

Kentucky Lung Cancer Research Program

UK-UL Lung Cancer Retreat (March 27, 2010) Abstracts

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Investigator: J. Anthony Brandon, C. Darrell Jennings, Donald Cohen, Alan M. Kaplan and J. Scott Bryson, University of Kentucky

Research Title: Allogeneic Immunotherapy: An Effective Treatment for Lung Cancer

Therapeutic strategies for non-small cell lung carcinoma (NSCLC) include surgery, chemotherapy and radiation. Many NSCLC patients present with advanced disease that is not treatable by surgery or does not respond to chemotherapy or radiation treatment. An alternative therapeutic approach is the use of allogeneic hematopoietic stem cell transplantation (HSCT) that utilizes the responsiveness of a donor T lymphocyte graft to respond against histocompatibility antigens present on lung cancer cells. It has been shown that a beneficial graft-versus-tumor (GVT) response is associated with a major complication of HSCT, graft-versus-host disease (GVHD). Allogeneic HSCT has been utilized to treat a number of hematologic malignancies and recently selected epithelial solid tumors. The use of this procedure resulted in regression of renal cell carcinoma lung metastases and a case report demonstrated the elimination of NSCLC following HSCT. Given these findings, we utilized major histocompatibility antigen mismatched murine bone marrow transplantation (BMT) models and bioluminescence imaging to test the hypothesis that the allogeneic immune response that develops following allogeneic HSCT will be effective in controlling growth of lung cancer. Our results demonstrated that while GVHD did not develop following reduced intensity (RIC) conditioning and allogeneic donor lymphocyte infusion (DLI) 28 days after BMT, DLI failed to limit the growth of lung tumors. In contrast, when DLI was given after lethal myeloablative conditioning and BMT, a significant reduction in lung tumor growth was observed in the absence of GVHD. In line with a reduction in tumor growth, trafficking studies demonstrated that allogeneic donor cells migrated at an increased frequency to the lungs of myeloablated recipients compared to those conditioned with the RIC regimen. These studies demonstrate the potential efficacy of allogeneic immunotherapy and provide a model system to study alternative therapeutic options in the treatment of malignancies of the lung.

Investigator: Guangrong Zheng¹, Sangeetha P. Sumithran¹, Roger L. Papke², Linda P. Dwoskin¹, Peter A. Crooks^{1*}, University of Kentucky

Research Title: NOVEL *TRIS*-QUATERNARY AMMONIUM ANTAGONISTS AT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS MEDIATING NICOTINE-EVOKED DOPAMINE RELEASE

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bis-3-Picolinium-1,1'-dodecanediyl dibromide (bPiDDB) and its analogs have been shown to potently and selectively inhibit neuronal nicotinic receptors (nAChRs) mediating nicotine-evoked [³H]dopamine ([³H]DA) release from superfused rat striatal slices. The present study investigated the synthesis and activities of a sub-library of novel structurally-related 1,3,5-tri-(pent-1-ynyl-5-azaaromatic quaternary ammonium)-benzene salts and 1,3,5-tri-(*n*-pentyl-5-azaaromatic quaternary ammonium)-benzene salts to inhibit acetylcholine-evoked responses of rat $\alpha 4\beta 2$ $\alpha 3\beta 2$ $\alpha 3\beta 4$ or $\alpha 7$ nAChRs expressed in *Xenopus* oocytes and to inhibit nAChRs mediating nicotine-evoked [³H]DA release from superfused rat striatal slices. These compounds were prepared from corresponding azaaromatic free bases by reaction with 1,3,5-tri-(5-bromopent-1-ynyl)-benzene or 1,3,5-tri-(5-bromopentyl)-benzene. 1,3,5-Tri-(5-bromopent-1-ynyl)-benzene was synthesized by initial Sonogashira coupling of 1,3,5-tribromobenzene with 4-pentyn-1-ol followed by PPh₃/CBr₄ bromination. 1,3,5-Tri-(5-bromopentyl)-benzene was prepared by catalytic hydrogenation of the Sonogashira coupling product and then bromination. Twenty *tris*-quaternary ammonium salts were prepared. Preliminary results show that GZ-551A (1,3,5-tri-[pent-1-ynyl-5-(3-*n*-butyl-pyridinium)]-benzene tribromide), GZ-550B (1,3,5-tri-[pent-1-ynyl-5-(3-phenyl-pyridinium)]-benzene tribromide) and GZ-552A (1,3,5-tri-(pent-1-ynyl-5-quinolinium)-benzene tribromide) were relatively selective for inhibiting $\alpha 7$ nAChR responses compared to the other subtypes tested. GZ-550A (1,3,5-tri-[pent-1-ynyl-5-(3-picolinium)]-benzene tribromide), GZ-554A (1,3,5-tri-[pent-1-ynyl-5-(2-picolinium)]-benzene tribromide) and GZ-555A (1,3,5-tri-[*n*-pentyl-5-(3-picolinium)]-benzene tribromide) exhibited high potency for inhibiting nAChRs mediating nicotine-evoked [³H]DA release. In conclusion, these *tris*-quaternary ammonium salts are considered to be exciting new leads as subtype-selective nAChR antagonists, and may be of value for therapeutic development.

Supported by USPHS Grant U19DA017548

Investigator: Guangrong Zheng, Zhenfa Zhang, Marharyta Pivavarchyk, Andrew Smith, A. Gabriela Deaciuc, Linda P. Dwoskin and Peter A. Crooks*, University of Kentucky

Research Title: NEW LEADS FOR THE TREATMENT OF NICOTINE ADDICTION: NOVEL *BIS*-, *TRIS*-, *TETRAKIS*-TERTIARY AMINO ANALOGS AS ANTAGONISTS AT NEURONAL NICOTINIC RECEPTORS THAT MEDIATE NICOTINE-EVOKED DOPAMINE RELEASE

Neuronal nicotinic acetylcholine receptors (nAChRs) which mediate nicotine-evoked dopamine (DA) release have been suggested to be target receptors of interest in the development of potential smoking cessation therapies. *N,N'*-Dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB), 1,2-*bis*-(5-isoquinolinium-pent-1-ynyl)benzene dibromide (bPyiQB), 1,3,5-*tri*-{5-[1-(3-picolinium)]-pent-1-ynyl}benzene tribromide (tPy3PiB), and 1,2,4,5-*tetrakis* -{5-[(3-(3-hydroxypropyl)-pyridinium)] -pentanyl}benzene tetrabromide (tkP3HPPB) have been shown to potently and selectively inhibit nAChRs mediating nicotine-evoked [³H]DA release from superfused rat striatal slices. However, these quaternary ammonium compounds may not be suitable for further clinical development due to their poor oral and brain bioavailability, and toxicity at high dose. In searching for more drugable compounds for the development of potential tobacco cessation therapy, a series of tertiary amino analogs derived from these lead azaaromatic quaternary ammonium salts has been designed and synthesized. Preliminary structure-activity relationships of these new analogs suggest that such tertiary amino analogs, which are protonated in the biological environment, potently inhibit nicotine-evoked [³H]DA release from striatal slices *in vitro*, may act similarly to the parent molecules in this respect. In contrast to the parent compounds, these tertiary amino derivatives are expected to cross the blood-brain barrier easily through passive diffusion and thus have better brain bioavailability. Further, the tertiary amino derivatives have the potential for an oral method of delivery as a result of the improved membrane permeation capabilities when compared to the parent molecules. Thus, such tertiary amino analogs may provide a new strategy for the design of drugable ligands which are potential smoking cessation therapies.

