy, and chemotherapy are treatment options, but generally have little influence on the outcome. The cure rate for 5-year (1950-2000) survival is between 5-20%. Gene expression studies revealed that there exists more than one pathway regulating growth inhibition and apoptosis processes. Of these, one pathway mediated through TGF- β signaling for negative growth regulation is pivotal in inhibiting tumor progression. Our recent preliminary experiments in non-small cell lung carcinomas showed that there exists aberrant expression of genes downstream to TGF- β pathway, particularly, in TGF- β type II (RII) receptor and DPC-4. In addition, we also observed that the loss of RII expression contributed to enhanced radiation resistance. Influence of aberrant TGF- β pathways on radiation treatment response remains to be understood. Based on our preliminary findings, we hypothesize that the dysregulation of TGF- β signaling due to lack of RII or DPC-4 expression will cause an impact on radiation response.

To test this hypothesis, we propose the following specific aims: a) determine the functional and regulatory role of TGF- β signaling on radiation-induced clonogenic inhibition and apoptosis in lung cancer cell lines harboring aberrant expression of RII and/or DPC-4; b) determine the basal and radiation-inducible expression levels of TGF- β effector genes, p21^{waf1/cip1} and Bax as a function of radiation dose by reporter assays, immunohistochemistry, Western blot, reverse transcription-polymerase chain reaction and RNase protection assay. Data obtained on TGF- β effector genes (basal and radiation-inducible) will be compared to those corresponding to clonogenic survival (analyzed by colony-forming assay) and apoptosis (analyzed by TUNEL and flow cytometry) profiles; and c) determine the incidence of dysregulation in TGF- β signaling genes such as RI, RII, RII and DPC-4 in paraffin-embedded and frozen specimens of lung carcinoma. In this way, we can elucidate the functional role of TGF- β signaling in radiation treated lung cancer cells.

Principal Investigator: **Douglas A. Andres, Ph.D., University of Kentucky**

Research Title: Novel Ras-related GTPase in lung cancer

Extensive studies have established a crucial contribution of oncogenic Ras to the development of human lung cancer. Mutationally activated K-Ras is found in 20-30% of lung adenocarcinoma and 15-20% of all non-small cell lung carcinomas (NSCLC) and results in a poor prognosis in both early and late stage non-SCLC. K-Ras mutations result from G-T transversions that arise from DNA damage caused by chronic exposure to tobacco smoke carcinogens. Oncogenic K-Ras contributes to the uncontrolled growth and invasive properties of lung tumor cells by chronically activating downstream signaling pathways. The elucidation of the biochemical pathways which oncogenic K-Ras proteins stimulate is critical to our understanding of normal cell growth as well as the defective regulation of these pathways in Ras-induced tumors.

We have recently identified Rit as the founding member of a novel and evolutionarily conserved Ras-like protein family. Like oncogenic Ras, expression of constitutively active Rit causes tumorigenic transformation. In addition, we have shown that the activation of Rit is a direct downstream consequence of the expression of oncogenic Ras and that dominant-negative Rit inhibits oncogenic Ras-dependent signal transduction pathways. These studies suggest that Rit is an important downstream target for Ras-mediated signaling. We therefore hypothesize that oncogenic K-Ras stimulates aberrant Rit function and that Rit-mediated signaling plays a critical role in the development of K-Ras-dependent lung tumors. Therefore, questions regarding the mechanisms by which Rit promotes cellular transformation and how Rit contributes to K-Ras-dependent human lung cancer, remain to be answered. We propose two specific aims to delineate the transforming actions of Rit in lung cancer. Specific Aim 1 will determine if activated Rit can induce tumor progression in lung carcinoma cells, and whether Rit function is necessary for K-Ras mediated lung cancer transformation. Specific Aim 2 will determine the extracellular stimuli that regulate Rit function and determine whether the autocrine/paracrine systems at work in lung cancer result in Rit activation. Taken together, these studies will add significantly to our understanding of K-Ras-mediated signaling in lung cancer, the role of aberrant Rit activity in this disease, and might make Rit a target for the development of novel chemotherapeutic agents for lung cancer.

