THE CHEMOPREVENTION OF LUNG CANCER USING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

DISSERTATION

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By

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ABSTRACT

A number of chronic inflammatory conditions are associated with increased risks of cancer. One of the key features of chronic inflammation is over-expression of the inducible enzyme, cyclooxygenase-2 (COX-2), which is responsible for conversion of arachidonic acid into molecular mediators of inflammation called prostaglandins. A host of recent studies suggest that COX-2 and its byproducts play a role in the development of certain types of cancer. Epidemiologic investigations have consistently shown that regular use of COX-2-inhibiting, non-steroidal anti-inflammatory drugs (NSAIDs) decreases the risk of colon cancer and breast cancer, and selective studies have noted NSAID-associated risk reductions for several other malignancies. However, one major type of cancer that has not been thoroughly investigated for NSAID effects is lung cancer.

To better understand the effect of regular use of NSAIDs on lung cancer development we undertook a case-control study of lung cancer, matching cases and controls for pack-years of cigarette smoking, as well as age and gender. In all, data for 384 cases from the Arthur G. James Cancer Hospital (Ohio State University, Columbus, Ohio) and 384 controls comprising participants in cancer screenings (breast and prostate) at the same institute were compiled for study. Using the methods of logistic
regression, we observed an overall protective effect for NSAIDs against the development of lung cancer (adjusted OR=0.52, 95% CI=0.38 – 0.72). A decrease in risk was observed in both men (adjusted OR=0.58, 95% CI=0.38 – 0.87) and women (adjusted OR=0.44, 95% CI=0.26 – 0.74). Likewise, significant protective effects of NSAIDs were observed for squamous cell carcinoma (adjusted OR=0.56, 95% CI=0.34 – 0.93) and large cell carcinoma (adjusted OR=0.32, 95% CI=0.14 – 0.72), and a suggestive effect was present for adenocarcinoma (adjusted OR=0.68, 95% CI=0.43 – 1.07). There was also a significant dose response of decreasing relative risk with amount of NSAID use (trend test, p<0.001). These results point to possible chemopreventive effects of NSAIDs against lung cancer in cigarette smokers and suggest a need for further molecular and clinical investigations of the role of COX-2 in lung carcinogenesis.
Dedicated to all of my loving family, my former teachers
and cancer survivors and victims
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CHAPTER 1

INTRODUCTION

1.1) Lung Cancer Facts

In 2001, an estimated 169,500 cases of lung cancer were diagnosed in the United States. This accounted for 13% of all cancers diagnosed. In fact, lung cancer is the second most commonly diagnosed cancer in men and women behind prostate and breast cancer respectively in this country (1).

The 5-year survival rate for lung cancer (all stages and all types) is currently only 14%. Thus, at the current rate, of the 169,500 persons diagnosed with lung cancers last year, 145,770 will be dead in 5 years. In fact, deaths due to lung cancer account for approximately 30% of all cancer deaths in men and women thus making lung cancer the current number one cause of cancer death in both American men and women (2).

Much of the high mortality associated with lung cancer is due to the fact that early detection of the disease is rare. It is estimated that only 15% of lung cancers are detected while the disease is still localized when the 5-year survival rate is as high as 49%. At this time however, early detection is difficult as examination of at risk sites such as bronchial
airways with chest x-rays, sputum analysis of cells and fiberoptic exams have shown to be minimally effective in at risk persons (1).

Until such time as early detection of lung cancer improves, one of the best methods to decrease lung cancer mortality is cancer prevention. While many argue that decreased tobacco use is the logical point at which to attack this problem, the fact is that while the link between tobacco use and cancer has been known for thirty-plus years, people continue to smoke. Currently, approximately 47 million people in the United States or roughly 25% of the countries’ population smokes. While this percentage has declined from its high of 42% in 1965, following the first report by the Surgeon General of the United States warning of the dangers of cigarette consumption, this number has held steady since 1990. In fact, in some segments of the population, tobacco use has increased, most notably in teenagers (1). Along these lines, worldwide tobacco consumption has increased at an alarming rate, most notably in China where over 300 million persons, or one third of the total world’s smokers now live (2). Thus, alternative means to decrease cancer incidence must be explored. One prevention strategy that is gaining acceptance is cancer chemoprevention. Cancer chemoprevention is defined as “the prevention, inhibition or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet” (3).

There are four major histological subtypes of lung cancer. These are squamous cell lung cancer, small cell lung cancer, large cell lung cancer and adenocarcinoma of
the lung. The most common type of lung cancer in the United States is adenocarcinoma, which accounts for approximately 35% of lung cancer cases. This is followed by squamous cell, small cell and large cell lung carcinomas, which account for roughly 30%, 15% and 10% of all lung cancers respectively. Lung cancers not falling into one of these four major categories make up the other 10% of lung cancers found (4). This group includes neuroendocrine tumors, bronchoalveolar tumors, plasmacytomas, spindle cell tumors and mixed cell tumors (5). Interestingly, while over 98% of squamous cell and small cell lung cancers are found in smokers or previous smokers, only 75% of persons with adenocarcinoma have a history of smoking. (4)

1.2.1) Risk Factors Other than Cigarette Smoking for Lung Cancer Development

The most common risk factor known to cause lung cancer is the smoking of cigarettes or other related tobacco products. It is generally accepted that cigarette smoking raises the risk of developing lung cancer 20-25 times that of the risk of a non-smoker (4). It has been estimated that 87% of lung cancers in the world are due to smoking tobacco products (6). However, this estimate still leaves 13% of lung cancers to be accounted for. Of this 13% there are both known and unknown risk factors. Of the risk factors that are thought to be known, a number are related to occupational exposures. These include high-level exposures to asbestos, arsenic, radon and silica (7).

Increased lung cancer risk as a result of exposure to high levels of asbestos has been well established since the 1960’s and the use of asbestos products in the United
States has dropped dramatically since the 1970’s. One should note however, that in many third world countries the use of asbestos continues, most notably in the production of cement products and friction materials. Among the occupations that have been linked to asbestos induced lung carcinogenesis are those involved in the mining and milling of asbestos, and the manufacture of friction products, insulation products, cement products and textiles containing asbestos (7). Interestingly, it has been noted that lung cancer risk in those exposed to asbestos may occur only in those persons developing lung fibrosis (asbestosis) due to asbestos exposure (8). This suggests that chronic inflammatory processes are inducing the cancerous phenotype.

An elevated risk of lung cancer due to long term exposure to arsenic also exists. Occupational arsenic exposure was first reported as a possible lung carcinogen in 1949 in workers exposed to arsenic containing pesticides. Since that time a number of other studies have been conducted implicating arsenic as an occupational hazard that seemingly raises the risk of lung cancer. These studies have been performed on copper smelters who are exposed to arsenic during the smelting process, miners of copper and other ores containing arsenic, pesticide manufacturers (the #1 use of arsenic in the world), agricultural workers exposed to arsenic containing pesticides and in glass and semiconductor manufacturing workers in which arsenic is used (7).

High level exposure to the radioactive gas, radon, as a possible risk factor for development of lung carcinoma was first examined in the 1920’s. However, it was not until 1988 that radon was classified as a Group 1 carcinogen meaning that
sufficient evidence exists to classify it as carcinogenic to humans. Radon is a decay product formed from radium-226, which is present in most soils. Two of the decay products of radon, polonium-218 and polonium-214, emit alpha particles which are highly damaging to tissues. This damage can result in cancer causing derangement of lung cells after radon has been respired and retained by lung tissues. The occupation most associated with prolonged exposure to high levels of radon is mining in areas with high radium concentrations in the ore. Due to the fact that radium is found in almost all soil types, radon exposures can be found environmentally in different concentrations, depending on the radium content of the soil. For this reason, radon can also be found in homes as it seeps up through the soil and enters the house in its gaseous form. Whether this type of exposure is in high enough doses to cause cancer remains in question, but the Environmental Protection Agency recommends that all houses be tested for radon and has provided guidelines for acceptable levels (7).