Supported by USPHS Grant U19DA017548

Investigator: Guangrong Zheng, Zhenfa Zhang, Marharyta Pivavarchyk, Andrew Smith, A. Gabriela Deaciuc, Linda P. Dwoskin and Peter A. Crooks*, University of Kentucky

Research Title: NEW LEADS FOR THE TREATMENT OF NICOTINE ADDICTION: NOVEL *BIS*-, *TRIS*-, *TETRAKIS*-TERTIARY AMINO ANALOGS AS ANTAGONISTS AT NEURONAL NICOTINIC RECEPTORS THAT MEDIATE NICOTINE-EVOKED DOPAMINE RELEASE

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Our research is focused on developing subtype-selective nicotinic receptor (nAChRs) antagonists to inhibit nicotine-evoked dopamine (DA) release. We have previously found that *bis*-azaaromatic quaternary ammonium salts potently and selectively inhibit nicotine-evoked DA release. To enhance bioavailability the quaternary ammonium head groups were replaced with tertiary amine head groups and a series of tertiary amino analogs (r-bIQHxl, r-bIQDB, r-bIQDDB, r-bPiDI, r-bQDDB, bMecDD, bMecD, tMecBPY) was synthesized. Striatal slices were incubated with 0.1 μM [³H]DA for 30 min, superfused for 68 min with Krebs buffer containing nomifensine (10 μM) and pargyline (10 μM), superfused for 36 min in the absence or presence of analogs and then for 36 min with nicotine (10 μM). Nicotine-evoked [³H]DA release was inhibited by the *bis*-tertiary amino analogs with $\text{IC}_{50} = 9.36\text{-}83.5$ nM; $I_{\text{max}} = 47\text{-}74\%$ and by the Mec-analogs with $\text{IC}_{50} = 70\text{-}250$ nM; $I_{\text{max}} = 63\text{-}90\%$. Co-application of a maximal concentration of ether bMecDD, tMecBPY or r-bPiDI with a maximally inhibitory concentration of α -conotoxin MII (α -CtxMII), a selective $\alpha 6\beta 2$ - nAChR antagonist, revealed a lack of additivity inhibiting nicotine-evoked DA release, indicating that these analogs interact at $\alpha 6\beta 2$ nAChRs. The results demonstrate that bMecDD, tMecBPY and r-bPiDI are highly potent $\alpha 6\beta 2^*$ nAChR antagonists.

Supported by USPHS Grant U19DA17548

Investigator: Andrew M. Smith¹, Marharyta Pivavarchyk¹, Zhenfa Zhang¹, Guangrong Zheng¹, J. Michael McIntosh², Peter A. Crooks¹ and Linda P. Dvoskin¹, University of Kentucky

Research Title: DISCOVERY OF b3,5L/3PiDDB AND r-b3,5L/3PiDDB: NOVEL $\alpha 6\beta 2^*$ SUBTYPE-SELECTIVE NICOTINIC RECEPTOR ANTAGONISTS

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The nAChR antagonist bPiDDB potently inhibits ($IC_{50}=2$ nM; $I_{max}=64\%$) nicotine-evoked striatal dopamine (DA) release and decreases nicotine self-administration; however, toxicity was found with repeated treatment. Here, the ability of two analogs of bPiDDB, b3,5L/3PiDDB and r-b3,5L/3PiDDB, to inhibit nicotine-evoked DA release was assessed. Rat striatal slices were superfused for 36 min with a single concentration of b3,5L/3PiDDB or r-b3,5L/3PiDDB. Nicotine (10 μ M) was added to the buffer and superfusion continued for another 36 min. Both b3,5L/3PiDDB and r-b3,5L/3PiDDB inhibited nicotine-evoked DA release with 4.7- and 34-fold higher potency ($IC_{50}=430$ pM and 57.5 pM; $I_{max}=76\%$ and 60%, respectively) than bPiDDB. To determine if these analogs interacted with $\alpha 6\beta 2^*$ nAChRs, striatal slices were co-exposed to maximally inhibitory concentrations of b3,5L/3PiDDB or r-b3,5L/3PiDDB and the $\alpha 6\beta 2^*$ -selective nAChR antagonist, α -conotoxin MII (α -CtxMII). Co-exposure of either analog with α -CtxMII resulted in inhibition not greater than that observed with either antagonist alone, suggesting that both analogs act at α -CtxMII-sensitive nAChRs. Inhibitory effects of the novel antagonists were also determined in nicotine-treated rats (0.4 mg/kg for 10 days). b3,5L/3PiDDB inhibited nicotine-evoked DA release in the repeated-nicotine group with a 1000-fold higher potency when compared to the control group, suggesting that b3,5L/3PiDDB has a higher affinity for those nAChR subtypes that are altered by repeated nicotine treatment. No shift in potency was found for r-b3,5L/3PiDDB in the repeated nicotine group, suggesting this analog may have greater selectivity nAChR subtypes not altered by repeated nicotine treatment. Thus, these novel small molecule antagonists appear to discriminate between subpopulations of $\alpha 6\beta 2$ -containing nAChR subtypes.

