

# Kentucky Lung Cancer Research Program

## Cycle 5 Grant Abstracts

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

Research Title: The Understanding and Manipulation of the Biosynthetic Pathway of Gilvocarcin V to Generate Novel, More Efficient Drugs against Lung Cancer

Historically, early stage (i.e. non spreading) non-small cell lung cancer (most common form) has been treated by surgical methods. However, survival with surgery alone is only 10-25% after 5 years. Patients who have more advanced cancer of the lungs have been treated with combination chemotherapy and radiation, because of the difficulty in completely removing the cancers surgically. While the combination of chemotherapy and radiation is powerful, many patients still die of their lung cancers or are severely debilitated by their treatments. One manner in which cancers evade the treatments and continue to progress is through a mechanism termed drug resistance. Drug resistance occurs after the continual addition of chemotherapy. Certain molecules in the cancers that are over produced and allow the cancer cells to grow in spite of the chemotherapy and radiation mediate another mechanism of resistance. We propose two different methods of reversing the development of resistance to therapy. One method uses known medicines that block a cancer cell's ability to get rid of the chemotherapy drug; the other method is a unique delivery of radiation at low doses, which prevents the signaling of cellular events that would otherwise cause the cells to continue to grow. By silencing these factors, the chemotherapy agent is more effective at killing the cancer cells. This well tolerated approach of lower doses of radiation combined with effective chemotherapy and using commonly prescribed drugs has the desired potential of improving survival and bettering the quality of life of patient afflicted with this deadly disease.

Principal Investigator: **Andre Baron, Ph.D., University of Kentucky**

Research Title: Evaluating Low Dose Computed Tomography and Serum Biomarkers for Lung Cancer Screening

Lung cancer remains a major health care concern in the commonwealth of Kentucky. More than 3,000 people die from lung cancer each year in Kentucky, yielding age-adjusted mortality rates for both men and women that exceed those in the United States (Hopenhayhn, Stump et al. 2001). The etiologic relationship between cigarette smoking and lung cancer is well established (Spivack, Fasco et al. 1997). Accordingly, the high prevalence of smoking in Kentucky, and, in particular the FIVCO Area Development District, contributes to the high incidence and presumably high prevalence of lung cancer in this geographical region. High disease prevalence in the screened population is known to impact positively on the test statistics of negative predictive value (NPV) and positive predictive value (PPV). Hence, the FIVCO population of Kentucky is ideal for assessing whether a screening test or a multimodal-screening program may be economically, clinically, and epidemiologically valid, feasible, and efficacious.

Surgical resection of early stage disease remains the most effective cure for lung cancer (Bach, Kelley et al. 2003; Bach, Niewoehner et al. 2003). Unfortunately, most patients have advanced stage disease at diagnosis. Therefore, screening or early detection represents a potentially practical approach for controlling mortality from lung cancer. Although low dose computed tomography (LDCT) might be a useful screening modality for lung cancer, LDCT detects a high proportion of non-calcified benign nodules leading to a high false-positive detection rate and low, test specificity. Adjuvant tests that supplement LCDT to differentiate non-calcified benign from malignant nodules, therefore, are urgently needed.

Immunoassay studies demonstrate that soluble isoforms of the ERBB proto-oncogene family are potentially useful screening and diagnostic biomarkers of lung cancer. In particular, serum sErbB1 and sErbB2 concentrations are altered in patients with lung cancer compared to healthy individuals (Brandt-Rauf, Luo et al. 1994; Brandt-Rauf 1995) Baron et al., unpublished observations, see Background and Rationale).

Well-designed screening programs that correctly apply multiple tests in parallel and/or in series can achieve a high degree of sensitivity, specificity, NPV, and PPV. We propose to perform a phase II, prospective case-control study in the FIVCO Appalachian region of Kentucky to assess the validity of using LDCT, sErbB1, and sErbB2 in a screening program for lung cancer. Our primary specific aims will allow us to determine how well serial or parallel testing with LDCT, sErbB1, and sErbB2 detects lung cancer among healthy individuals, and how well sErbB1 and sErbB2 discriminate patients with benign versus malignant nodules after initially being detected by LDCT. Secondary specific aims will allow us to determine whether periodic measurements of sErbB1 and/or sErbB2 may be useful as primary screening tests for lung cancer. Results from this case-control study are prerequisite for proceeding with a future nested case-control study, a cohort study, and a randomized trial that employ LDCT, sErbB1, and sErbB2 as screening modalities of lung cancer.