Increases in lung cancer risk due to exposure to silica has been debated for a long time. In fact, until the early 1980’s chronic silica exposure was thought by most not to increase the risk of developing lung cancer. However, since that time the thinking has changed. Exposure to high levels of silica is thought to be present in many occupations. Among these are miners of metal ores, which are commonly found in deposits with high amounts of silica and workers in granite, stone and tunneling industries including street masons, stone masons, stone quarriers and sandblasters. Additionally, those working with ceramics, pottery, porcelain and glass have occupational exposure to silica as do foundry workers who are exposed to other potential carcinogens such as arsenic.
Exposure to silica by itself, has not been definitively shown to increase the risk of lung cancer. Interestingly, persons with silicosis, a slowly progressing, nodular, fibrosing disease of the lung, have been reported to develop lung cancer at higher rates than persons without silicosis (7). This, much like asbestosis, suggests that chronic inflammation is a possible mechanism by which these agents may elevate the risk of lung cancer in persons chronically exposed.

1.2.2) Cigarette Smoking as a Risk Factor for Lung Cancer Development

As mentioned previously, the number one risk factor for lung cancer is cigarette smoking. It is estimated that 90% of male lung cancer deaths, 80% of female lung cancer deaths and 85% of deaths overall from lung cancer are due to smoking (9). The odds ratios calculated by metanalysis for squamous cell carcinoma of the lung, small cell carcinoma of the lung, adenocarcinoma of the lung and large cell carcinoma of the lung for individuals with greater than 56 pack year exposure to cigarettes (pack years = number of packs of cigarettes smoked a day multiplied by the number of years smoking) are 52.5, 23.9, 11.5 and 20.4 respectively. (10)

There are two main components that cigarette smoke can be divided into, gaseous and particulate. The gaseous phase, which composes 95% of cigarette smoke is comprised mostly of 3 gases: oxygen, nitrogen and carbon dioxide. In contrast, the particulate phase in contrast is composed of more than 3500 components, not all of which have been identified. To date, 55 of these components have been classified as
probable systemic carcinogens with 20 of these classified as pulmonary carcinogens (2). These components are listed in Tables 1 and 2 (See Appendix A).

Classically, cancer is thought to arise from mutations to the genetic code of a cell, which is made up of deoxyribonucleic-acid (DNA). Mutations in the genome arise when bases in the DNA code are altered or changed. Most often these changes in the genetic code are inconsequential and do not alter the cell. However, sometimes the mutations occur in critical areas of a DNA coding region. If this occurs and the cells DNA repair machinery of the cell does not repair the mistake it can become a permanent cell alteration. These alterations can lead to gain or loss in function of the protein that the damaged DNA codes for and can have serious consequences. Most commonly the mutations leading to a cancerous phenotype consist of either gain of function in an oncogene or loss of function in a tumor suppressor gene (11).

A number of the carcinogens in cigarette smoke, most notably 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo(a)pyrene (B[a]P) have been shown to form DNA and protein adducts within cells. These adducts produce mutations in the genetic code of the cell and can alter protein function. Mutations and interruptions due to NNK and B[a]P have been noted in a number of proto-oncogenes and tumor suppressor genes of affected cells (12). Among these is NNK induced mutation of the oncogene Ras. Ras is mutated in a number of tumor types. Among these are adenocarcinoma of the pancreas, colon and lung. Rates of Ras mutation in these tumor types differ, with 80% of pancreatic, 50% of colon and 30% of lung adenocarcinomas
having this type of mutation (11). Ras mutation in cancers of the lung and pancreas have been linked to the cigarette carcinogen, NNK (13,14). This mutation is characterized by a GC to AT transition in the second base of codon 12 of the K-ras gene created by methylation of the O-6 position of guanine (2). Normally, the Ras protein is activated for brief periods of time and then turned off. The mutation in codon 12 leads to an inability of the cell to turn off Ras once it is activated. This constitutive activation leads to uncontrolled cell replication and development towards a cancerous state (4). Likewise, the carcinogen B[a]P has been implicated in adduct formation in lung cancer. Its characteristic adduct creates a GC to TA transversion as well as a less common GC to AT transition (2). While the carcinogen NNK has been theorized and partially shown to be responsible for adenocarcinoma formation in the lung, B[a]P has been theorized and partially shown to be responsible for squamous cell carcinoma formation in the lung (15). Along these lines of thinking, GC to TA transversions are often found in the p53 tumor suppressor gene of persons with a past cigarette smoking history and lung cancer. This type of mutation (GC to TA) is created by the binding of the diol epoxide form of B[a]P to the N2 position of guanine (16). It is also of worth to note that over 66% of Ras mutations in adenocarcinoma are due to transversion mutations, which may be related to mutation by B[a]P (17). Models of lung cancer induction and cigarette carcinogen activation can be found in Figures 1 and 2 (Appendix A). Interestingly, new evidence has come forth that links the cigarette carcinogen NNK to lung cancers in a mechanism separate from DNA mutation. This evidence suggests that NNK may play a role in cell proliferation of the adenocarcinoma and small cell carcinomas of the lung in a cell-type-specific receptor-mediated fashion in which cell proliferation is enhanced (14).
1.3) Chronic Inflammation and Cancer

As mentioned above, it is becoming increasingly apparent that inflammation, especially chronic inflammation has a role in carcinogenesis. For many years it has been recognized that states of chronic immune activation in some instances can lead to cancer. These chronic inflammatory states can be brought about by either infectious or non-infectious disease processes. Among the chronic infections that can lead to cancer are those caused by viruses, bacteria and parasites (18). Viruses were first conclusively reported to cause cancerous lesions in the early 1900’s when Peyton Rous noted the cause of sarcomas in chicken by a filterable agent later discovered to be Rous-Sarcoma Virus (RSV) (19). Since that time a number of other viral induced cancers have been found. Among these are Human papilloma virus (HPV)-induced cervical cancer, Hepatitis B and C virus (HBV and HCV)-induced hepatocellular carcinoma and Epstein Barr virus (EBV)-induced lymphoproliferative and solid tumors (4). Cancer caused by chronic bacterial infections has also been recently discovered in the form of Helicobacter pylori induced gastric cancer (20). The parasite Schistosomiasis which can cause infection in the bladder has also been shown to increase the risk of bladder cancers in those chronically infected with it (21).

A number of other non-infectious conditions have been noted in which chronic inflammation results in increased occurrence of cancer. For example, Barrett’s esophagus (chronic esophagitis), which is caused by chronic acid reflux past the gastro-esophageal junction into the esophagus is known to increase the risk of esophageal
adenocarcinoma. Inflammatory bowel disease, a long-standing inflammatory disease of the large intestine, is associated with increased risk of colon cancer. Cirrhosis of the liver, a liver disease accompanied by chronic inflammatory processes, is associated with an increased risk of liver cancer. Primary sclerosing cholangitis, an inflammatory disease of intra and extrahepatic bile ducts is associated with an increased risk of cholangiocarcinoma, cancer of these ducts. Chronic pancreatitis is associated with an increased risk of pancreatic cancer. Also, as mentioned previously, chronic exposure to asbestos, resulting in the chronic inflammatory condition asbestosis, has been shown to increase the risk of lung cancer and also mesothelioma, a rare cancer of the pleural cavity (4). Additionally, as mentioned above, the chronic inflammatory condition silicosis may be a risk factor for development of lung cancer. Other long standing inflammatory diseases of the lung have been documented to increase the risk of lung cancer such as chronic bronchitis, emphysema and chronic infection with tuberculosis (22). Lastly, and most importantly, cigarette smoking, the number one risk factor for development of lung cancer has been shown to result in chronic inflammation of respiratory airways (23).