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

Research Title: Nucleolin: A novel marker and therapeutic target for lung cancer

The goal of this proposal is to test the hypothesis that a proliferation-related protein called nucleolin is a useful marker and therapeutic target for lung cancer. The known characteristics of this protein, as well as our own data regarding nucleolin levels in cancer cells and the antiproliferative effects of nucleolin-binding agents, support this hypothesis. We have discovered a novel oligonucleotide (GRO26B) that can bind to nucleolin and has potent antiproliferative and apoptosis-inducing activity against lung cancer cell lines (including fast-growing, metastatic NSCLC and SCLC) but little effect on a cell line derived from normal skin tissue. GRO26B is extremely stable in biological media and its mechanism of action is new and different from that of antisense oligonucleotides. Experiments using cancer cell lines also indicate that a high level of nucleolin on the cell surface (detected by immunofluorescence microscopy) is related to rapid cell proliferation and high sensitivity to GRO. The first aim of this proposal is to confirm the significance of our preliminary findings by examining these factors in a larger sample of lung cancer cell lines. In parallel, we will pursue our second aim, which is to evaluate the utility of nucleolin staining as a clinical marker for lung cancer. This will be achieved by examining patient lung biopsies, sputum samples, and resected lung tumors. Nucleolin staining will then be compared to a number of established diagnostic and prognostic markers. The third specific aim is to evaluate the ability of GRO26B to inhibit tumor growth and metastasis in nude mice bearing lung cancer xenografts and thereby assess the therapeutic potential of agents that target nucleolin. GRO26B has already shown remarkable activity against lung cancer cells in culture, and the proposed experiments will determine whether similar anticancer effects can be observed *in vivo*. In summary, this project represents a collaborative effort that will combine the expertise of basic scientists, pathologists, and clinicians to establish whether extremely promising laboratory results can be translated to create a new clinical marker and treatment to fight lung cancer.

Principal Investigator: **Haribabu Bodduluri, Ph.D., University of Louisville**

Research Title: Role of G-protein coupled receptor mediated motility in lung cancer

Two of the critical steps involved in tumor spread or metastasis, exit from primary tumor site and dissemination to other target organs, involve cell motility. For many tumors, laboratory evidence shows that cell motility correlates with invasiveness and clinical outcome.

This investigation will test the hypothesis that cell motility in lung cancer is regulated by G-protein coupled receptor activation through mechanisms similar to those that enable migration of white blood cells to sites of inflammation or infection. This laboratory has developed novel microscopy techniques for tumor cell imaging. They have identified several chemicals that are key intermediates in the induction of directed cell migration. The methods of cell imaging will be used to identify the cellular signals involved in tumor cell migration. Specifically, the study focus will be on determining the role of protein Cdc42, guanine nucleotide exchange factors (GEFs) and p21 activated kinases (PAKs). Experiments are designed to enhance or reduce motility through these chemicals in lung cancer cell lines.

These studies will likely identify several potential targets for inhibiting the motility of lung cancer cells, with the goal of blocking metastatic spread.

Principal Investigator: **Stephen A. Brown, Ph.D., University of Kentucky**

Research Title: Radioprotective agents in NSCLC therapy

Effective treatment of many lung cancers encompasses the triad of surgery, chemotherapy, and irradiation. However, the effectiveness of both chemotherapy and irradiation is limited by local and systemic toxicity against healthy tissues due to the production of free radicals. To counter these undesirable effects, extensive efforts have been directed at the development of cytoprotective agents capable of preserving normal cells without inhibiting the cytotoxic activity of these therapeutic agents. The only approved agent effective in reducing the untoward side effects of chemotherapy in lung cancer patients is Amifostine, i.e. EthoYL, WR-2721. It is primarily a scavenger of free radicals. The goal of our research is to develop a new generation of therapeutic agents that are both radioprotective for healthy tissue and cytotoxic for tumor tissue. Recently, we have identified the compound D609 as being both radioprotective and tumor toxic. The combination of *in vivo* irradiation and D609 therapy exhibits synergy yielding longer leukemia free survival in our A20 murine leukemia model system. Thus, D609 protects the normal host tissue while being cytotoxic for the tumor cells. Our goal in this pre-clinical study is to develop D609 as a radioprotective-tumor toxic agent for the therapy of lung cancer. Specifically, we will test the hypothesis that the radioprotective agent D609 is toxic for lung cancer cells while protecting normal cells. In turn, this radioprotection will preserve host lung immune function and increase resistance to *Pneumocystis carinii* lung infection following radiation therapy. These studies will use the murine non-small cell lung carcinoma (NSCLC) Lewis lung cancer model. Specifically, we will investigate the following specific aims: 1) to determine the cytotoxicity profile of D609 and D609 plus irradiation for murine Lewis lung cancer cells *in vitro*; 2) to determine *in vivo* if D609 is radioprotective for normal lung and esophageal tissue as well as host lung immune function and resistance to infection; and 3) to determine if D609 increases survival and the efficacy of irradiation therapy in the murine Lewis lung cancer cell model. In all studies, the effectiveness of D609 will be compared to Amifostine. We expect that the combination of radioprotection and tumor toxicity offered by D609 will result in more effective therapy for lung cancer.