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

Research Title: Parthenolides as Potential Treatments for Lung Cancer

More than 160,000 cases of non-small cell lung cancer are diagnosed every year in the USA and 150,000 die of this disease. Novel therapies attacking the molecular targets inducing anticancer drug resistance are needed. We have identified a new target called Nuclear Factor kappa B which we believe promotes resistance to anticancer drug therapy in lung cancer. We are currently investigating novel chemical entities for their ability to inhibit this new target. Such molecules should provide more effective and novel treatments for metastatic lung cancer. We have recently isolated a constituent from a herb called "feverfew", a relative of the *Chrysanthemum* family of plants. This constituent, called parthenolide, is a molecule that potently inhibits Nuclear Factor kappa B, and we have already shown in our recent research that parthenolide inhibits the growth of lung, breast and prostate cancer cells. Unfortunately, parthenolide has very poor water solubility, which limits its usefulness as a drug molecule. Our proposed studies plan to develop more water soluble analogs of parthenolide, and to evaluate them as anticancer agents in cultured lung cancer cells and in mice. We plan to test several hundred analogs and to identify the most promising candidates for development as anticancer agents for the treatment of lung cancer. Our overall goal is to take the most promising parthenolide analog into the clinic, and in so doing translate our preclinical findings to the lung cancer patient. However, the scope of this current proposal is limited to the initial development and evaluation of the parthenolide analogs in the research laboratory.

Principal Investigator: **Aly A. Farag, Ph.D., University of Louisville**

Research Title: Image Analysis for Early Detection of Lung Cancer

Lung cancer screening has been conducted in the US since 1970's and there has been no conclusive evidence to support the usage of CT or X-ray for regular chest screening. In November 1999, the Jewish Hospital Lung Screening Study was initiated [4][5]. It is a separate and distinct study from the study of the National Lung Screening Trials (NLST), but related in several ways. First, the Jewish Hospital study was the first randomized study ever in the US to compare spiral CT of the chest with chest x-ray for the early detection of lung cancer. The protocol was scrutinized and although not duplicated in the NLST, some elements persisted. In other words, it was helpful in the study design of the NLST. A more important connection between the studies is this: By virtue of the success of ACRIN component of the study. (NLST will enroll 50,000 subjects; ACRIN will enroll 30,000 of them). Dr. Robert Falk, consultant on this proposal, is thus one of only 20 radiologists nationwide who are running this study. Likewise, Jewish Hospital is one of 20 ACRIN sites nationwide, and the only non-university that is participating.

This project will focus on the image analysis of the CT and X-ray generated from the screening program at Jewish Hospital, in order to create an automatic diagnosis system for lung cancer screening. Dr. Farag, PI, and his collaborators at the CVIP Lab have over 15 years of experience with biomedical image analysis. Dr. Robert Falk, Director of Medical Imaging Division at Jewish Hospital, and Dr. Renato LaRocca, Co-PI, is the Director of the Kentuckiana Lung Cancer Institute, have considerable experience in diagnosis and treatment of cancer. They will provide the clinical expertise in evaluating the effectiveness of the medical imaging analysis system proposed in this project.

Overall, this proposal is expected to be a success in many fronts: 1) It will consolidate the ongoing activities of cancer research at the Louisville Medical Center; 2) The findings of this research will have a positive impact on answering some of the lingering questions about the use of CT for regular screening of cancer patients; and 3) Through the interaction with other national screening studies, such as ACRIN, the investigators of this proposal will be able to correspond with their peers nationwide on this important healthcare issue. The investigators will be pursuing major NIH R01 proposal based in the results of this project.

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

Research Title: Oncoprotein as a Modifier of Small Cell Lung Carcinogenesis

Small cell lung cancers (SCLC) are aggressive neuroendocrine lung tumors. Few SCLC patients survive beyond 5 years. While the growth and survival of aggressive neuroendocrine tumors such as SCLC rely on the autocrine production of neuroendocrine growth factors, little is known about the molecular underpinnings of the neuroendocrine phenotype(1). Our preliminary data indicate that the Growth-factor-independence- 1 (GFII) oncoprotein is important for human SCLC tumor formation. GFII is a transcriptional repressor that targets a cyclin dependent kinase inhibitor, p21<sup>waf1</sup>, and an inhibitor of the Rb tumor suppressor and basic-helix-loop-helix (bHLH) transcription factors, Inhibitor-of-DNA-binding-2 (ID2). The neuroendocrine phenotype of normal and transformed pulmonary neuroendocrine cells is critically dependent on the action of bHLH proteins. Our data indicate that GFII is also part of an evolutionarily conserved pathway that controls the neuroendocrine phenotype of normal and transformed cells. Our collaborator, Anton Berns, has generated a mouse model of lung cancer that closely resembles human SCLC in morphology, histology and metastatic behavior. We propose to determine the effect of Gfi1 deletion and forced GFI 1 expression on GFI 1 - mediated repression of p21<sup>waf1</sup> and ID2, and on the generation of SCLC tumors in this new *vivo* model. These experiments are expected to resolve the role of GFII in neuroendocrine carcinogenesis, as well as to provide a rationale for further clinical, and molecular/mechanistic studies.