1.4) COX-2 and Cancer

One of the possible mechanisms underlying the association between chronic inflammation and cancer is the upregulation of the enzyme cyclooxygenase. Cyclooxygenase (COX), also referred to as prostaglandin endoperoxide synthase, is the key enzyme that catalyzes the conversion of arachidonic acid into prostaglandins and other eicosanoids. An overview of the cyclooxygenase cascade can be seen in
Figure 3 (Appendix A). Two isoforms of COX have been identified to date, COX-1 and COX-2. COX-1 expression is constitutively active in a wide variety of cells whereas COX-2 expression is inducible by factors such as cytokines, growth factors and tumor promoters (24). The primary role of COX-1 is homeostatic production of prostaglandins, typified by balanced blood flow to the kidney and cytoprotection of the gastrointestinal tract. COX-2 expression is believed to be selectively expressed in the inflammatory response and its products are involved in pain and inflammation following injury (25). Non-selective inhibition of COX enzymes with long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been shown to increase the risk of gastric ulcer and, to a lesser extent kidney failure via their inhibition of COX-1. Selective COX-2 inhibitors do not exhibit these side effects (26).

Previous studies have shown that breast, colon and certain lung neoplasms produce greater amounts of prostaglandins than normal tissue derived from the same organ (27, 28, 28). Studies have also shown that in a number of cancerous tissues such as breast, ovarian, prostate, colon, esophageal, pleural, lung and bladder, the associated tumors exhibit increased COX-2 expression. Furthermore, when these cancers are treated with NSAIDs, their growth is inhibited in-vitro (30, 31, 32, 33, 34, 35, 36, 37). Adding to this picture is the fact that in a number of these cancers, COX-2 levels of non-tumorous tissue surrounding the cancerous tissue are found to be the same as tissue in noncancerous patients, while the cancerous tissue exhibits increased levels of COX-2 (32, 34, 35, 37). Additional reports have implicated COX-2 expression and Prostaglandin E2 (PGE2) production with increased mitogenesis, cell differentiation to
malignancy, increased metastasis, decreased immune responses and decreased apoptosis as well as linking COX-2 expression with tumor angiogenesis. These findings suggest a role for COX-2 in the development of cancer and will be discussed in more detail below (38, 39, 40, 41, 42, 43, 44). COX-2 expression has also been correlated to increased formation of reactive oxygen species (ROS), due primarily to the peroxidase action of COX on xenobiotics. ROS molecules have been shown to result in increased oxidative DNA damage, which is thought to lead to tumorigenesis (45). Furthermore, cyclooxygenase has the ability to activate the cigarette carcinogens NNK and B[a]P. Normally, these two carcinogens do not mutate DNA until they are metabolized within the cell to forms that can react with DNA. This is usually carried out by P-450 enzymes, but can also occur via a reaction with the COX enzyme (46, 47).

For cancers where COX-2 expression is specifically upregulated and is important to tumor survival and tumor progression, selective inhibition of COX-2 enzymes may prove to be beneficial in the prevention and treatment. Knockout studies of COX-2 have given credence to this theory showing that MIN (multiple intestinal neoplasia) mice with homozygous COX-2 deletion have 75-80% less tumor formation than wild type MIN-mice (48). In tumors not showing COX-2 expression this may also be true as it has been shown that colon cancer cell lines lacking COX-2 are induced to undergo apoptosis with COX-2 inhibitors (49).

The use of COX-2 inhibitors in preventing lung cancer in animal models has shown to be quite promising. One of the more prevalent animal systems used as a
model for lung cancer is the A/J mouse model. A/J mice have a genetic polymorphism in the K-Ras gene. This polymorphism makes them highly susceptible to lung cancers, especially when administered lung carcinogens. The most common type of cancer derived from administration of the carcinogen NNK to A/J mice is adenoma, a precursor lesion to adenocarcinoma. Studies show that the adenomas and the adenocarcinomas of these mice have high COX-2 contents (50, 51). Treatment of A/J mice with COX-2 inhibitors (both specific and non-specific), prior, during and after administration of NNK results in an approximately 60% decrease in tumor multiplicity (52, 53). These decreases are associated with decreases in COX-2 levels, decreases in PGE2 levels, increases in natural killer (NK) cell cytotoxicity and increases in apoptotic indices in lung tumor cells (52, 54).

Other studies in different models systems such as nude mice xenografted with non-small cell lung carcinoma (NSCLC) show that COX-2 blocking drugs such as aspirin, indomethacin and ibuprofen decrease tumor growth by over 75% (36). In yet another model system, C57BL/6J mice injected with NSCLC cells in their paws, tumor growth has been shown to be decreased by over 75% with the use of the COX-2 inhibitor indomethacin. Treatment with indomethacin is also shown to decrease the number of metastases by over 70% and increase the number of tumor cells arrested in the G1 phase of the cell cycle (55)

COX-2 inhibitors have also been shown to substantially decrease the cell growth rate of human lung tumors in-vitro as well as increasing apoptosis, providing further
evidence of the role of COX-2 in tumorigenesis (36, 55). This hypothesis is strengthened by epidemiologic evidence that shows inverse relationships between intake of NSAIDs and risk of prostate, ovarian, gastric, breast, skin and colon cancer in humans (reviewed in Section 12 below). In fact, celecoxib, a COX-2 inhibitor, has recently been approved by the Food and Drug Administration for the chemoprevention of colon cancer in familial adenomatous polyposis patients (56).

1.5) COX-2 and Lung Cancer

COX-2 is expressed in human lung tumors. Adenocarcinomas of the lung exhibit COX-2 expression in over 85% of tumors examined (57, 58). Likewise, squamous cell carcinomas of the lung also demonstrate COX-2 expression, but to a lesser extent than adenocarcinomas, ranging anywhere from 50% to 100% depending on the study (57, 58, 59, 60, 61). Adenocarcinomas of the lung also stain the most intensely for COX-2 protein as compared to other histologic lung tumor types. COX-2 expression levels in adenocarcinomas are followed by squamous cell carcinomas in terms of intensity of COX-2 immunohistochemical staining, while small cell carcinomas show virtually no COX-2 expression or mRNA production (57, 58). Much like small cell carcinoma, normal human lung tissue, when examined by quantitative mRNA expression, exhibits significantly lower quantities of COX-2 enzyme than that found in adenocarcinomas and squamous cell carcinomas of the lung. Adenocarcinoma COX-2 mRNA levels are also found to be statistically higher than squamous cell carcinomas. (59, 57). In addition to being found in high levels in adenocarcinomas and squamous cell carcinomas of the lung,
the COX-2 enzyme is expressed in high percentages of precursor lesions of these two cancer types (58). COX-2 expression is also linked in one study to poorer survival time in persons with Stage I non-small cell lung carcinoma and in another study to poorer survival in persons with stage I adenocarcinoma (62, 63).