Supported by USPHS Grants U19DA17548, R01 MH53631 and T32DA016176

Investigator: Shafiqur Rahman¹, Nichole M. Neugebauer¹, Zhenfa Zhang², Peter A. Crooks², Linda P. Dwoskin² and Michael T. Bardo¹, University of Kentucky

Research Title: EFFECTS OF NOVEL N,N'-DODECANE-1,12-DIYL-BIS-3-PICOLINIUM DIBROMIDE (bPiDDB) ON NUCLEUS ACCUMBENS DOPAMINE RELEASE IN RATS SENSITIZED TO NICOTINE

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The mesolimbic dopamine (DA) system has been implicated in the rewarding effects of many drugs of abuse, including nicotine (NIC). We have shown that the novel nAChR antagonist N,N'-dodecane-1,12-diyl-bis-3-picolinium dibromide (bPiDDB) reduces intravenous NIC self-administration in rats and inhibits acute NIC-induced DA release and DA metabolites in the rat nucleus accumbens (nACB). The present study examined the effects of bPiDDB after repeated NIC treatment on nACB DA release in Sprague-Dawley rats, using *in vivo* microdialysis with HPLC-EC. Rats were pretreated with daily injections of NIC (0.4 mg/kg, once daily sc) or saline for 5 days and then were challenged with 0.4 mg/kg NIC or saline. Measurements of DA release were performed approximately 24 hr after the last injection of NIC. Results revealed increased basal levels of DA in the nACB dialysate following repeated NIC, compared to saline. The NIC challenge increased DA release in nACB, and the NIC-induced effect was blocked by pretreatment (15 min prior to NIC challenge) with the non-selective nAChR antagonist mecamylamine (4 mg/kg, sc). Similarly, pretreatment (15 min prior to NIC injection) with the novel nAChR antagonist bPiDDB (1 or 3 mg/kg, sc) dose-dependently attenuated the NIC-induced DA release in nACB. These results indicate that repeated pretreatment with NIC enhances basal DA levels in microdialysate from nACB, and bPiDDB likely acts as an antagonist at neuronal nAChRs to inhibit the effect of NIC in nACB. The ability of bPiDDB to inhibit the effect of NIC in nACB in rats previously sensitized to NIC and provides further support for bPiDDB as a lead compound with potential utility for the treatment of NIC dependence.

Supported by USPHS Grant U19DA017548

Investigator: Thomas E. Wooters¹, Jason T. Ross¹, Zhenfa Zheng², Peter A. Crooks², Linda P. Dwoskin² and Michael T. Bardo¹, University of Kentucky

Research Title: EFFECTS OF THE NOVEL NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONIST *N,N'*-DECANE-1,10-DIYL-*BIS*-3-PICOLINIUM DIIODIDE (bPiDI) ON NICOTINE SELF-ADMINISTRATION AND FOOD-MAINTAINED RESPONDING IN RATS

¹Psychology and ²College of Pharmacy, University of Kentucky

Although nicotine replacement products, the monoamine uptake inhibitor bupropion (Zyban®) and the nicotinic acetylcholine receptor (nAChR) partial agonist varenicline (Chantix®) are FDA-approved smoking cessation pharmacotherapies, the limited efficacy and potential side effects of these agents warrants further development of alternative medications. We have reported previously that the novel nAChR antagonist, *N,N'*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB), decreases nicotine-evoked dopamine release and attenuates nicotine self-administration. In the present study, we determined the effects of the bPiDDB analog, bPiDI, on nicotine self-administration and food-maintained responding; this analog has reduced affinity for peripheral nAChRs but also decreases nicotine-evoked dopamine release. Adult male Sprague-Dawley rats were trained to respond for either intravenous nicotine (0.03 mg/kg/infusion) or food pellets in two-lever operant conditioning chambers during daily 60-min sessions. Rats were then assigned to receive acute or repeated (7 days) pretreatment with bPiDI (0.58-5.8 µmoles/kg) prior to nicotine self-administration or food-maintained responding. In the acute dose effect experiment, bPiDI (1.94 µmoles/kg) reduced nicotine self-administration to ~40% of the saline control level without altering food-maintained responding; the highest dose (5.8 µmoles/kg) reduced both nicotine self-administration and food-maintained responding. With repeated administration, bPiDI reduced nicotine self-administration without any loss of effect across repeated treatments. In contrast, bPiDI initially decreased food-maintained responding, but this effect dissipated completely across repeated treatments, an effect indicative of tolerance. Thus, these findings indicate that bPiDI has a specific effect on nicotine self-administration with repeated treatment, suggesting that bPiDI represents a new lead compound in ongoing efforts to develop novel smoking cessation agents.

Supported by USPHS Grant U19DA017548

Investigator: Nichola C. Garbett, Ph.D.¹, C. William Helm, MB.Bchir.^{1,2}, A. Bennett Jenson, M.D.¹ and Jonathan B. Chaires, Ph.D.¹, University of Louisville

Research Title: Differential Scanning Calorimetry: A novel tool for discriminating the extent of intraepithelial neoplasia and invasive carcinoma of the cervix

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Invasive cervical cancer (IC) can be prevented if pre-cancerous lesions on the cervix (CIN) are detected and treated. Current screening methods cannot reliably differentiate women with significant precancerous lesions (HSIL) that require treatment from those with lesions that do not require treatment. This means that many women have to undergo additional investigations. Once an invasive carcinoma of the uterine cervix is detected the principal need is to determine whether or not the disease is confined to the cervix without metastasis. Differential Scanning Calorimetry (DSC) provides profiles of the behavior of plasma biomolecules in response to heat yielding a denaturation thermogram that is sensitive to the weighted sum of proteins in solution and their interactions with binding ligands. We have found specific changes in plasma can be detected in several types of cancer and are evaluating the potential of DSC as a clinical diagnostic tool in cervical neoplasia. The method is rapid and requires only a small blood sample. Our results have shown that DSC can discriminate plasma from healthy individuals from those with HSIL and IC with no significant effect of age, ethnicity, smoking or parity. Additional specimens are being collected and analyzed to define a DSC signature characteristic for each stage of pre-cancerous lesion through cancer progression. The method has clear potential in screening for HSIL and IC. The method is also useful for studies of lung cancers.

Supported by the James Graham Brown Cancer Center.

Investigator: Sung Gu Han, Isabel Mellon, Nathaniel Holcomb, Mamta Goswami, David K. Orren and C. Gary Gairola, University of Kentucky

