

# Kentucky Lung Cancer Research Program

## Cycle 10 Grant Research Abstracts

<u>UK Principal Investigators</u>	<u>UL Principal Investigators</u>	<u>Grant Research Title</u>
Younsoo Bae, Ph.D.	Jason Chesney, M.D., Ph.D.	Controlled Inhibition of the Glycolytic Pathway for Lung Cancer-Targeted Therapy
Rolf Joseph Craven, Ph.D.	Teresa W. M. Fan, Ph.D.	Stable isotope-derived metabolomics to elucidate the mechanism of a tumor-associated cytochrome in lung cancer growth and metabolism
Edward Hirschowitz, M.D. John R. Yanelli, Ph.D.	Goetz Kloecker, M.D. Jun Yan, M.D.	Combined Orally Administered Yeast derived $\beta$ -Glucan with 1650 Tumor Vaccine in the Treatment of NSCLC
Vivek M. Rangnakar, Ph.D. C. Gary Gairola, Ph.D. (Co-PI)	Ramesh C. Gupta, Ph.D.	Activation of the Par-4 Extrinsic Pathway for Suppression of Lung Cancer
Zhigang Wang, Ph.D.	W. Glenn McGregor, M.D.	Rev1 and Carcinogen-Induced Lung Cancer

**Principal Investigator: Younsoo Bae, Ph.D., University of Kentucky, Jason Chesney, M.D., Ph.D., University of Louisville**

**Research Title: Controlled Inhibition of the Glycolytic Pathway for Lung Cancer-Targeted Therapy**

Lung tumors take up 10-fold more glucose than adjacent normal tissues in vivo and oncogenic proteins converge on glycolysis by activating the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) to produce fructose-2,6-bisphosphate, an allosteric activator of 6-phosphofructo-1-kinase (PFK1). Although there are four PFKFB family members, PFKFB3 is activated in human lung cancer cell lines and required for the growth of lung xenograft tumors in mice. We recently reported the computational identification of a small molecule antagonist of PFKFB3, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), which suppresses glycolysis, is selectively cytostatic to lung cancer cells relative to normal epithelial cells, and reduces the growth of established lung tumors in vivo. Although these data support the development of optimized derivatives and formulations of 3PO as chemotherapeutic agents, 3PO has limited bioavailability, including poor water-solubility and short plasma retention time. Our initial exploration has proven that block copolymer micelles (BCM) may be a drug nanocarrier platform (<100 nm) suitable for the delivery of 3PO. In preliminary studies, we demonstrate that block copolymer micelles (BCM) with poly(ethylene glycol)-poly(amino acids) possessing either methyl sulfoxide moieties or butyric acid linkers markedly improved the solubility of 3PO in aqueous solutions and decreased the anchorage-independent growth of lung cancer cells as soft agar colonies. We have previously demonstrated that BCMs can achieve prolonged plasma retention time, tumorspecific accumulation, in vivo stimuli-responsive release, reduced systemic toxicity, and enhanced therapeutic efficacy of other cytotoxic agents. We now propose to test the hypothesis that the BCM formulations of 3PO will significantly improve bioavailability and reduce toxicity while causing regressions of lung adenocarcinomas in vivo. We anticipate that the results to be generated in this proposed project should facilitate dosage formulation design, GMP/NSF production, and pre-clinical pharmacokinetic and toxicity studies needed for an IND application and phase I clinical trial of BCM formulated 3PO in advanced lung cancer patients.

**Principal Investigator: Rolf Joseph Craven, Ph.D., University of Kentucky,  
Teresa W. M. Fan, Ph.D., University of Louisville**

**Research Title:** Stable isotope-derived metabolomics to elucidate the mechanism of a tumor-associated cytochrome in lung cancer growth and metabolism

Lung cancer and other smoking related diseases kill approximately 4-5 million people annually worldwide. Kentucky has one of highest smoking and lung cancer rates in the United States. The long-term goal of the research is to develop pharmacological inhibitors that target growth pathways in lung cancer and to individualize the therapeutics by identifying mechanistically-linked biomarker combinations that distinguish likely responders to the treatments. The subject of this proposal is Pgrmc1 (progesterone receptor membrane component 1), a cytochrome that drives lung cancer cell growth, invasion, tumor formation and metastasis. Furthermore, pgrmc1 is inhibited by a small molecule that disrupts tumor growth in vitro and in vivo and is well tolerated in animals. In a collaboration between the laboratories of Rolf Craven at the University of Kentucky and Teresa Fan at the University of Louisville, we have found that pgrmc1 promotes glycolysis, which is an energy-generating pathway that is elevated in cancer cells. Glycolysis is associated with growth under-hypoxic conditions and is also employed by tumor cells to limit the production of reactive oxygen species. We will determine the extent to which (1) glycolysis is regulated by Pgrmc1 in vivo and (2) Pgrmc1 is required for anaerobic growth in vivo. The UK group have also found that pgrmc1 is secreted in small lipid microvesicles called exosomes, while the UofL group has been analyzing in human blood the patterns of the lipids that define the exosome itself. Together, we will determine the extent to which Pgrmc1 levels in exosomes, and the exosomal structure, from cancer patients correlate with other markers of lung disease. Together, the experiments will test a hypothesis regarding a new, synergistic interaction between therapeutics for lung cancer and will potentially identify patients with a high likelihood of responding to the treatments.