COX-2 levels are also correlated to tumor differentiation status. Two such studies report increased levels of the COX-2 mRNA in poorly differentiated adenocarcinoma and squamous cell carcinoma tumors as compared to more well differentiated NSCLC tumors (59, 61). Likewise, another study performed by Hida et al. shows increased amounts of COX-2 immunostaining in poorly differentiated NSCLC tumors as compared to well differentiated tumors (58). In conjunction, the same study showed that lymph node metastases exhibited increased COX-2 immunostaining as compared to their primary tumor source. Previous studies also show that poorly differentiated non small cell lung cancers (NSCLC’s) of the lung have increased rates of metastasis that corresponded to decreased rates of survival (64, 65). This finding is in agreement with the findings above in which COX-2 positive Stage 1 lung cancers were associated with a poor prognosis. However, in contrast to the two differentiation studies mentioned above, a report citing increased COX-2 mRNA in well-differentiated adenocarcinoma and squamous cell carcinoma tumors as compared to poorly differentiated tumors has been published (57).
1.6) COX-2, PGE2, the Immune System and Cancer

There are two distinct patterns of immunity that can result following activation of T-helper cells, a TH-1 or a TH-2 response. These two patterns are dictated by the types of cytokines secreted by cells of the immune system. The TH-1 response, referred to as cell mediated immunity (CMI), is typified by an activation of CD8+ cytotoxic T-cells and macrophages while the TH-2 response, known as the humoral response, is typified by activation of B-cells (66). The cytokines that regulate these two branches of immune system activation have been well studied and characterized. Those cytokines that activate TH-1, CMI related responses are the interleukins; 2, 1-alpha, 1-beta and 12 (IL-2, IL-1-alpha, IL-1-beta, IL-12), interferon-gamma (IFN-gamma) and tumor necrosis factors (TNF’s). Those cytokines which activate the TH-2 humoral response are IL-4, IL-5, IL-6, IL-10 and IL-13 (67).

TH-1 (CMI) responses have been shown to play a major role in the ability of the immune system to monitor and rid the body of tumors (66). In many types of cancers, there is a suppression of TH-1 type cytokine production and cell mediated immune response, with a concomitant upregulation of TH-2 type cytokines and humoral immune response (67). Along this line of thinking, delayed type hypersensitivity, a marker of CMI or TH-1 type immunity is found to be reduced in a variety of tumors and tumor infiltrates, including small cell lung cancer (67). Additionally, dominant TH-2 responses are associated with increased fatal outcomes in potentially curable tumors (68).
Among the more studied cytokines in lung cancers are the cytokines IL-10 and IL-12. IL-10, secreted by lymphocytes and macrophages, is a TH-2 promoting cytokine and IL-12, secreted by macrophages, is a TH-1 promoting cytokine (67). IL-10 inhibits antigen presentation, and T-cell proliferation in response to antigens and TH-1 cytokine production (69, 70). Treatment with IL-10 makes tumors more resistant to lysis by cytotoxic T-lymphocytes (71). IL-12 in contrast to IL-10 induces the production of TH-1 cytokines and has been demonstrated to be needed for effective antitumor responses by the immune system (72). IL-12 is also necessary to increase IFN-gamma production which is implicated in macrophage activation, part of the TH-1 response (73). Reports indicate that IL-10 is increased in TH-1 immunosuppressive states associated with non-small cell lung cancer (adenocarcinoma, squamous cell carcinoma, large cell carcinoma), whereas IL-12 in these instances is decreased (29).

As previously discussed, COX-2 and its byproduct PGE2 are found at increased levels in many cancer types, including lung cancer. PGE2 is secreted by macrophages as well as lung tumor cells. One of the postulated mechanisms of PGE2 involvement in tumorigenesis is immunosuppression, allowing for the cancerous cells to evade the immune system (29). PGE2 levels are increased in bronchial lavage fluid of lung cancer patients (74). PGE2 has also been shown to decrease the production of TH-1 cells and increase the production of TH-2 cells (75). In conjunction, PGE2 affects the production of the cytokines IL-10 and IL-12 by lymphocytes and macrophages (increase IL-10 and decrease IL-12). This effect in lung cancer is mediated in-vitro by an increase in COX-2 in tumor cells that then produce and secrete PGE2 (29).
The two different branches of T-cell responses are also linked to angiogenesis. As mentioned previously, TH-2 responses and their associated cytokines are upregulated in cancerous conditions while at the same time, TH-1 responses and their associated cytokines are downregulated. Similarly, in studies of upregulated tumor angiogenesis, TH-2 cytokines are increased while TH-1 cytokines are downregulated (67). Angiogenesis is important in tumorigenesis in that tumor cells can only grow to sizes of approximately 2mm$^3$ before they outgrow the capacity of their surrounding blood vessels to supply nutrients, oxygen and to remove waste products generated by the cells. It is generally thought that these cancers exist for some time at this size until such time as increased amounts of blood vessels are formed to facilitate increased growth and progression of the malignancy (76). In fact, high microvessel counts are related to poor prognosis in a number of cancers including that of the lung (77, 78, 79). Studies also show that some of the TH-2 cytokines have direct affects on angiogenesis, specifically IL-6 which increases VEGF expression while conversely indicating that certain TH-1 cytokines have antiangiogenic properties (80).

Chronic disease states are also associated with loss of CMI (TH-1 response) and increases in humoral immunity (TH-2 response). These include chronic hepatitis and inflammatory bowel disease among others (67). Increases in IL-6 a TH-2 associated cytokine are reported in relation to a number of chronic inflammatory processes including asbestosis, ulcerative colitis, chronic hepatitis and cirrhosis of the liver (81, 82, 83, 84). It should be noted that all of these chronic inflammatory conditions have been linked to increased risk of cancer in their respective organs as discussed previously.
Byproducts of cigarette smoke also have an effect on the immune system and angiogenesis. Hypoxia, which is brought about by the increased CO2 content of tobacco smoke suppresses CMI responses (TH-1) and increases angiogenesis (85, 86). Additionally, nicotine, another component of tobacco smoke stimulates DNA synthesis in endothelial cells as well as reducing the production of the TH-1 cytokines (IL-2 and IFN-gamma) and upregulating the production of the TH-2 cytokines IL-4 and IL-10 (87, 88).

1.7) COX2, PGE2, MMP’s, Metastasis and Angiogenesis.

As mentioned above, COX-2 expression alters the profile of the immune system in such a way that it might favor the induction of angiogenesis. In addition to this, a number of findings are available which further serve to identify the COX-2 protein as an inducer of angiogenesis and tumor metastasis. These findings include the induction of matrix metalloproteinases (MMP’s) and various angiogenesis inducing molecules. MMP’s are a family of zinc dependent molecules that are normally secreted in a proenzyme form. These molecules are not activated until cleaved by MMP’s that have already been activated or other proteases such as plasmin (89).

The MMP family of proteins has the ability to degrade basement membranes. This serves as a means for endothelial vessels to reach tumors in the process of angiogenesis. Degradation of the basement membrane also serves as a means by which tumor cells can metastasize to other tissues in the body (89).
Increased production of MMP’s is noted in a number of cancer cell lines in-vitro including lung cancer (90, 91). It has been shown that the amount of MMP expression tends to increase as the disease progresses to a more malignant and aggressive stage. In NSCLC specifically, the MMP family members 2 and 9 exhibit much higher levels of mRNA in Stage 3 disease as compared to Stage 1 and 2 disease (92, 93). Likewise, levels of MMP expression (2,7 and 9) also increase as lung lesions progress from the stages of benign hyperplasia to dysplasia to carcinoma in-situ to malignant carcinoma (94, 95). Thus, as one might expect, the expression level of MMP’s relate to a poorer prognosis in lung cancer (96).