Research Title: CIGARETTE SMOKE INHIBITS THE NUCLEOTIDE EXCISION REPAIR PATHWAY IN HUMAN LUNG CELLS

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Exposure to cigarette smoke introduces DNA damage that likely contributes to the pathogenesis of lung cancer in smokers. Although the DNA damaging properties of cigarette smoke have been well documented, relatively few studies have examined the effect of tobacco smoke on DNA repair pathways. This is especially true for the nucleotide excision repair (NER) pathway which recognizes and repairs many structurally diverse DNA lesions, including those introduced by cigarette smoke constituents. The aim of the present study was to determine if cigarette smoke influences NER in human lung cells. Human lung fibroblasts were exposed to cigarette smoke condensate (CSC, a surrogate for whole smoke exposure) or vehicle (DMSO only) for 24 h and then irradiated with UV-C. UV introduces 2 major DNA lesions, cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs), that are exclusively repaired by NER in mammals. Cells were harvested either immediately after UV irradiation (0 h) or after incubation for additional periods of time to monitor removal of 6-4 PPs and CPDs using the method described below. The amount of 6-4PPs decreased rapidly over a 9 h time interval in untreated (control) cells, reflecting their removal by NER. In contrast, the amount of 6-4PPs persisted longer in CSC-pretreated cells and this inhibition of 6-4 PP removal was pronounced at the 120 and 240 µg/ml concentrations. Thus, the removal of 6-4PPs was inhibited by CSC in a dose-dependent manner. In addition, CSC inhibited the removal of CPDs, the other major UV photoproduct, in IMR90 cells. CSC also inhibited NER in WI38 lung fibroblasts, demonstrating that the inhibitory effects were not specific to IMR90 cells. The abundance of XPC protein which is required for DNA damage recognition in NER was significantly reduced in IMR90 cells treated with CSC for 24hrs. The NER pathway is required to remove many of the carcinogenic DNA adducts formed by cigarette smoke. Hence, the inhibition of NER by cigarette smoke may reflect a novel mechanism underlying cigarette smoke induced lung carcinogenesis. (*Supported in part by the Kentucky Lung Cancer Research Program*).

Investigator: **Manana Melikishvili, Michael G. Fried, Ph.D., University of Kentucky**

Research Title: COOPERATIVITY, CROSSLINKING AND SUPERCOILING IN THE INTERACTION OF AGT WITH DNA

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The mutagenic and cytotoxic effects of many endogenous and exogenous alkylating agents are mitigated by the actions of O^6 -alkylguanine-DNA alkyltransferase (AGT). In humans this protein protects the integrity of the genome, but it also contributes to the resistance of tumors to DNA-alkylating chemotherapeutic agents. Here we report properties of the interaction between AGT and short DNAs and between AGT and covalently closed circular plasmids. We show that while AGT sediments as a monomer in the absence of DNA, it binds with high cooperativity to both single-stranded and double-stranded DNAs. This result is surprising in view of the 1:1 binding mechanism found in crystalline AGT-DNA complexes. Crosslinking analysis allows identification of protein residues juxtaposed in the cooperative complex. The simplest models consistent with the data place the protein in a three-start helical structure with a net pitch similar to that of B-form DNA. Topoisomerase assays test this notion and reveal that AGT unwinds DNA by only ~ 7.3 deg/protein molecule. These results have significant implications for the mechanisms by which AGT locates and interacts with O^6 -alkylguanine lesions to effect DNA repair.

Supported by NIH grant GM 070662.

Investigator: **ShouWei Han, MD, PhD, University of Louisville**

Research Title: Division of Pulmonary, Critical Care and Sleep Disorders
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Research interests include several aspects:

1. Investigate how extracellular matrices (ECMs) such as fibronectin and its splicing variants affect tumor growth, progression and metastasis.
2. Examine the signaling mechanisms that control NSCLC cell growth, and are exploring novel strategies designed to modulate these signals. Some of this work focuses on the actions of peroxisome proliferator activated receptors (such as PPAR α and β/δ) ligands, PGE2 and Tobacco
3. Studying how dietary compounds (such as EPA/DHA and NAC, among others) prevent and control human lung carcinoma cell survival.
4. Exploring the mechanisms by which target pre-oncogenic factors in the control of the pre-malignant phenotype of human lung non-cancer cells.

Expertise in most of the state of art techniques in molecular biology and in animal models.

Investigator: **Chuan Hu, Ph.D., University of Louisville**

Research Title: Vesicle Trafficking of Integrins in the Migration of Cancer Cells

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Integrins are principal receptors for cell adhesion to the extracellular matrix (ECM). During metastasis, cancer cells invade surrounding tissues and migrate into the vascular system. Integrins mediate adhesion at the cell front and serve as traction points to move the cancer cells forward. As transmembrane proteins, integrins are synthesized in the endoplasmic reticulum, transported in vesicles, and delivered to the cell surface by vesicle trafficking. Like other receptors, integrins at the cell surface are continually endocytosed, transported into endosomal compartments and then recycled back to the surface. Therefore, the density of integrins at the cell surface is ultimately determined by the balance between two vesicle trafficking events: the delivery of newly synthesized integrins and the recycling of endocytosed integrins. Despite the key roles of integrins in cancer progression and metastasis, vesicle trafficking of integrins is poorly understood at the molecular level. Elucidating the molecular mechanism of integrin trafficking will likely lead to new drug targets for the treatment of cancer metastasis.

It is now well established in the vesicle trafficking field that SNARE proteins mediate vesicle fusion whereas Rab proteins regulate vesicle targeting and tethering. A primary focus of the research in my lab is to identify the SNARE and Rab proteins important in integrin trafficking, with current emphasis on the endocytic recycling of integrins. We show that the SNARE proteins VAMP2 and VAMP3 colocalize with endocytosed $\beta 1$ integrin. siRNA silencing of VAMP2 markedly reduces cell surface $\alpha 5\beta 1$ and inhibits cell adhesion and chemotactic migration to fibronectin, the ECM ligand of $\alpha 5\beta 1$, without altering cell surface expression of $\alpha 2\beta 1$ or $\alpha 3\beta 1$ integrins, indicating that VAMP2 mediates the trafficking of $\alpha 5\beta 1$ specifically. Interestingly, silencing of VAMP3 reduces cell surface $\alpha 3\beta 1$ without affecting $\alpha 5\beta 1$, and also inhibited chemotactic migration to fibronectin. Surprisingly, knockdown of VAMP3 but not of VAMP2 inhibits endocytic recycling of $\alpha 5\beta 1$. Collectively, these data indicate that distinct pathways exist to deliver $\alpha 5\beta 1$ integrin to the cell surface and that VAMP2 and VAMP3 play different roles in integrin trafficking. In addition, we show that Rab25, which is overexpressed in lung cancer, is involved in the trafficking of both $\alpha 5\beta 1$ and $\alpha 3\beta 1$ integrins. Using confocal microscopy, live-cell imaging, FACS, cell-based and *in vivo* metastasis assays, we are investigating how SNARE and Rab proteins function together to mediate and regulate integrin trafficking.