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

Research Title: Pro-inflammatory and clastogenic actions of smoke-borne free fatty acids

The lungs of long-term smokers accumulate abnormally large amounts of iron and this iron may contribute to the chronic inflammation, progressive pulmonary damage, and predisposition to neoplasia seen in smokers. Tobacco and tobacco smoke contain free fatty acids that cause the solvation of iron in non-polar organic solvents, cause intact human cells to accumulate iron and promote phagocyte adherence and oxidant production. The proposed work will further define the importance of fatty acid-mediated iron delocalization in the genesis of smoking-related diseases. Specifically, we shall determine: (1) the effectiveness of smoke condensates and free fatty acids in delivering iron into whole cells; (2) the consequences of such iron loading on the oxidation of lipids in target cell membranes and on oxidative destruction of iron-loaded target cells by oxidants of reagent, enzymatic and phagocytic origin; and (3) whether this 'delocalization' of iron might play a part in the carcinogenic activities of cigarette smoke, especially through exerting either clastogenic or (through non-transferring dependent iron delivery) growth promoting effects.

Principal Investigator: **H. Leighton Grimes, Ph.D., University of Louisville**

Research Title: Involvement of the GFI1 oncoprotein in human lung cancer

Human neuroendocrine (NE) lung cancers are driven to proliferate by multiple autocrine and paracrine neuropeptide loops. The NE phenotype is not only associated with NE-tumor biology, but also with poor patient prognosis. The expression of the *achaete-scute homolog-1*, *ASH1*, transcription factor may be causal in initiating both the neuroendocrine phenotype in normal lung, and NE-tumor oncogenesis. However, no known targets of *ASH1* possess oncogenic activity. Clues to the mechanism of *ASH1*-mediated tumorigenesis can be found in the fruit fly, *Drosophila melanogaster*. The *Drosophila* orthologs of *ASH1* are transcription factors of the *achaete-scute* complex (*AS-C*). *AS-C* factors regulate the development of the fly peripheral nervous system. In *Drosophila*, *AS-C* factors activate the *Senseless* gene, and *AS-C* factors require *Senseless* for neural development. *Senseless* is the ortholog of the GFI1 oncoprotein. In general, *Drosophila* is a well-accepted model system in which to study mammalian gene function; *Drosophila* orthologs generally perform parallel functions in the mammalian system. If *AS-C* factors activate *Senseless*, then *ASH1* should activate the GFI1 oncoprotein. Our central hypothesis is that GFI1 is a direct downstream target of *ASH1* in NE tumors, and that expression of GFI1 is prognostic for human lung tumors and critical for tumor maintenance. We will test our hypothesis by experiments designed to: 1) determine the level and pattern of GFI1 expression in human lung tumors, and its prognostic value; 2) determine if intervention into GFI1 expression is deleterious to NE tumor growth, and/or survival; and 3) determine the role of GFI1 in a transgenic model of lung tumorigenesis. This proposal is designed to validate the GFI1 oncoprotein as a novel therapeutic target for intervention in human NE-lung tumors.