In-vitro studies exhibit the fact that increased levels of MMP secretion lead to the increased capacity for tumor cells to degrade basement membrane and invade (97). Likewise, increased expression of COX-2 leads to an increased ability of tumor cells to degrade basement membranes and invade. This upregulation of COX-2 is reported to increase the levels of MMP’s secreted by these cells (98). Further, treatment of tumor cells with COX-2 blocking agents such aspirin, ibuprofen, NS398 or phospholipase A2 inhibitors decrease the production of MMP’s and concomitantly the invasiveness of the treated cells (99, 100).

MMP expression is also related to angiogenesis in addition to tumor invasiveness. However, unlike in metastasis where MMP expression by the tumor cell leads to invasion, MMP expression in angiogenesis is in part related to production by endothelial cells (101). A number of other factors, in addition to MMP’s are considered
important in the process of tumor angiogenesis. These are angiogenic inducing factors which act both as inducers of angiogenic growth and chemotactic factors to guide the growing blood vessel to the tumor. These angiogenic factors include, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiopoetins (102).

The angiogenic growth factor thought to be the most responsible for tumor angiogenesis is VEGF (102). This factor is secreted by a number of tumor cell types and is found to have increased mRNA levels in various tumors, including lung cancer (103, 104). Increases in secretion of VEGF are reported to mirror the microvessel density within the tumor cell. Increases in microvessel density are have been shown to relate to poorer prognosis (105). Additional reports indicate that blocking the actions of VEGF inhibit the growth of lung carcinomas in nude mice. This inhibition of growth is consistent with a lack of growth of blood vessels within these tumors (106). These results are consistent with the view that VEGF is a key player in the initiation of tumor angiogenesis (107).

One of the regulators of VEGF expression is hypoxia (108). Hypoxia results if there is not adequate blood supply to a tissue. Thus, it is necessary for ever-growing tumor masses to attain an increase in blood supply. This increase supports their continued growth past a certain critical point after which they exhaust the oxygen capacity of their local supply networks (102). The COX-2 protein is also upregulated by hypoxic states (109). The expression of COX-2 protein is correlated to expression
of VEGF (110). Likewise, studies show that COX-2 specific inhibitors can inhibit angiogenesis in tumor models in-vivo as well as mutations of the COX-2 gene and the PGE2 receptor (111, 112). These results imply that induction of VEGF may be, at least in part, COX-2 dependent.

1.8) COX-2, NSAIDs and Apoptosis

Increased expression of the COX-2 gene is associated with increase in the number of cancer cells. Evidence suggests that these increases are not only due to enhanced cell proliferation, but also decreased death of cancer cells. The process by which most cells undergo death is apoptosis (programmed cell death) (113).

The mechanism by which COX-2 overexpression might inhibit apoptosis is still being elucidated. However, a growing evidence points to alterations in the bcl-2 family of proteins as the end product of increased COX-2 expression. Studies using forced expression of COX-2 protein, which increase inhibition of apoptosis in colon adenocarcinoma cells in vitro, show statistically significant increases in expression of the antiapoptotic protein, bcl-2 (114). It is further shown that in-vitro administration of PGE2, a COX-2 metabolic byproduct, leads to a 4.5 fold increases in bcl-2 expression 8 hours after administration. Further, this expression of bcl-2 is preceded by a 4 to 5 fold induction of MAPK (MAP kinase) activation 3 to 6 hours after administration of PGE2 leading to speculation that the MAPK pathway is involved in COX-2, PGE2 mediated apoptosis resistance (115). Similar phenomena are also shown in-vivo in
transgenic Min mice forced to overexpress COX-2. In addition to increased bcl-2 expression, the anti-apoptotic protein bcl-xl is upregulated and the pro-apoptotic protein bax is reduced in expression in the studies (113). Going one step further, in non-neoplastic intestinal epithelial cells, forced overexpression of COX-2 in-vitro leads to resistance to apoptosis and a concurrent increase in the expression of bcl-2 (116).

Based upon the observed findings mentioned above, it would seem most likely that NSAIDs, which block the action of the COX enzymes and hence block production of prostaglandins, the effectors of COX, bring about increased apoptosis by reducing the levels of bcl-2 and bcl-xl and raising the levels of bax. This type of mechanism has been shown to be the case in certain studies. Aspirin is able to reduce bcl-2 levels and induce apoptosis in an esophageal adenocarcinoma cell, NS398; a COX-2 inhibiting drug, reduces bcl-2 levels and induces apoptosis in an androgen-sensitive prostate cancer cell line (LNCaP); and, the NSAID, nabutemone, increases apoptosis correlating with a decrease in bcl-2 protein expression in a colon adenocarcinoma cell line (HT-29) (117, 118, 119). Additionally, NSAIDs induce apoptosis and downregulate bcl-xl levels in another colon cancer cell line (HCT-116), while indomethacin upregulates bax levels in three different esophageal adenocarcinoma cell lines (Flo-1, Bic-1 and Seg-1) (120, 121).

Interestingly however, the upregulation of bax by indomethacin in the esophageal adenocarcinoma cell lines is independent of COX-2 protein expression indicating that other pathways other than the COX pathway may be affected by certain types of NSAIDs (121). In fact, multiple mechanisms have been shown for NSAIDs increasing the
level of apoptosis in cancer cells. In one instance, NS398 was found to induce apoptosis in an esophageal squamous cell cancer line expressing high amounts of COX-2 (TE-8) but not in an esophageal squamous cell cancer line not expressing COX-2. The apoptosis in the TE-8 cells was shown to be reversed by administration of PGE2 and the mechanism of this COX-2 dependent apoptosis was reported to be due to cytochrome c release and not related to alterations of bcl-2, bcl-xl or bax protein levels (122). In another study, on the COX-2 expressing prostate cancer cell lines, PC-3 and LNCaP, it was shown that the COX-2 inhibitors, celecoxib, NS398, rofecoxib and DuP697 were able to promote apoptosis while the non-selective COX inhibitor, naproxen, and the COX-1 specific inhibitor, piroxicam, were not able to induce apoptosis. However, while a COX-2 specific mechanism seemed to promote apoptosis, it was further reported that celecoxib induced apoptosis in the cell lines in mere hours while the other COX-2 inhibiting compounds took 5 days to bring about apoptotic change. Further, the mechanism of celecoxib mediated apoptosis in the prostate cancer cell lines was shown to be bcl-2 independent and was correlated with a blockage of ERK-2 activation, a blockage of AKT activation and an increase in intracellular calcium. The actions of the other three slow acting COX-2 blocking NSAIDs (NS398, rofecoxib and DuP697) were shown not to be correlated to these measures or to bcl-2. Interestingly, it was shown that a dose of celecoxib in excess of three times the amount needed to completely block COX-2 activity was required to stimulate apoptosis (123, 124).