Investigator: Marie Wehenkel, Jee-Eun Kim, Kim Cornish & Kyung-Bo Kim,
University of Kentucky

Research Title: A selective inhibitor of the immunoproteasome subunit LMP2 suppresses proliferation of non-small cell lung cancer cells

Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky

The approval of the proteasome inhibitor bortezomib for the treatment of recurring multiple myeloma and mantle cell lymphoma has validated the proteasome as an anticancer target. However, the toxicity of this broadly acting proteasome inhibitor to non-tumor cells is a major clinical concern. The immunoproteasome, which is normally expressed in cells of hematopoietic origin, is an alternative form of the constitutive proteasome. Recent studies have shown that the immunoproteasome is up-regulated in some diseases such as cancer and inflammatory diseases. Thus, considerable attention has been paid to the immunoproteasome as a potential therapeutic target. While there have been reports demonstrating promising pharmacological effects for the treatment of multiple myeloma and arthritis by immunoproteasome inhibitors, it is not clear whether the immunoproteasome can be targeted for the treatment of solid tumors, such as lung cancer. We recently found that LMP2, a major catalytic subunit of the immunoproteasome, is highly expressed in most NSCLC cells (Fig. 1). We also found, for the first time, that the inhibition of LMP2 by UK-101, a LMP2-specific inhibitor developed by us, suppresses proliferation of NSCLC cells. These studies indicate the important roles of LMP2 in cancer cell proliferation and provide a rationale for targeting LMP2 for the treatment of solid cancers. Given that the immunoproteasome is not critical for survival of normal cells, we propose that targeting LMP2 provides a novel non-toxic approach for the treatment of lung cancer.

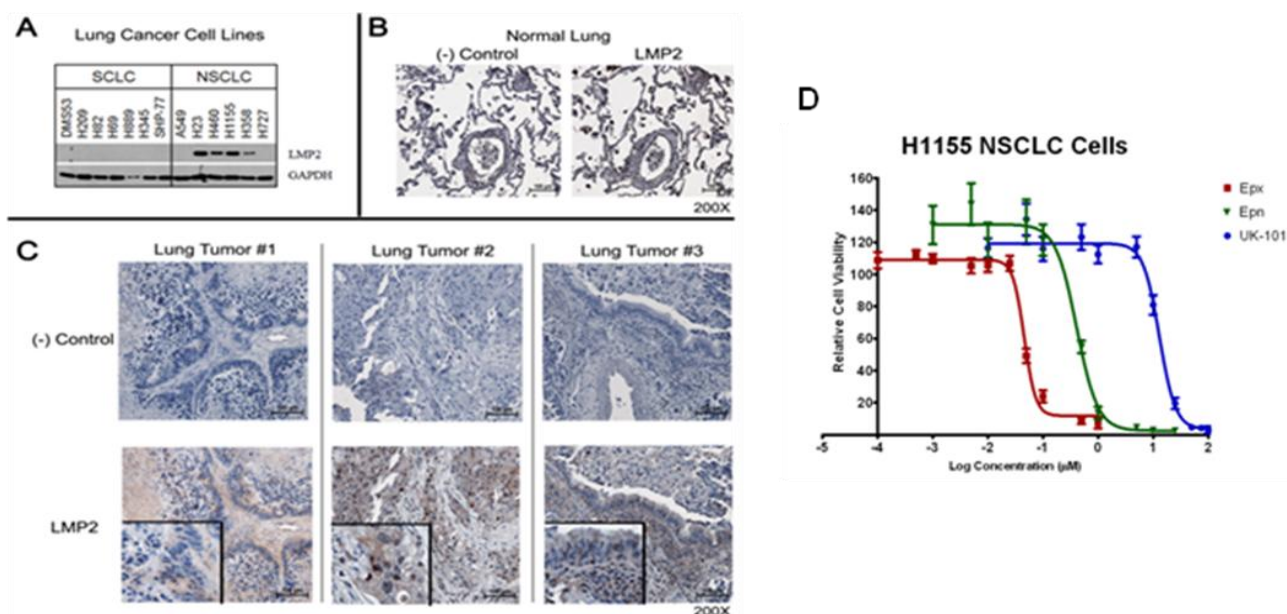


Figure 1. A. Western blotting analysis shows that LMP2 is expressed in NSCLC cell lines. B & C: Immunohistochemical analysis of LMP2 expression (positive signals visualized in dark brown by diaminobenzidine staining). LMP2 is minimally expressed in normal lung tissue. In contrast, the NSCLC tissues express LMP2 at substantially higher level than normal lung tissue. The inset figures are the magnified images. D. IC₅₀ of H1155 cells.

Investigator: **Ann J. Bryan, Heinz Kohler, M.D., Ph.D., University of Kentucky**

Research Title: Second-generation anti-EGFR Antibodies for Lung Cancer

Our laboratory has discovered homophilic antibodies in mice and primates and also developed methods to convert antibodies of choice into homophilic antibodies using chemical and recombinant methods. Compared to conventional antibodies homophilic antibodies form lattices on targets leading to enhanced binding via polyvalent attachment. We generated homophilic Herceptin (Trastuzumab) and demonstrated its superior anti-tumor activity against a human lung cancer cell line. In addition we investigated some biophysical properties of homophilic antibodies. Dimer formation and viscosity of a homophilic antibody is greater at physiological temperature than at 4⁰C. Furthermore, dimer formation is enhanced by reducing the antibody concentration supporting the notion that preformed dimers in solution are the effective molecular species responsible for polyvalent target binding and enhanced therapeutic potency.

Investigator: Jun Li,^{*}† Job C. Tharappel,^{*} Sung Gu Han,[‡]§ Austin H. Cantor,[§] Eun Y. Lee,[{] C. Gary Gairola,[‡] and Howard P. Glauert^{*,‡,1},
University of Kentucky

Research Title: Effect of Dietary Selenium and Cigarette Smoke on Pulmonary Cell Proliferation in Mice

^{*}Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, Kentucky 40506; [†]Health Supervision Institute of Chongqing Municipal Health Bureau, Chongqing, China; [‡]Graduate Center for Toxicology; [§]Department of Animal and Food Sciences; and [{]Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington, Kentucky 40506

The objective of this study was to determine if dietary selenium could inhibit pulmonary cell proliferation in control and cigarette smoke-exposed female A/J mice. Selenium in the form of sodium selenite was supplemented to purified diets similar to the AIN-93M diet to yield 0.15, 0.5, or 2.0 mg selenium/kg diet. After 3 weeks, mice in each dietary group were divided into two subgroups; one used as control, whereas the other was exposed to cigarette smoke for five consecutive days. Mice from both groups were euthanized 3 days later. Mice were administered bromodeoxyuridine in the drinking water starting 5 days before the initiation of the smoke exposure and continuing until they were euthanized. After euthanasia, the left lung lobe was processed for histology and cell proliferation analysis. Cigarette smoke increased cell proliferation in the terminal bronchioles and large airways, but not in alveoli. High-selenium diets inhibited cell proliferation in the alveoli, terminal bronchioles and large airways areas in both control and smoke-exposed mice. Increasing the dietary selenium level led to increased selenium levels in the blood and lung, and increased glutathione peroxidase (GPx) activity in the lung. Cytochrome P-450 1A1 protein levels in the lung were increased by cigarette smoke but were not affected by dietary selenium. It is concluded that dietary selenium inhibits pulmonary cell proliferation in both control and cigarette smoke-exposed mice, indicating that selenium is inhibiting cell proliferation independently of smoke exposure, and that this inhibition may be related to selenium concentration and GPx activity in the lung. Key Words: selenium; cigarette; smoke; cell proliferation; lung.