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

Research Title: Etiology and prevention of lung cancer: Biomarker development in clinical studies

Human smokers are at increased risk for lung cancer. The proposed studies are based on the hypothesis that interplay between various host predisposing factors and complex chemical mixture inhaled during smoking determines the susceptibility to tobacco carcinogenesis. The factors reducing the conjugation and excretion of toxic metabolites from the body increase formation of DNA lesions. Adverse effects on the DNA repair capacity further increase the fixation of mutations thus increasing the chances of neoplasm development. We propose to determine the metabolic capacity, DNA repair capacity and the ability to form DNA lesions by using cells from smokers and nonsmokers. Furthermore, genotypic analysis will be performed to identify the polymorphisms in biotransformation enzymes, which relate to increased or decreased DNA damage. Both lipophilic and polar DNA adducts will be analyzed in the lung tissues of smokers and nonsmokers to discern their relationship to different types of damage. These studies are expected to identify those early biomarkers, which impact on the development of lung cancer.

Principal Investigator: **David A. Hein, Ph.D., University of Louisville**

Research Title: Environmental genomics and molecular epidemiology of lung cancer:
Functional characterization of N-acetyltransferase-1 and -2 genetic polymorphisms

Despite general appreciation for a role of genetics and environment in cancer risk, genetic predisposition to lung cancer following environmental exposures from cigarette smoking remains very poorly understood. We hypothesize that the aromatic and heterocyclic amine carcinogens present in cigarette smoke initiate lung cancer in those individuals with genetic predisposition. Testing this hypothesis requires understanding the functional genomics of lung enzymes critical in the activation and metabolism of these chemical carcinogens. N-acetyltransferases-1 (NAT1) and -2 (NAT2) are two enzymes that catalyze critical steps in the activation and metabolism of carcinogens present in cigarette smoke. To properly design and interpret molecular epidemiological investigations of lung cancer risk in cigarette smokers, we propose to fully characterize expression of NAT1 and NAT2 as a function of individual and combinations of *NAT1* and *NAT2* single nucleotide polymorphisms (SNPs) and genotypes using genetically-engineered mammalian cells and human lung samples. The results will yield a comprehensive and mechanistic understanding for the role of SNPs in *NAT1* and *NAT2* on the activation and metabolism of cigarette smoke carcinogens within the lung and will enhance the accuracy and validity of lung cancer risk assessments in individuals and families.

Principal Investigator: **Louis B. Hersh, Ph.D., University of Kentucky**

Research Title: A gene therapeutic approach for the treatment of lung cancer

Small cell lung carcinomas and many non-small cell lung carcinomas have been shown to be dependent on bombesin-like peptides as growth factors. The bombesin-like peptides are normally metabolized by the peptidase neprilysin found in the lung. Neprilysin levels decrease and bombesin-like peptide levels increase in lung cancer. Neprilysin has been shown to be inactivated by cigarette smoke, providing a link between smoking and lung cancer. In this proposal we will test the ability of recombinant neprilysin, delivered to the lungs through a lentivirus vector, as a method of treating ethyl carbamate induced adenocarcinomas in mice. In this system, we will test the ability of cell surface and secreted forms of neprilysin to hydrolyze the bombesin-like peptide growth factors leading to arrest of growth of the lung cancer. We also propose to use structural data to produce by genetic engineering a form of neprilysin that is more specific for bombesin-like peptides and use this form of the enzyme in the mouse model.