This evidence leads to speculation that not all NSAIDs bring about apoptosis by COX-2 specific mechanisms. This was indeed shown in a study of the COX-2
specific inhibitor NS-398 on two different colon carcinoma cell lines, one that exhibited COX-2 expression (HT-29) and the other not exhibiting COX-2 expression (S/KS). Both cell types underwent apoptosis as a result of NS-398 administration (125). It was additionally apparent in further studies that PGE2 administration was not able to reverse apoptosis in the treated cell lines (126). A mechanism for non-prostaglandin repression mediated NSAID induced apoptosis has been reported by Kinzler et al. In their work, two colon cancer cell lines (SW480 and HCT116) were shown to undergo apoptosis in response to the COX inhibiting drug, sulindac sulfide. The mechanism by which these cells underwent apoptosis was shown to be due to an arachidonic acid increase that stimulated neutral sphingomyelinase, which converted sphingomyelin into ceramide, a known inducer of apoptosis. Ceramide levels were shown to increase up to 10 fold and this induction of apoptosis occurred in immortalized keratinocytes and primary fibroblasts treated with sulindac sulfide, however not to the degree of the colon cancer cells (127). In yet another report arachidonic acid accumulation as a mechanism for apoptosis induction was also found. In this report, increases in arachidonic acid were brought about in COX-2 expressing colon cancer cells (HT-29) by either a COX inhibiting drug, sulindac sulfide or by a triascin C, a drug which blocks the action of fatty acid CoA ligase, an enzyme that like COX, utilizes arachidonic acid. Apoptosis was induced by using either sulindac or triascin C, however in a non-ceramide increasing manner that resulted in the activation of caspase-3 (114). Lastly, it has been reported that very high doses of NSAIDs inhibit the activity of the NF-kappa B pathway and could bring about non-prostanoid mediated cell death in this manner (128).
1.9) Other Possible Mechanisms of COX-2 Induced Tumorigenesis

The upregulation of the COX-2 enzyme is postulated to induce the formation of cancer, including lung cancers through mechanisms other than those listed above (decreased tumor surveillance, increased angiogenesis, increased metastasis, decreased apoptosis, activation of carcinogens in cigarettes). One such mechanism is through the induction of telomerase. Telomerase is an enzyme necessary for a tumor cell to become immortalized. It is responsible for adding hexameric repeats to the end of chromosomes. In normal adult cells, with every cell division there is a shortening of the DNA strands at the end of the chromosomes (telomeres). As the telomeres become shorter, the lifespan of the cell is thought to decrease as apparently once the telomeres shorten past a specific length, the cell is no longer able to replicate itself. In normal cells, telomerase activity is reported to be absent. However, in over 80% of tumor cells, telomerase activity is present (129, 130). The activity of telomerase in essence allows a tumor cell to divide an infinite amount of times without its telomeres ever shortening. Hence, it prevents the chromosomes from ever shortening to a point at which the cell will not divide again (130).

A number of studies exhibit the ability of NSAIDs to inhibit the activity of the telomerase enzyme in tumor cells, both in-vitro and in-vivo. However, whether this effect is due to inhibition of the cyclooxygenase cascade remains to be completely elucidated with reports of telomerase induction being both COX-2 dependent and COX-2 independent events (131, 132, 133).
1.10.1) Adenocarcinoma of the lung and its growth regulation

Adenocarcinoma of the lung, as discussed above, is currently the most prevalent histological subtype of lung tumor in the United States, comprising approximately 35% of all lung tumors diagnosed. This is in stark contrast to the histological distribution of lung cancers up until the 1950’s during which time adenocarcinoma was not a particularly common cancer type. As shown by Heath Jr. et al. who looked at the rise in lung cancers in Connecticut over the last 50 years, the incidence of adenocarcinoma increased nearly 17-fold in women and nearly 10-fold in men during the time period 1950-1991. The dramatic rise in this histological subtype is most closely correlated to the introduction and subsequent usage of filtered cigarettes that are inhaled more deeply by smokers than the non-filtered cigarettes that were used previous to the 1950’s. Adenocarcinomas of the lung are more likely to be found in African-Americans, women and non-smokers than any other lung cancer subtype. The reasons for this are currently unknown (134).

The lung cell thought to be the cell of origin for adenocarcinomas is the Clara cell (135). Clara cells are found in the terminal bronchioles of the lung. These cells are devoid of cilia and contain secretory granules in their apex. It is thought that within these secretory granules are a secondary source of surfactant for the lungs, the primary source being provided by type II alveolar cells (136). Studies indicate that the secretory activity of Clara cells can be stimulated by the catecholamines epinephrine and norepinephrine. Further studies have shown this stimulation to be beta-adrenergic receptor mediated.
It has been noted that conditions of chronic stimulation of cellular secretion often result in cellular proliferation of the stimulated cell. Thus, it may be reasonable to assume that chronic stimulation of Clara cells by norepinephrine, epinephrine or other beta agonists may lead to promotion of an adenocarcinoma of the lung (138).

Solidifying this hypothesis, work by Schuller et al. demonstrates that proliferation of human adenocarcinoma cells of the lung are under the control of beta-adrenergic dependent signal transduction pathways. In their studies, it is reported that beta agonists and cyclic AMP (cAMP) increasing agents increase the growth rate of lung adenocarcinoma cells by statistically significant margins. Furthermore, pretreatment of these cells with beta antagonists or cyclooxygenase inhibitors, before treatment with beta agonists, inhibits the growth rate of these cells. Dr. Schuller’s group also demonstrates that the tobacco specific carcinogen, NNK, is a beta agonist that is able to bind to the beta receptor with high affinity (138). Based on other signal transduction pathway work in other cell types, one can begin to speculate on the complexity of the pathway that will surely arise with further research on the adenocarcinoma cells of the lung.

There are 3 known subtypes of beta receptors in humans. These are beta-1, beta-2 and beta-3. Currently, beta-1 and beta-2 are known to reside in the lung, with the beta-3 subtype having not been found in lung tissue to date (139). More importantly, the beta-1 and beta-2 receptor subtypes are found on the surface of human lung adenocarcinoma cells in a 60% / 40% distribution respectively (137). Binding of a beta-agonist to a beta receptor causes activation of receptor coupled GTP binding proteins (G-
proteins). G-proteins are proteins with 7 transmembrane regions that may activate a number of downstream effectors (139). In the case of G-protein coupled beta receptors, these include adenylate cyclase, Src, Ras, calcium channels and Phospholipase A2 (139, 140, 141, 142). Classically, both beta-1 and 2 receptor subtypes are coupled to adenylate cyclase. Depending on the G-protein receptor subtype, adenylate cyclase can either be turned on or turned off (known as Gs and Gi respectively). If adenylate cyclase is turned on by the Gs subset of G-proteins, ATP is converted into cAMP. cAMP is an activator of protein kinase A (PKA). cAMP acts to activate PKA by binding to the two regulatory units that prevent the two catalytic components of the molecule from functioning. Once the regulatory subunits are bound by cAMP, the catalytic subunits are released and allowed to act. Active PKA preferentially phosphorylates certain substrates. Among the most important are the cAMP response element binding proteins (CREB). Activated CREB proteins are able to bind to certain sequences in promoters of DNA call cAMP response elements (CRE). Binding of the CRE elements by CREB proteins alters the transcriptional activity in the gene that contains the CRE within its promoter (143). One such gene with a CRE in its promoter is the COX-2 gene (144). In addition to affecting CREB proteins, PKA also can interact with the Ras/Raf/MAPK signal transduction pathway. PKA can affect this pathway in multiple manners, depending on the cell type and situation. In some instances, PKA phosphorylates Raf and inhibits its ability to be activated by the Ras protein and activate the MAP kinase cascade. In other instances, PKA phosphorylates and activates Raf (specifically B-Raf), which in turn activates the MAP kinase cascade. In yet another instance, PKA can activate the protein Rap-1, which can activate the MAP kinase cascade irrespective of Ras or Raf, the classical
upstream activators of MAP kinases (AR). The MAP kinase can also be activated by the
proteins Ras or Src, both of which can be activated by beta-2 receptors. Ultimately,
activation of the MAP kinase cascade leads to increases in the activity and availability of
the transcription factors, specifically, c-jun and c-myc which activate the transcription of
genes needed for cell growth and cell division (146, 147).