National Cancer Institute (grant number CA125788)

Investigator: **Xiao-Feng Li, MD, Ph.D., University of Louisville**

Research Title: Department of Radiology, University of Louisville School of Medicine

Research interests:

- Animal models of metastases to lung liver bone etc.
- Study hypoxia, angiogenesis and cancer cells-stroma interaction in metastases of lung cancer, colorectal cancer and breast cancer.
- ¹⁸F-FDG uptake mechanism in metastases relating to tumor hypoxia, GLUTs, cellular proliferation and blood perfusion.
- Molecular imaging cancers and metastases using multi-modalities molecular imaging techniques (Micro-CT/PET, MRI and optical imaging devices), imaging tumor hypoxia and glucose metabolism.
- Development of novel therapeutic strategies to cure micrometastases: targeting hypoxic cells, proliferative cells and neovasculature.

Investigator: **Ronald C. McGarry, M.D. Ph.D., University of Kentucky**

Research Title: Department of Radiation Medicine, University of Kentucky

Research involves writing and administration of clinical trials in Radiation Oncology. Stereotactic Body Radiation Therapy is an emerging standard of care in the treatment of lung cancer and other localised therapies. Having had extensive experience with SBRT, my main interests are to expand the indications and participate in trials of SBRT. I currently participate in the Lung Committee at RTOG. Current local initiative in SBRT trials at UK Lexington:

**Principal Investigator,
Investigator Initiated Trial;**

Stereotactic Body Radiation therapy for Post-chemoradiation Residual Disease in Stage II/III Non-small Cell Lung Cancer
Open to accrual 10/2007

**Principal Investigator,
Investigator Initiated Trial;**

Stereotactic Body Radiation Therapy for Asymptomatic Metastatic Disease to the Thoracic and Lumbar Spine
Open to accrual 01/2010

I would welcome referrals and/or collaborators on these trials.

Secondly we have multiple RTOG trials open and are accruing to them as follows:

07-RAD-01	Stereotactic Body Radiation therapy for Post-chemoradiation Residual Disease in Stage II/III Non-small Cell Lung Cancer
RTOG04-33	RTOG 04-33: A Phase III International Randomized Trial of Single Versus Multiple Fractions for Re-Irradiation of Painful Bone Metastases
RTOG06-14	RTOG 06-14: A Randomized, Phase III, Double-Blind, Placebo-Controlled Trial Of Memantine For Prevention Of Cognitive Dysfunction In Patients Receiving Whole-Brain Radiotherapy
RTOG06-17	RTOG 06-17: A RANDOMIZED PHASE III COMPARISON OF STANDARD- DOSE (60 Gy) VERSUS HIGHDOSE (74 Gy) CONFORMAL RADIOTHERAPY WITH CONCURRENT AND CONSOLIDATION CARBOPLATIN/PACLITAXEL +/- CETUXIMAB (IND #103444) IN PATIENTS WITH STAGE IIIA/IIIB NON-SMALL CELL LUNG CANCER
RTOG06-18	RTOG 06-18: A Phase II Trial of Stereotactic Body Radiation Therapy (SBRT) in the Treatment of Patients with Operable Stage I/II Non-Small Cell Lung Cancer
RTOG08-13	RTOG 08-13: Seamless Phase I/II Study Of Stereotactic Lung Radiotherapy (SBRT) For Early Stage, Centrally Located, Non-Small Cell Lung Cancer (NSCLC) In Medically Inoperable Patients

Relevant current publications:

1. Lo, S.S., Fakiris, A.J., Chang, E.L., Mayer, N.A., Wang, J.Z., Papiez, L., Teh, B.S., **McGarry, R.C.**, Cardenes, H. R., Timmerman, R.D., Stereotactic Body Radiation Therapy: A Novel Cancer Treatment Modality. 2010. Nature Reviews, Clinical Oncology. 7: 44-54
2. Lo, S.S., Fakiris, A.J., Teh, B.S., Cardenes, H. R., Henderson, M.A., Forquer, J.A., Papiez, L., **McGarry, R.C.**, Wang, J.Z., Li, K., Mayer, N.A., Timmerman, R.D. 2009. Stereotactic Body Radiation Therapy for Oligometastases. Expert Rev. in Anticancer Ther. 9(5): 621-35.
3. Fakiris, A.J., **McGarry, R.C.**, Yiannoutsos, C., Papiez, L., Williams, M., Henderson, M.A., Timmerman, R. 2009. Stereotactic Body Radiation Therapy for Early-Stage Non-Small-Cell Lung Carcinoma: 4 Year Results of a Prospective Phase II Study. Int. J. Radiat Oncol. Biol. Physics. 75(3):677-682.
4. Forquer, J.A., Fakiris, A.J., Timmerman, R.D., Lo, S.S., Perkins, S.M., **McGarry, R.C.**, Johnstone, P.A.S. Brachial Plexopathy from Stereotactic Body Radiotherapy in Early Stage NSCLC: Dose-Limiting Toxicity in Apical Tumor Sites. Radiotherapy and Oncology, in press.
5. Henderson, M.A., Hoopes, D., Fletcher, J., Lin, P., Tann, M., Yiannoutsos, C.T., Williams, M., Fakiris, A.J., **McGarry, R.C.**, Timmerman, R. A Pilot Trial of Serial FDG-PET in Patients with Medically Inoperable Stage I Non-Small Cell Lung Cancer Treated with Hypofractionated Stereotactic Body Radiotherapy (SBRT). Int. J. Radiat Oncol. Biol. Physics. 76(3):789-795.