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

Research Title: Therapeutic investigation of dendritic cell vaccines in NSCLC

Tumor vaccines may have a role in non-small cell lung cancer (NSCLC). Tumor vaccines are intended to enhance recognition of NSCLC antigens and augment effector mechanisms directed against antigen-bearing tumor cells. A logical strategy is to provide antigen presenting cells (APCs) with NSCLC antigens *ex vivo*, which, when given back to individuals, will induce an immune response to those antigens *in vivo*. This proposal is built around the use of dendritic cells (DC), one of the most potent antigen presenting cells, as components of a lung cancer vaccine. A relevant concern is whether tumor-induced immunosuppression is a major hurdle for successful vaccination. Although host tumor environments have been shown to suppress immune reactivity, including the development and normal function of dendritic cells, preliminary data indicates that dendritic cells grown from peripheral blood precursors, and matured in *ex vivo* culture, can bypass the potential hurdle of tumor-induced APC dysfunction. When delivered back into the tumor environment, however circulating tumor-derived cytokines, such as PGE-2, and monocyte-derived IL10 may significantly suppress antigen presentation and proliferation of NSCLC specific T cells as well as lead to premature death of dendritic cells. Modifying the host environment either by removing tumor or with pharmacological agents, specifically COX-2 inhibitors, can enhance CTL responses to DC vaccines. To address these questions, this proposal will compare immune responses to autologous DC vaccines, similarly pulsed with allogeneic tumor from a panel of well-characterized NSCLC cell lines, in two immunologically distinct groups of NSCLC patients. One group will be post complete resection of NSCLC and will be assumed to be immune competent. The other group will be comprised of stage 3 unresectable NSCLC who will be likely to have impaired immune responsiveness based on persistent tumor burden. Both groups can potentially benefit from this approach. The former has a high risk of recurrence and risk of second primary lung cancers and the latter, difficulties with progressive disease. The proposal will focus the first two years on the relevant differences in immune response between advanced stage and fully resected NSCLC patients to define the importance of tumor-induced immunosuppression. The third year of the proposal will incorporate pharmacological manipulation of the host environment with COX-2 inhibitors to enhance responses to vaccines in stage 3 unresectable candidates. This could support the use of pharmacological manipulation as a routine adjuvant to vaccine therapy.

In this context, the study will explore the effects of NSCLC tumor environment on immune reactivity to a therapeutically relevant vaccine. In addition the study will evaluate approaches to enhance NSCLC-antigen specific lymphocyte responses *in vivo*. Correlative studies will define whether poor responses to vaccines can be predicted by the nonspecific markers of immunosuppression PGE-2 and monocyte-derived IL10 in peripheral blood and perhaps suggest who might most benefit from pharmacological intervention to complement tumor vaccines. Information obtained from this proposal will lead to improved understanding of the immunobiology of NSCLC.

Principal Investigator: **W. Glenn McGregor, M.D., University of Louisville**

Research Title: Mechanisms of BPDE-induced mutagenesis and mutation avoidance

Chemicals in cigarette smoke that cause cancer act primarily by damaging DNA. Such damage can lead to permanent changes in the genetic code, called mutations, which can lead to abnormal cell division and ultimately to cancer development. Understanding the mechanisms by which mutations occur is essential in order to improve cancer control strategies. The goal of this project is to elucidate the mechanism by which benzo[a]pyrenedioloepoxide (BPDE), one of the carcinogens in cigarette smoke, induces mutations in DNA of human cells.

We propose to examine the biological function of a variety of proteins that have recently been discovered in human cells, which appear to be required to replicate damaged DNA. These proteins are named after similar proteins that are found in baker's yeast cells, and are called RAD18, REV1L, REV3L, REV7L. The overall goal of this project is to examine the hypothesis that these proteins are required for the replication of DNA that has been damaged by BPDE, and that reducing the levels of these proteins has an anti-tumor effect.

The study will first investigate if the proteins listed above are produced and mobilized to cell-replication sites in presence of the BPDE-induced DNA adducts. Specifically, the role of RAD18 and the REV-complex in the replication of BPDE-damaged DNA will be tested. If mutations induced by BPDE are dependent on the REV complex, inhibition of this activity could become a therapeutic target for lung cancer prevention.

Principal Investigator: **Marcos A. Oliveira, Ph.D., University of Kentucky**

Research Title: A novel chemo/radio sensitizing target: PARP-1 activation.domain

Poly(ADP-ribose) polymerase (PARP-1) is a natural DNA damage biosensor whose activity triggers cellular responses leading to DNA repair or cell death by apoptosis or necrosis. Knockout experiments in mice have provided compelling evidence for PARP-1 as a target for the development of radio- and chemosensitizing agents for cancer therapy including lung cancer. In the past two years, it has become apparent that PARP-1 is but one of a family of enzymes with PARP activity, all containing a conserved NAD⁺ binding site and known inhibitors of PARP-1 inhibit these enzymes. Thus, while PARP-1 remains a promising therapeutic target, the discovery of multiple PARPs raises questions of inhibitor selectivity. We propose a paradigm shift in PARP-1 inhibitor discovery by targeting its unique mechanism of activation for the design of selective inhibitors. The hypothesis to be tested is that PARP-1 activation, resulting from DNA strand break recognition, is mediated by a Src-homology 3 module (SH3). We will focus on the identification of the protein interactions involved in PARP-1 activation and in particular whether this interaction involves an SH3 module. Specific Aim 1: To utilize co-expression of GST/His tagged PARP-1 domains along with pull down assays and surface plasmon resonance techniques to identify protein interactions between PARP-1 domains necessary for PARP-1 activation. Specific Aim 2: We will use a structure-based approach to screen for point mutations that generate activation deficient PARP-1. These mutations are designed based on the known structure of a typical SH3 domain and are predicted to disrupt the interaction between PARP-1-SH3 module and/or the PARP-1-SH3 ligand domains.