Activation of Phospholipase A2, as stated above, can also occur when a beta-2
receptor is activated by an agonist (some evidence suggests that this activation may be
dependent upon an influx of calcium created by beta receptor mediated opening of
calcium channels). This phospholipase is responsible for the cleavage of membrane
phospholipids to produce arachidonic acid. The arachidonic acid can then be acted on by
cyclooxygenase and lipoxygenase enzymes to form various second messengers of the
prostaglandin / thromboxane / leukotriene pathway (142). One of the key prostaglandins
produced is prostaglandin E2 (PGE2). Prostaglandin E2 is released from the cell and
bind prostaglandin E2 receptors in an autocrine / paracrine fashion. Upon activation of
these PGE2 receptors, which are G-protein receptors, adenylate cyclase is activated
resulting in cAMP production (AU). Arachidonic Acid can also be part of the MAP
kinase cascade by either activating MAP kinase directly or via protein kinase C (PKC)
activation (AV). Additionally, 15-HETE and 12-HETE, products of the leukotriene
pathway, have been shown to activate the MAP kinase pathway (146, 147).
1.10.2) Small Cell Carcinoma of the Lung and its Growth Regulation

Small cell carcinomas of the lung (aka oat cell carcinomas), as discussed above, account for approximately 15% of lung cancer cases in the United States. This histological subtype of lung cancer has an extremely strong association with cigarette consumption with over 98% of these cancers occurring in persons with a history of smoking. Small cell carcinomas have been identified as the most aggressive of the lung tumors with a propensity towards metastasis leaving little chance of a cure by surgical means. In addition to their malignant potential, small cell carcinoma cells are known for their ability to secrete various neurotransmitters and hormones into the body. For this reason small cell carcinomas of the lung are sometimes referred to as neuroendocrine tumors of the lung (4).

The theorized cell of origin for small cell carcinomas of the lung are neuroendocrine argentaffin (Kulchitsky) cells also known as pulmonary neuroendocrine cells (PNEC’s). These cells are found in larger numbers in the fetus and neonate and seem to play a role in oxygen adaptation of the lung in the newborn, most notably in the act of spontaneous breathing. These cells begin to degenerate around four weeks after birth and are sparsely found in healthy adults. However, in animal studies in which animals are exposed to hypoxic environments, the number of PNEC’s is found to be higher than normal. This is likewise seen in humans with pulmonary disease that reduce the oxygenation of the lung such as chronic obstructive pulmonary disease (COPD), asthma, chronic bronchitis and others. Moreover, consumption of cigarettes leads
to decreased oxygen levels and increased CO2 levels in the lung and has been linked to several pulmonary disease states that further reduce oxygenation of the lungs, such as COPD. Studies by Schuller et al. identify that in-vitro, PNEC cells only proliferate in environments with higher CO2 levels than that seen in healthy adult lungs. They further exhibit that these cells express nicotinic acetylcholine receptors that when activated by an agonist (such as nicotine or its nitrosated derivatives NNN or NNK) in the presence of elevated levels of CO2, further increase the rate of cell growth of PNEC cells (150).

Small cell carcinomas of the lung proliferate in a similar manner to PNECs, with nicotinic agonists or increasing CO2 levels increasing the rate of their proliferation. However, unlike PNEC’s, small cell carcinoma cells are not dependent on nicotinic agonists or higher than normal CO2 levels in order to proliferate (150). Further studies by Schuller et al. have lead to the elucidation of the pathway by which PNEC’s and small cell carcinomas of the lung proliferate in response to nicotinic agonists. The signal transduction system described by their work involves the activation of alpha-7 nicotinic acetylcholine receptors, which when activated open up calcium channels in the cell. This influx in calcium induces the release of neurosecretory products from the agonist activated cell (151, 152). Two of the more studied neurosecretory products released by SCLC's are gastrin releasing peptide (GRP) and serotonin. These two peptides bind to cellular receptors and act in an autocrine fashion once released from the SCLC cell. Binding to cellular receptors results in activation of the MAP kinase cascade in a PKC / Raf-1 dependent manner. The MAP kinase cascade, once activated in this type of cancer, has been shown to activate a number of transcription factors including c-myc, a
transcription factor highly associated with SCLC in humans (153). The fact that GRP is only found in 30% of SCLC's and that serotonin is found in all SCLC's leads to the assumption that serotonin is the more important of the neurotransmitters in the pathogenesis of SCLC (154).

Studies on the pharmacokinetics of nicotine and its derivatives NNN and NNK show that NNK has the highest affinity to the alpha-7 nicotinic acetylcholine receptor found in PNEC's and SCLC's with more than 1000-fold greater affinity than nicotine or NNN. These studies also report that NNK does not show high binding affinity to nicotinic acetylcholine receptors other than alpha-7, while nicotine and NNN do. When these findings are coupled to the fact that over 90% of the nicotinic ACh receptors in the body are not alpha-7 receptors, NNK, in the presence of elevated CO2, presents as the candidate chemical leading to development of small cell lung cancer even though nicotine is found in concentrations of cigarette smoke 5,000-30,000 greater than NNK (155).

1.10.3) Squamous Cell Carcinoma of the Lung and its Growth Regulation

Up until the early 1980’s, before adenocarcinoma of the lung passed it, squamous cell carcinoma of the lung was the most common type of lung cancer seen. As discussed previously, at present, squamous cell carcinomas of the lung account for approximately 30% of lung cancer cases in the United States. Much like small cell carcinoma of the lung, squamous cell carcinoma of the lung has an extremely strong association with
cigarette smoking with over 98% of these cancers occurring in persons with a history of smoking. Although squamous cell carcinomas tend not to metastasize as often as the other histological types of lung cancer, its rate of growth is usually faster (4).

Squamous cell carcinoma of the lung is thought to arise from basal bronchial epithelial cells (156). Unlike adenocarcinoma of the lung and small cell carcinoma of the lung, the growth-regulating pathway controlling squamous cell carcinoma of the lung has not been linked to a receptor of the autonomic nervous system. In fact, the pathway regulating growth of squamous cell carcinoma of the lung is not entirely clear. One receptor that has been linked to squamous cell carcinoma is the epidermal growth factor receptor (EGFR) (157).

Increased EGFR expression has been shown to correlate to metaplasia of bronchial epithelium (a premalignant change) and squamous cell lung cancer (157). Additionally, in some epithelial tumors EGFR has been shown to be non ligand binding, however constitutively activated (EGFRvIII oncogene). Squamous cell carcinomas of the human lung, much like adenocarcinomas and small cell carcinomas of the lung, regulate their growth in an autocrine fashion via the production of the EGFR ligands, most notably TGF-alpha (158).

The EGFR is a member of a superfamily of receptors with tyrosine kinase activity. It can be activated by at least five different ligands, which include epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), heparin binding
EGF and a number of virally encoded factors. EGFR is present on all epithelial and stromal cells as well as some smooth muscle and glial cells. Upon binding by a ligand, the tyrosine kinase activity of the EGFR increases and it begins to phosphorylate itself as well as downstream effectors, in turn, activating them. These effectors include signal transducer and activator of transcription isoforms (STATs), phospholipase C (and consequently PKC), Ras (and consequently MAP kinase), and others. These pathways can carry out multiple actions including mitogenesis, activation or inhibition of apoptosis and cell migration (158). Only recently has work begun to detect the actions of the effectors of these pathways. Early work shows that at least in some types of squamous cell carcinoma, Stat3 (a STAT isoform) signaling acts to block apoptosis. The mechanism for this blockage is thought to most likely occur by upregulation of the antiapoptotic protein bcl-xl, whose gene promoter has been shown to be regulated by Stat3 and whose mRNA levels rise with increased Stat3 activation (159). Stat3 activation is also linked to production of TGF-alpha and EGFR expression, thus forming an autocrine loop (160). Other work shows that activation of Ras and the MAP kinase cascade is critical for squamous cell carcinoma proliferation, but not invasion (161). Invasion rather, seems to be mediated by the PLC activated PKC pathway (158).