Investigator: Joseph P. McGillis¹, Nichole Frantz¹, Donald Cohen¹, and Peter Laslo², University of Kentucky

Research Title: The Role of Genetic Regulatory Networks in Macrophage Polarization in Lung Cancer

1. Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY, 2. Section of Experimental Hematology, Institute for Molecular Medicine, University of Leeds, Leeds, Great Britain

Macrophages are highly plastic cells with functions in inflammation, immune responses and tissue repair. To work effectively in specific environments, macrophages can be 'polarized' to unique functional phenotypes. A nomenclature has evolved in which classically or alternatively activated macrophages are referred to as "M1" or "M2" macrophages. M1s produce pro-inflammatory mediators, promote immune responses and have increased 'killing' activity. In contrast, M2s produce immunosuppressive mediators and are involved in tissue repair and remodeling. In many solid tumors, macrophages are the most abundant cell type in the stroma and there is a strong correlation between macrophage abundance and prognosis. There is substantial evidence for communication between neoplastic cells and tumor infiltrating macrophages which resemble the alternatively activated M2 phenotype. By inducing an M2-like phenotype, neoplastic cells are thought to suppress tumor specific immune responses and promote macrophage functions that support tumor growth and development (e.g., promotion of angiogenesis). Our working hypothesis is that there are genetic regulatory networks that specify unique sets of genes that are expressed in the M1, M2 and tumor-induced macrophage phenotypes, and that the tumor-supportive M2-like tumor-induced phenotype can be suppressed and/or converted to an M1-like phenotype by targeting genetic regulatory molecules. M1 and M2 macrophage functions have been well characterized in the laboratory but most macrophages *in vivo* probably exist in states along a continuum between prototype M1 and M2 phenotypes. This has made biochemical and genetic studies on polarized macrophages difficult leading to confusing and contradictory results in the literature. To study M1 and M2 polarization, we developed an *in vitro* system where we first differentiate a common myeloid progenitor (CMP) to a naïve macrophage that can subsequently be polarized to relatively pure M1 and M2 phenotypes. These *in vitro* polarized macrophages express M1 and M2 specific genes and have M1 and M2 specific functions. Using genome wide microarray analysis we recently identified 383 and 55 genes uniquely expressed in M1 and M2 phenotypes, respectively, as well as 72 and 180 genes specifically repressed in M1 and M2 states. In addition to transcriptional regulators, the list of uniquely expressed genes includes cytokines, growth factors, matrix proteases and angiogenic factors. To confirm the function of candidate transcriptional factors in M1/M2 polarization, we are 1) using shRNA technology to knock down their expression and 2) forcing continuous ectopic expression. If a candidate transcriptional regulator drives M1/M2 specific gene expression, knocking down its expression should result in a loss of the polarized phenotype in response to polarizing stimuli while forced expression should result in the gain of the polarized phenotype in the absence of polarizing stimuli. Finally, we are also doing studies to confirm the expression of M2-specific genes in a murine lung adenocarcinoma model. These studies will confirm that M2-specific factors are expressed in lung tumor M2-like macrophages. Collectively, these results may provide the basis for novel therapeutic approaches to M1/M2 polarization in tumors.

Investigator: **Daniel J. Noonan, Ph.D., University of Kentucky**

Research Title: TSC Gene Impact On The Pathogenesis Of Female-Specific Lung Cancer Lymphangiomyomatosis (LAM)

The Disease: Lymphangiomyomatosis (LAM) is a female-specific lung cancer that has been genetically linked to mutations in the TSC1 and TSC2 genes, which encode the proteins hamartin and tuberin respectively. These proteins have been classified as tumor suppressors and it is hypothesized that mutations in the TSC genes disrupt their growth suppressing functions resulting in localized tumor development.

The Problem: Currently the only viable treatment for LAM is lung transplantation. Unfortunately, even here a significant number of LAM patients prove to be refractory to transplantation due to the presence of lesions that generate large amounts of chylous effusion. These effusions have been characterized as being rich in triglycerides and cholesterol esters.

Our research: Our interest in this area emanated from a discovery that the TSC2 gene product tuberin could significantly modulate the transcription events mediated by a small family of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), intimately associated with lipid synthesis and storage. In this poster we further investigate the role tuberin plays in basic lipid synthesis and storage parameters of a human embryonic kidney cell line modified to either stably knockdown or stably over-express the TSC2 gene in a doxycycline-inducible manner. The results of this study demonstrate:

- 1) Tuberin expression modulates the growth parameters of protein and non-polar lipid expression,
- 2) Tuberin expression modulates the activities of PPARs, and
- 3) Tuberin expression modulates the expression of lipid uptake and transport proteins.

Clinical Significance: These data would suggest that mutations in tuberin could be linked to chylous production in LAM pathology, and this in part could be mediated by tuberin's impact on PPAR activities. Furthermore, these findings suggest chylous production might be therapeutically targeted through the PPARs and/or cholesterol synthesis inhibitors, proteins for which a variety of small molecule agonists and antagonists have been designed.

Investigator: Daniel J. Noonan, Ph.D., Thomas Roszman, Ph.D., University of Kentucky

Research Title: Inhibition Of Lung Cancer Cell Migration/Invasion By Cell Penetrating Peptides

This proposal examines the hypothesis that inhibition of the morphological alterations obligatory for lung cancer cell invasion and proliferation can be achieved using peptide inhibitors specifically designed to abrogate activation of the Ca²⁺-dependent cysteine protease, calpain II (CpII), upon which these features of malignancy are contingent. More specifically, it is proposed that blocking CpII engagement with its regulatory subunit (Rs), endoplasmic reticulum (ER) and/or Golgi apparatus (GA), will block lung cancer cell proliferation and migration (i.e. metastatic potential) and could have significant therapeutic value in adjunctive treatment of these tumors. To investigate this hypothesis 2 Specific Aims are proposed that will: 1) identify amino acids within the CpII protein that are required for its interactions with the Rs, ER and GA; and 2) develop a cell penetrating peptide (CPP) approach for delivering peptide inhibitors of CpII-Rs, -ER and/or -GA interactions in these cells. Accordingly, recombinant technologies will be used to mutate key amino acid domains within the CpII molecule that facilitate CpII interactions with its Rs and organelles. These mutant CpII structures subsequently will be analyzed for their impact on A-549 cell proliferation, migration and programmed cell death. Information from these studies will be used to develop peptide inhibitors of CpII activities. The efficacy of these inhibitors will be investigated using a CPP delivery approach to analyze the impact these peptides have on lung cancer cell proliferation, migration and survival. Data accrued from this study should provide insight into the role of Cp in lung cancer cell biology and provide a template for the subsequent design of in vivo models that explore the true therapeutic potential of these inhibitors.