Principal Investigator: **Stephen C. Peiper, M.D., University of Louisville**

Research Title: Role of G-protein coupled receptors in lung cancer biology: Novel approaches to block proliferation and spread

Lung cancer mortality remains very high, partly due to an unchanged poor response of lung cancer to current therapies. The ultimate goal of this project is to elucidate the biological mechanisms underlying tumor metastasis to enable development of therapies to block tumor growth and dissemination.

The central hypothesis of this research is that chemokines secreted by cells in lung and other common metastatic sites stimulate receptors on the tumor cell surface to promote spread within the lung and dissemination to target tissues. Therefore, blocking this activation may decrease tumor spread.

This group found that CXCR4, a receptor implicated in metastatic breast cancer spread, is also expressed by lung cancer cells. SDF-1 is an exclusive product of this type of cells and is expressed at high levels in lung tissue. This proposal seeks to determine if SDF-1 from lung cancer cells promotes spread of tumor cells through activation of CXCR4. If the investigators can link the function of CXCR4 in directing the migration and spread of lung cancer cells, the findings can then be tested in a mouse model.

A second, more long-term objective is to characterize the role of a genetically-related receptor called GPR84, in the biologic behavior of lung cancer. The expression of this protein was identified through NCI's Cancer Genome Project and is a potential candidate for new cancer blocking treatments.

Principal Investigator: **Gordon D. Ross, Ph.D., University of Louisville**

Research Title: Oral adjuvant immunotherapy of lung carcinoma

Oral and intravenous (i.v.) fungal β -glucans have been used for adjuvant therapy of cancer for >18 years in Japan. In US trials, inconsistent results and a lack of understanding of mechanisms dampened enthusiasm. β -glucans are now known to bind to leukocyte CR3 (CD11b/CD18; α M β 2-integrin), priming this iC3b-receptor of neutrophils, macrophages, and NK cells to mediate cytotoxicity of iC3b-opsonized tumor cells. Therapy of syngeneic tumors in mice given i.v. fungal β -glucans caused a 70-95% reduction in tumor weight. Successful therapy required tumor-reactive antibodies (Abs) that activated complement and deposited iC3b on tumor cells. Therapy failed in SCID mice, but was reconstituted with i.v. tumor-reactive Abs. The requirement of C3 on tumors and CR3 on leukocytes was highlighted by therapy failures in C3- and CR3-knockout mice. Despite these data, patient trials were delayed because of problems in obtaining sufficient amounts of a CR3-binding fungal β -glucan. Current research has shown that barley β -glucans, that are readily available, bind avidly to CR3 and prime the receptor for tumor cytotoxicity in a similar manner as fungal β -glucans. Moreover, barley β -glucans given orally to nude or SCID mice with human tumors mediated significant tumor regression when given in combination with tumor-specific Abs that deposited iC3b on tumors. The proposed research will investigate the ability of barley β -glucan to function as an oral adjuvant for the immunotherapy of lung cancer. Pre-clinical studies will be carried out in syngeneic and xenogeneic murine lung tumor models to characterize mechanisms and efficacy. For aim 1, it will be determined if oral barley β -glucans given to mice prime CR3 or circulating leukocytes in vivo to kill iC3b-opsonized lung tumors in vitro. In aim 2, it will be determined if intravenous or oral β -glucan therapy is effective in mediating tumor rejection responses against Lewis lung carcinoma (LL/2) in syngeneic C57BL/6 mice or NCI-H69 (human) small cell lung carcinoma in nude mice. Aim 3 will be to determine whether C3a and C5a are required for the recruitment of CR3-bearing neutrophils and macrophages into the lung tumors of mice receiving oral barley β -glucan. Since this therapy requires the recruitment of CR3-bearing leukocytes into tumors, it is hypothesized that complement activation plays a major role in leukocyte recruitment. The relative roles of C5a and C3a will be explored through immunotherapy of lung tumors in mice that are deficient in either C3a- or C5a-receptors (C3a-R or C5a-R).