Principal Investigator: **Andrew N. Lane, Ph.D., University of Louisville**

Research Title: Biophysical Characterization of the CXCR4 Receptor and its interactions with Ligands

Membrane proteins account for 20-30% of all proteins, and are involved in a wide variety of fundamental biological processes such as cell-cell communication, up to 50% of current drug targets are membrane proteins. Yet compared with the soluble proteins, there is little structural information about them. We are interested in the G-protein coupled chemokine receptors CXCR4 and CCR5 that are also associated with HIV entry into cells. It has been recently demonstrated that CXCR 4 is over expressed on numerous cancer cells and is associated with breast cancer metastasis to lung, liver and bone marrow by chemoattraction to SDF-1 secreted by these target tissues. Blocking CXCR4 has already been shown to greatly inhibit the metastatic spread of cancer to the lungs.

We are currently investigating small molecule inhibitors of these proteins using molecular biology, synthetic chemistry, molecular modeling and NMR. Recent state of the art advancements in NMR methodology and instrumentation have made feasible structure determination of such proteins in solution. This requires the production and purification of large amounts (milligrams) of pure protein in a functional form. We propose to express and purify CXCR4 for biophysical analysis in our new protein expression laboratory. Different fusion and expression strategies will be pursued to optimize protein yield. The purified protein will be characterized functionally by sensitive fluorescence-based ligand binding experiments using available tight-binding peptide antagonists, and used to map the binding surface of vMIP2. These results will then be used to optimize the system for structural analysis by NMR, using the state-of-the-art 800 MHz NMR spectrometer housed in the newly established Brown Cancer Center NMR facility.

Principal Investigator: **Catherine A. Martin, M.D., University of Kentucky**

Research Title: Breaking the Cycle: Smoking Intervention with Youth in Families Dealing with Lung Cancer

This study asks the question: Does having a family member with smoking-related lung cancer open a window of opportunity where parents and teens are highly motivated to either prevent or stop early experimentation with cigarette smoking in the teens in the family? Early and mid-teens are making decisions about smoking behavior that are likely to effect their smoking behavior for the rest of their lives. Eighty percent of adult smokers report starting smoking in adolescence, with the first cigarette usually being consumed at age 12. Early and mid-teens are particularly vulnerable to becoming dependent quickly, even when they are in the early stages of experimenting. This proposal is a clinical study that meets the Kentucky Lung Cancer Research Program priority of familial relationships of lung cancer and will help investigators develop preliminary data for a National Cancer Institute (NCI) grant submission. Evidence suggests that patients are more responsive to a health provider talking to them about examining their drug use after a drug-related illness or accident in their family. This is called a “teachable moment” when the patient is forced to acknowledge the consequences of drug or alcohol related activity. Some teachable moments are dramatic ones: for example, an alcohol related car wreck can be a good time to talk to young adults about their alcohol use, and there is evidence that smokers change their behavior when family members or friends become cancer patients. A further impact on early and mid teen smoking is their parents’ attitudes about smoking and this is true whether or not the parent smokes. These elements set the stage for a smoking abstinence strategy: 1) a teachable moment (lung cancer in the family); 2) parents’ influence on early and mid adolescents smoking beliefs and behaviors; and 3) early and mid teens are at a critical point in making decisions about smoking behavior that will have a lifelong impact. This smoking abstinence strategy will be evaluated by recruiting teens between the ages of 11-15 and their parents to participate in a smoking abstinence intervention. One group of adolescent/parent dyads will be recruited from families with a member who is currently being treated for smoking-related lung cancer. A second group of adolescent/parent dyads will be families that do not have a member who has been diagnosed or treated for lung cancer. Half of the lung cancer family dyads will receive the intervention first and be followed for 6 months. The other half will be put on a waiting list for 6 months and will then receive the intervention. Family dyads with no lung cancer will be similarly divided in half. These four groups will allow us to determine if adolescents in families dealing with lung cancer who receive the smoking abstinence intervention will be less likely to start smoking or more likely to stop experimentation than adolescents who do not receive the intervention. With adolescent smoking being a pediatric epidemic and with the evidence that early smoking causes genetic changes in the lung that put teens at lifelong risk for lung cancer, it is important to identify new strategies that will help early and mid teens change their smoking-related beliefs and behaviors so that they will not smoke.

Principal Investigator: **Jurgen Rohr, Ph.D., University of Kentucky**

Research Title: Novel Gilvocarcin-type Natural Products against Lung Cancer by Combinatorial Biosynthesis

Cancer chemotherapy is one of the most important and effective ways to treat various types of cancer, and the only way to treat metastatic settlements of undetectable cancer spreads. However, the use of current existing chemotherapeutics is compromised by cross-resistance of several tumors against established drugs and the severe side effects of the available agents. Several novel natural products, so far not established as clinically used antitumor drugs, are available as lead structures, among them the gilvocarcins, which show promising results in anticancer assays. In contrast to antibiotics, which are often evolutionary optimized by natural organisms to fight competitors, natural anticancer drugs are not designed by nature to fight cancer cells, and thus are far away from being optimal.