Investigator: Xiuling Li, Hsin-Hsiung Tai, Ph.D., University of Kentucky

Research Title: Thromboxane Receptors and Lung Tumorigenesis

Thromboxane A₂ (TXA₂) is a key metabolite of arachidonic acid through cyclooxygenases (COXs) pathway. Thromboxane receptors (TPs) are implicated in mediating tumor cell growth, metastasis and angiogenesis. Molecular mechanisms involved in activating these processes by TXA₂ remain unclear. Our previous studies indicated that a TXA₂ mimetic, I-BOP, induced GSK-3 phosphorylation, β-catenin/TCF/LEF signaling activation, cyclin D1 expression and cell morphology change leading to cell proliferation and transformation. We recent found that I-BOP also induced orphan nuclear receptor Nurr1 expression and stimulated cell proliferation of human lung cancer H157 cells. The induction of Nurr1 expression by I-BOP appeared to be mediated through protein kinase A (PKA)/CREB, protein kinase C (PKC) and ERK pathways and not related to epidermal growth factor receptor (EGFR) pathway. Further studies revealed that Nurr1 mediated cyclin D1 expression and I-BOP-induced cell proliferation since siRNA of Nurr1 blocked both processes. TP-mediated tumor angiogenesis was also investigated in our group. A549-TPα cells induced greater tumor growth and increased vascularization in tumors than in the control A549 cells. Increased angiogenesis could be due to induction of VEGF expression since I-BOP stimulated VEGF synthesis in A549-TPα cells. Our future research plan is to study the role of TPs in lung cancer metastasis. We start with TP-mediated lung cancer invasion step using *in vitro* transwell invasion assay. We will be glad to collaborate with other groups interested in cancer metastasis study.

Investigator: E. Bensadoun, M. Brooks, A. Baron, D. Mannino, E. Hirschowitz, T. Weaver, A. Khan, J. Castle, JD. Miller, S. M. Arnold, University of Kentucky

Research Title: Marty Driesler Lung Cancer Project: Preliminary Report of Prevalence Lung Cancers and Lung Nodules

Purpose: Southeastern Kentucky has one of the highest incidence rates of lung cancer in the United States, as well as a very high prevalence of smoking compared to the national average. Computed tomography (CT) scan screening for lung cancer is controversial, and results of the large, randomized National Lung Screening trial are pending. To improve the efficacy of CT screening, we undertook to enrich the screened population by: 1) targeting a region with a documented high incidence of lung cancer, and 2) further selecting smokers with poor pulmonary function (a known risk factor for lung cancer). This study, known as the Marty Driesler Lung Cancer Project was developed to assess the feasibility of this approach in rural Kentucky. This preliminary report provides data regarding the prevalence of pulmonary nodules and lung cancers found in rural Southeastern Kentucky.

Methods: Participants were screened for eligibility by telephone. Qualifying individuals were required to be 55 to 75 years of age with a history of >40 pack-years of smoking or were former smokers (<10 years of cessation) and were invited to one of four participating regional centers. Those subjects who had an FEV1/FVC<70% (GOLD 1 or higher COPD) underwent non-contrasted, low-dose spiral CT scans and biospecimen collection yearly for three years, and were interviewed with an extensive epidemiologic questionnaire. All research procedures were performed at the community hospital centers.

Results: 955 individuals were screened for eligibility by telephone, and 626 (65%) were eligible. 533 subjects presented to the testing centers and 254 of these (47%) had FEV1/FVC<70% and underwent CT scans yearly for three years. The prevalence rate for lung cancer (lung cancer discovered on the first CT scan) was 1.2% (3 of 254). The incidence of pulmonary nodules was 105 out of 254 (41%): 53 (21%) were calcified, 51 (20%) were non-calcified and in 1 (0.4%) nodule characteristics not recorded. To date, over 5,000 biospecimens (blood, urine and exhaled breath) have been collected during this study.

Conclusions: CT screening for lung cancer in a rural, high-risk population using regional community hospital partners is feasible. The prevalence rate of lung cancer in this population is 1.2%, which was lower than expected. The prevalence of pulmonary nodules was 41%, while the nodule rate requiring further investigation (non-calcified nodules) was 20%. CT screening in Southeastern Kentucky yields a high prevalence of pulmonary nodules and a low prevalence of lung cancer. The study is ongoing, and mature results regarding lung cancer incidence are anticipated in 2012.

Investigator: J.K. Hartsfield, Jr., DMD, PhD, FACMG, ^{1,2,4} L.A. Morford, PhD¹, D.W. Fardo, PhD³, University of Kentucky

Research Title: Genetic Investigation of Disease Etiology, Risk and Treatment Response

The completion of the Human and Mouse Genome Projects and Haplotype Map Project have provided the critical foundation for an explosion in the analysis of genetic and environmental factors on growth, development and disease. The kind of genetic investigation employed often depends on the type of inheritance, the relative contribution, and variation involved in the genetic factor(s). Members of The Hereditary Genomics Laboratory at the University of Kentucky have or are currently involved in primary and collaborative studies on the genetic factors involved in a wide range of areas; including normal and abnormal skeletal growth, dysmorphic syndromes, non-syndromic developmental anomalies, cancer, interaction between bacterial and human genotypes, and variation in response to treatment.

The Lab Director (Dr. Hartsfield) is a Diplomate of the American Board of Medical Genetics, and a Founding Fellow in the American College of Medical Genetics. With the expertise and resources found in The Hereditary Genomics Laboratory (located in MN 279) and collaborators in the University of Kentucky College of Public Health, and the Indiana University School of Medicine Department of Medical and Molecular Genetics (where Dr. Hartsfield was for 15 years and now is an adjunct professor), genetic linkage analysis, linkage disequilibrium and mutation studies may be undertaken in any area of investigation.

Genomic or mitochondrial DNA may be obtained from individual blood samples, cell culture, buccal cells in whole saliva, or pathology samples. Advances in technology have led to the increasing use of DNA obtained from whole saliva, particularly in field studies, or mailings to subjects and family members, where obtaining a saliva sample is often easier to obtain than a blood draw. The use of the Oragene® whole saliva collection containers for extraction of DNA is easy, facilitates transport back to the lab (including by mail), and allows the samples to be stored at room temperature for months without immediate processing.

Studies have shown that this method of DNA collection provides a sufficient quantity and quality of DNA for the analysis of microsatellites, also called single variable number of tandem repeats (VNTR), as well as single nucleotide polymorphism (SNP) genotyping in single applications or microarrays; e.g., candidate gene studies, whole genome linkage panels, or whole genome association studies (GWAS). We also perform whole genome amplification (WGA) for increasing the amount of DNA available from a subject for analysis.

Genetic variation influences practically all areas of biology. Investigation of this variation has become an expected component of many research studies. If you would like to discuss how the Hereditary Genomics Laboratory may help in showing how genetic variation may affect your area of interest, please contact Dr. Hartsfield @ James.Hartsfield@uky.edu or 859-323-0296.