Principal Investigator: **Sandra Sephton, Ph.D., University of Louisville**

Research Title: Psychosocial effects in lung cancer outcomes

In the next century, tobacco-related diseases will become the number one cause of preventable death throughout the world. The psychological and social (psychosocial) characteristics of the lung cancer patient are important determinants of disease outcomes including survival time and quality of life. More research is needed to characterize how psychosocial variables influence disease course and response to treatment. Moreover, there is a pressing need for well-honed interventions to help patients and their families confront the precipitous changes imposed by a rapidly fatal disease course. Such interventions need to ameliorate the profound effects of medical treatment on quality of life. This project will utilize two research designs to address these needs. First, a naturalistic study will characterize the effects of psychosocial factors on lung cancer outcomes including functional status, quality of life, and survival, and explore the influence of adherence with medical treatment, health-related behavior, and neuroendocrine function in these associations. Second, a randomized controlled study will determine the benefits of a psychosocial intervention for alleviating distress among lung cancer patients. We will characterize the effects of psychosocial intervention on survival time, emotional distress, treatment choice and treatment adherence, health behavior, neuroendocrine function, functional status, and quality of life. This project will greatly improve our understanding of how patient-centered factors influence a) the prognosis and progression of lung cancer, b) the quality-of-life standards that patients experience, and c) disparities in disease outcome. By gathering data on treatment adherence, health behavior, and neuroendocrine function, we gain a powerful opportunity to test the mediating effects of psychosocial factors on lung cancer outcomes. This project will provide tremendous information for the design of interventions that address lung cancer patient's psychological, social, and behavioral needs in ways that more specifically provide a positive impact on health outcome.

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

Research Title: A novel approach to tumor vaccination

The main objective of this proposal is to use a novel approach to express immunostimulatory molecules on tumor cells and use these cells for vaccination to induce anti-tumor immunity. T-cell mediated immune responses play a critical role against tumors. A productive T-cell response requires three distinct signals: Signal 1, 2, and 3. Signal 1 is generated by T-cell recognition of foreign tumor antigens in context of major histocompatibility complex molecules. Signal 2 is mediated by the engagements of costimulatory molecules, such as B7/CD28 and CD40/CD40L, on T cells and antigen-presenting cells (APCs). Signal 3 is transduced via cytokines elaborated by T cells and APCs that have received both Signal 1 and 2. The transduction of these 3 signals drives T cells and APCs to proliferation, differentiation, and maturation into effectors for the generation of a productive immune response. Tumors generally evade the immune system by down-regulating one of these three signals.

B7.1 and CD40L costimulatory molecules and IL-2 cytokine will be expressed rapidly (~1 hr) on several tumor cells at the protein level using a novel technology designated as ProtEx. Tumor cells expressing immunostimulatory molecules will then be used to vaccinate rodents against primary as well as established tumors of different origins. We hypothesize that vaccination of animals with cancer cells displaying these three immunostimulatory molecules will activate not only T cells but also APCs, such as dendritic cells, macrophages, and B cells, which will in turn perpetuate the response by the synthesis and elaboration of a battery of cytokines and chemokines for the generation of a protective anti-tumor response. A proof of concept in animal models may allow immediate application of this technology into clinics since tumors can be resected from patients, modified to express proteins of interest using ProtEx, and injected back into the patients in a regular hospital setting within a day, if not hours.