Hypothesis: Starting from the natural product antitumor agent gilvocarcin V as a lead structure, it should be possible to improve this drug to become a superior lung cancer chemotherapeutic, with less side effects than common lung cancer chemotherapeutics.

Methods: Since traditional ways of natural drug optimizations (chemical derivatization) is very limited on natural products, due to their complex multifunctional chemical structures, we propose to apply recently developed techniques, which aim on the manipulation of the genes encoding the natural product biosynthesis. This technique is called combinatorial biosynthesis, and will allow the targeted modification of structural moieties of the natural product drug in order to improve its interaction with known cellular targets (here DNA, and certain histone proteins involved in the control of cell division). Preliminary tests have shown a very promising activity of gilvocarcin V against NCI-H460 (a non-small cell lung cancer cell line). From previous biosynthetic studies on gilvocarcin V in our laboratory, the genes of the biosynthetic pathway leading to this natural product are available and have been identified and characterized. The anticipated 'biological derivatization' approach will lead to new gilvocarcin derivatives with altered structural moieties, which will be characterized by (i) structure elucidation using spectroscopic methods and (ii) through MTS-anticancer activity and mice model assays (using various human lung cancer cell lines available in the co-investigator's laboratories). The most interesting derivatives will be further analyzed with respect to its mode-of-action, taking into consideration cell cycle analyses and by comparison with the NCI data bank.

The advantage of such an molecular biological approach is: once an optimal new active natural product has been discovered, a producing organism has been constructed simultaneously, and the biotechnological production of such a new drug can start right away. Since the optimization will be performed with the most important lung cancer cell lines, a selection towards lung cancers will be achieved.

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

Research Title: Repairing RNA transcripts linked to lung cancer

It is estimated that 171,900 new cases of lung cancer will be diagnosed in the United States, with 157,200 estimated deaths (2003). Of these cases, 3,500 Kentuckians will be diagnosed with lung cancer and 3,200 will die. Although lung cancers make up only 13% of overall new cancer cases, they account for 28% of cancer deaths. Clearly, new and more effective medical strategies for combating lung cancer are needed. Perhaps surprisingly, the root cause of many lung cancers are known. An example of a gene linked to lung cancer is k-ras. Its mutated form is found in approximately 20% of lung cancers. A mutation in the k-ras gene leads to production of a protein that does not function properly and is linked to initiation and continuation of cancerous tumors. Fortunately, technologies for the identification and early detection of such mutations are progressing relatively rapidly. Unfortunately, technologies to combat these mutations, especially at the causative gene level, are lagging far behind.

We propose to design, engineer, and test catalytic RNA molecules, called ribozymes, that can replace known cancer-causing mutations in the RNA of mutant k-ras genes, thus restoring the coding potential of the gene back to normal. Initial tests of this new experimental strategy will involve the development of a cell-free model system for which the number of variables can be controlled. We will use a small model of the mutated k-ras gene to analyze the ability of our engineered ribozymes to replace the designated mutation. Success in this mutant k-ras RNAs and then involving cellular systems. We feel that the potential benefits could be tremendous, both for cancer research in general and for patients who will (or have) come face to face with this disease, and could far outweigh the inherent risk of attempting to put into practice this novel idea.

Principal Investigator: **Jun Yan, M.D., Ph.D., University of Louisville**

Research Title: Development of a Novel Immunotherapy for Lung Carcinoma

Lung cancer is the leading cause of death among both men and women. Unfortunately, our region has the highest number of lung cancer cases in the nation. Cancers are caused by the progressive growth of the progeny of a single transformed cell. Therefore, controlling cancer requires that all the malignant cells be killed or contained. The big obstacle is that the immune system views a growing tumor as normal self tissue and therefore establishes tolerance. The goal of this proposal is to develop a novel immune therapy to overcome host non-responsiveness (“immune tolerance”) to naturally expressed tumor antigens that are likely to exist in patients with cancer. The basis for this proposal is largely relied on our previous studies on autoimmune disease. Tumor immunity and autoimmunity are two sides of a coin. We have demonstrated that B lymphocytes are critical autoantigen presenting cells and break host immune tolerance to “self”. In this proposal, we will target lung cancer tumor antigen human MUC1 glycoprotein to B lymphocytes to further test this concept. Our preliminary data have been successful in inducing anti-tumor T cells, as well as antibodies and preventing tumor growth in a murine model of mammary carcinoma. The proposed studies will extend our current observations to lung cancer. This project takes an innovative strategy to developing anti-tumor vaccine. The results of these experiments will be the basis for future patient clinical trials.