**Principal Investigator: Edward Hirschowitz, M.D., University of Kentucky, John R. Yannelli, Ph.D., University of Kentucky, Goetz Kloecker, M.D., University of Louisville, Jun Yan, M.D., University of Louisville**

**Research Title: Combined Orally Administered Yeast -derived  $\beta$ -Glucan with 1650 Tumor Vaccine in the Treatment of NSCLC**

Since 2003, Drs. Yannelli and Hirschowitz at the University of Kentucky have developed and tested both antigen pulsed dendritic cells (DC) and tumor cell vaccines for non-small cell lung cancer (NSCLC), a major killer of men and women across the Commonwealth of Kentucky. Recent studies have demonstrated that a simplified version of a DC vaccine, 1650G (1650 apoptotic bodies + recombinant GM-CSF), designed to be transportable and less expensive, resulted in similar immunologic responses following immunization (55% compared to 67%). In the following study proposed by the combined clinical translational teams at both Louisville and Kentucky, the Investigators plan to expand patient accrual and test a novel approach combining the 1650G vaccine with an oral adjuvant,  $\beta$  -glucan, developed by Dr. Jun Yan at the University of Louisville. We hypothesize that particulate  $\beta$  -glucan may serve as a potent adjuvant for tumor antigen based vaccine in NSCLC to elicit augmented anti-tumor T cell responses. The goal is to develop a novel vaccine which results in anti-tumor immunity in greater than 90% of patients immunized.

It is anticipated that the combined efforts of the 4 Investigators listed will achieve this goal within a 2 year time period. The Investigators anticipate in year 1 obtaining pre-clinical data to apply for regulatory approval both locally and with the FDA as an amendment to an existing IND held for the 1650G vaccine. In year 2, the Investigators will test the hypothesis in 12 NSCLC patients that orally administered  $\beta$ -glucan enhances the immunologic response to antigens contained in 1650G, above and beyond what was observed in historical controls. This study demonstrates the compatibility of both basic science and translational medicine being conducted at the two premier Universities in Kentucky.

**Principal Investigator: Vivek M. Rangnekar, Ph.D., University of Kentucky, Gary Gairola, Ph.D., University of Kentucky, Ramesh C. Gupta, Ph.D., University of Louisville**

**Research Title:** Activation of the Par-4 Extrinsic Pathway for Suppression of Lung Cancer

Lung cancer deaths in women in the U.S. currently exceed those from breast cancer. Estrogen receptors  $\alpha$  and  $\beta$  are expressed at detectable levels in non-neoplastic and neoplastic lung tissue from both women and men at a frequency of 85 and 15%, respectively. Administration of estrogen during hormone replacement therapy greatly increases the risk of lung cancer especially in women who smoke cigarettes. Consistently, exposure of mice to the synthetic estrogen diethylstilbestrol increases the incidence and multiplicity of lung tumors induced by urethane administration.

Despite a decrease in the overall cigarette use over the past decades, the incidence of lung cancer in women continues to increase at a steady pace. This risk may be due, in part, to the extent to which women can convert estrogen to carcinogenic metabolites. Systemic alterations associated with lung cancer risk following estrogen replacement therapy are not fully understood. We noted that levels of the tumor suppressor protein Par-4 are down-modulated by estrogen or smoking. As Par-4 is secreted by both normal and cancer cells but induces apoptosis selectively in cancer cells by binding to its cell surface GRP78 receptor, we hypothesize that restoration of Par-4 levels in circulation will reduce the risk of lung cancer progression and metastasis. The objective of this study is to utilize diverse approaches to up-regulate and maintain adequate levels of Par-4 in circulation in animal models and test the effect on lung cancer progression associated with estrogen and cigarette smoke. We anticipate that the findings will have therapeutic significance and can be translated for treatment of lung cancer.

**Principal Investigator: Zhigang Wang, Ph.D., University of Kentucky, W. Glenn McGregor M.D., University of Louisville**

Research Title: Rev1 and Carcinogen-Induced Lung Cancer

Tobacco smoking is the major cause of lung cancer, and one of the most potent mutagens and carcinogens in cigarette smoke is benzo[a]pyrene (B[a]P). Mutations in critical growth-control genes induced by B[a]P that activate oncogenes and inactivate tumor suppressor genes are key mechanisms of lung carcinogenesis. However, how genes are mutated in lung is not known, although this is relatively well understood in model cell systems in that mutagenesis is produced mainly by the REV1 pathway. In this pathway, mutations are produced as a result of error-prone translesion synthesis, which frequently inserts a wrong base opposite the DNA lesion. We found that B[a]P-induced gene mutations are predominantly produced by the REV1 pathway in yeast and in cultured human and mouse cells. Therefore, we hypothesize that REV1 is essential for lung mutagenesis and carcinogenesis induced by B[a]P and other carcinogens in cigarette smoke. Supporting this hypothesis, we found that a ribozyme aerosol is able to suppress B[a]P-induced lung tumorigenesis by inhibiting REV1 expression. We anticipate that the tumor-resistance effect would be much more dramatic if the REV1 function is completely inactivated, as in a Rev1-knockout mouse model. We have created such Rev1 knockout transgenic mice. Combining the expertise and resources of the Wang and McGregor laboratories together, our hypothesis will be definitively tested. Our specific aims are (1) to determine the effect of Rev1 knockout on B[a]P-induced lung mutagenesis; (2) to test the hypothesis that REV1 is essential for B[a]P-induced lung tumorigenesis; and (3) to investigate the effectiveness of a Rev1 siRNA aerosol in suppressing B[a]P-induced mutagenesis and carcinogenesis in lung. These studies should elucidate how genes are mutated to drive B[a]P-induced lung cancer, and provide support for lung cancer prevention by inhibiting REV1 function in mutagenesis.