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

Research Title: Pre-clinical studies of novel Ras function inhibitors to treat lung cancer

Our goal is to develop novel inhibitors of Ras, mutated forms of which are found in 20% of lung cancers. Interference with oncogenic Ras function forms the basis of effective disease treatments by blocking its action in cellular signal transduction cascades. Covalent modification of Ras with the farnesyl lipid farnesyl pyrophosphate (FPP) by the enzyme protein farnesyltransferase (FTase) is essential for its oncogenic function. Potent inhibitors of FTase (FTIs) have been developed and a number have shown promise as anti-neoplastic agents in clinical trials. However, isoforms of Ras, most notably K-Ras, evade the action of FTIs because it is alternatively modified by the related prenyl transferase GGTase I, which is not inhibited by FTIs. We propose to develop an alternative approach to block Ras function by modifying the structure of the farnesyl lipid to interfere with downstream aspects of Ras processing or metabolism.

We have synthesized FPP analogs that are efficiently transferred to oncogenic Ras but fail to support transformation. These molecules serve as lead compounds for a unique class of potential anti-cancer therapeutics we term RFIs (Ras function inhibitors). Based on these promising results, we propose the hypothesis that prenylation of Ras proteins with unnatural prenyl analogs will lead to significant, biologically relevant effects on the activity of the modified protein, and that these unnatural analogs will be lead compounds for novel therapeutic agents. Two specific aims are proposed: 1) to synthesize structurally diverse farnesyl pyrophosphate analogs using combinatorial methods to generate new RFIs; and 2) to characterize these molecules as a series of high throughput biochemical and cellular based screens for anti-Ras activity. This work will identify and define the molecular features of unnatural FPP analogs that give rise to anti-Ras activity and lay the foundation for future clinical therapy development.

Principal Investigator: **Willam St. Clair, M.D., Ph.D., University of Kentucky**

Research Title: Novel anticancer agents to promote the efficacy of contemporary or GRID radiation therapy for treatment of lung cancer

The goal of this project is to translate basic biologic knowledge into a practical application for enhancing the efficacy of radiation therapy for the treatment of lung cancer. Lung cancer represents one of the great challenges of oncology. Radiation, surgery, and chemotherapy alone or in combination have been employed in attempts to improve treatment outcome. Yet over the past 30 years, the five-year survival of lung cancer patients has only improved modestly. Advances in the understanding of molecular pathways of cancer have progressed to the point that new biological targets for therapy and potential therapeutic agents have been identified. Proteasome inhibitors and COX-2 inhibitors are prominent candidates for new therapeutic agents that may enhance radiation response of tumors. Both proteasome inhibitors and COX-2 inhibitors are associated with tumor apoptosis and limit tumor angiogenesis. Cell cultures study demonstrate both these agents preferentially increase tumor cell sensitivity to radiation compared to non-tumor cells. Thus, combining proteasome inhibitors and/or COX-2 inhibitors with radiation is expected to enhance the tumor control without enhancing normal tissue toxicity. GRID radiation is a method of delivering spatially fractionated radiation using multiple pencil beams with unirradiated segments between. The advantage of delivering spatially fractionated radiation is the ability to kill large numbers of tumor cells while leaving islands of normal unirradiated tissue for recovery. The killing of unirradiated tumor is thought to be mediated by the by-stander effect. *In-vitro* studies have found increased cytokine production and apoptosis in cells neighboring the irradiated field after GRID treatment. Combining biologic agents that preferentially sensitize tumor to the process of apoptosis may enhance the efficacy of radiation treatment, particularly with GRID therapy through its known association with apoptosis and the by-stander-effect.

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

Research Title: Use of dendritic cells to present non-small cell lung cancer associated antigens

The most potent antigen presenting cell in humans are dendritic cells (DCs). These cells are derived from bone marrow or peripheral blood and schemes exist to get adequate numbers to educate T cells against viral, tumor, or self-antigens. The current proposal aims to further characterize and investigate the biology of DCs derived from normal donors and patients with non-small cell lung cancer (NSCLC). The major goal is the use of DCs in the generation of both CD4 and CD8 restricted NSCLC specific lymphocytes. These lymphocytes, once derived, are a potent effector mechanism for future immunotherapy trials in NSCLC and also are a tool in identifying NSCLC specific antigens. The PI, in a timely fashion, will also apply what is learned about DCs to a clinical trial submitted to the Kentucky Lung Cancer Research Board with Dr. Edward Hirschowitz "Autologous DC Vaccines in NSCLC". The current proposal is written for a three-year time-period. The PI is well poised with expertise, space, equipment, and preliminary data to achieve the goals stated in the specific aims.