Another tumor associated factor found in squamous cell carcinomas is the COX-2 enzyme, which is found to be up-regulated in these tumors (57). Increased levels of COX-2 in these tumors can be induced by TGF-alpha or EGF binding of EGFR. This upregulation in squamous cell carcinomas is linked to induction of the MAP kinase cascade (162). As noted earlier, MAP kinase activation can lead to COX-2 upregulation.
Additionally, the COX-2 gene has a Stat3 enhancer element in its promoter, thus providing yet another possibility for EGFR activation to increase COX-2 levels (163).

1.10.4) Large Cell Carcinoma of the Lung and its Growth Regulation

Large Cell Carcinomas of the lung account for a little less than 10% of all lung cancers seen in the United States (165). It is thought that these carcinomas may represent squamous cell carcinomas and adenocarcinomas that are so undifferentiated that they can no longer be recognized (4). To date little research has been done on this type of cancer and possible signal transduction pathways regulating its growth and other malignant features.

1.11.1) Chemoprevention Possibilities for Adenocarcinoma of the Lung

Using the above findings, a potential model for lung adenocarcinoma growth signaling can be created (Figure 4, Appendix A). Based on the speculated and known signaling molecules within the adenocarcinoma growth pathways, a number of potential chemopreventives could be used to target areas within the pathways where inhibition of growth may take place. Chief among these are the beta-blocker agents which would inhibit the activation of the pathway at the very source from where it is thought to originate. Beta-blockers are used therapeutically to treat hypertension, prevent ischemic heart disease, treat certain types of heart arrhythmia’s and various other heart related diseases as well as in the treatment of migraines (165). Another set of potential
chemopreventive drugs are the HMG-CoA reductase inhibitors. These drugs are used therapeutically to lower cholesterol levels by inhibiting the rate-limiting enzyme in cholesterol production, HMG CoA reductase. This limitation works to block the conversion of HMG-CoA into mevalonate and hence other downstream cholesterol synthesis intermediates (165). One of the key cholesterol synthesis intermediates that might be inhibited from being produced is farnesyl. Farnesylation allows Ras to be introduced into the cell membrane where it can interact with cell membrane receptors that can activate it. Failure of Ras to reach the cell membrane would result in its inability to be activated and upregulate certain signal transduction pathways, specifically the MAP kinase cascade (166). As mentioned previously, approximately 30% of adenocarcinomas of the lung have gain of function mutations of the Ras gene that allow it to be activated constitutively. Coupled to the fact that Ras may play a role in the signal transduction pathway of the adenocarcinoma cell even without Ras mutation, blockage of this signal transduction cascade could prevent cell growth in the adenocarcinoma system and could inhibit adenocarcinoma development. Yet another potential chemotherapeutic agent to be tested are cyclooxygenase inhibitors. The cyclooxygenase inhibitors would act to prevent the conversion of arachidonic acid into prostaglandins, which as described previously can inhibit proper immune responses to tumors, inhibit apoptosis and promote metastasis, invasion, angiogenesis and carcinogen activation. Additionally, they have been shown in-vitro, as described above, to inhibit the growth of adenocarcinoma cells. These drugs, specifically aspirin and NSAIDs are the most commonly taken drugs in the United States (167). Therapeutically they are taken to reduce pain and inflammation as well as in the prevention of heart attacks and strokes (165). Calcium channel blockers
may be another subset of drugs that could inhibit adenocarcinoma cell growth by blocking the activation of Phospholipase A2 and hence production of arachidonic acid. These drugs are used most commonly in the treatment of hypertension, angina, congestive heart failure and heart arrhythmia’s (supraventricular tachycardia) (165). Another class of drugs that also blocks phospholipase A2 action, however in a more direct manner, are the glucocorticoids. These drugs selectively block the COX-2 enzyme. Glucocorticoids are commonly used as antiinflammatory drugs and in the treatment of asthma (165).

There are also a number of drugs that may increase the risk of adenocarcinoma of the lung by activating the growth regulation of these cells. Chief among these are the beta-agonists. These drugs are used therapeutically to increase large airway diameter in those with asthma and COPD, increase the heart rate in those persons suffering from heart failure, act as over the counter decongestants and, are an active ingredient in some diet pills (165). These drugs would act to increasingly stimulate the proliferation of adenocarcinoma precursor cell by activating beta receptors, which are the start of the signal transduction pathway mentioned above. Other drugs that may potentially increase the risk of adenocarcinoma of the lung are glucocorticoids, drugs that increase cAMP levels within cells, tricyclic antidepressants (TCA’s) and monoamine oxidase inhibitors (MAO inhibitors). Glucocorticoids as mentioned above are used primarily as antiinflammatory drugs and in the treatment of asthma. While glucocorticoids as previously discussed have been demonstrated to block the action of phospholipase A2 and COX-2, they have also been shown to potentiate the action of beta agonists and upregulate
beta-2 receptors via glucocorticoid response elements (GRE’s) in the beta-2 receptor gene promoter (138). Predominance of the latter two effects might allow for glucocorticoids to potentiate adenocarcinoma development rather than protect against it. The most commonly known drugs to increase cAMP in cells are the methylxanthine drugs. Among these are theophylline, theobromine and caffeine, which are found in tea, cocoa and coffee respectively. Theophylline is also prescribed as treatment for asthma. The postulated mechanism of action of these drugs is the inhibition of the enzyme phosphodiesterase. This enzyme is responsible for the breakdown of cAMP into AMP (165). Blockage of this enzyme would theoretically cause a rise in cAMP leading to increased activation of PKA and would potentiate the downstream effects (aka cell growth) of this signal transduction pathway. Tricyclic antidepressants, which are used in the treatment of depression, panic disorder and obsessive compulsive disorders to name a few, block the reuptake pumps of the neurotransmitters serotonin and norepinephrine (165). Use of these drugs would allow norepinephrine, a classic beta agonist, to increasingly activate the beta-receptors and hence stimulate the growth of adenocarcinomas for longer periods of time. MAO inhibitors are also used for a similar spectrum of ailments as the tricyclic antidepressants. These drugs act to inhibit the function of the oxidase that degrades norepinephrine, serotonin and tyramine, MAO-A (165). It is proposed that the MAO-A inhibitors will allow greater amounts of norepinephrine to build up in the cells that secrete it. Thus, excitation of these cells will result in larger amounts of norepinephrine than normal to be released resulting in the stimulation of the growth of adenocarcinomas or their precursor cells to a greater degree.
Other risk factors for adenocarcinoma may be states of chronically high epinephrine and/or norepinephrine (beta-agonists) content in the body (pheochromocytoma, hypertension) (4). Chronically high levels of beta agonists in the body would lead to increased activation of the signal transduction pathways necessary in the growth of lung adenocarcinoma cells.

1.11.2) Chemopreventive Possibilities for Small Cell Lung Carcinoma

Using the above findings, a potential model for small cell lung cancer growth signaling can be created (Figure 5, see Appendix A). Based on the speculated and known players within the SCLC growth pathways, a number of potential chemopreventives can be used to target areas within the pathways where inhibition of growth may take place. Chief among these drugs are the selective serotonin reuptake inhibitors (SSRI's), which were shown in-vitro to inhibit the growth of SCLC's (150). These drugs are commonly prescribed to treat psychiatric problems encompassing depression, panic disorder, and obsessive compulsive disorder to name a few. These drugs work to inhibit the reuptake of serotonin once it is released (165). While in theory one might expect that blockage of reuptake might allow serotonin to act for longer periods of time by continually being able to activate serotonin receptors, this has shown not to be the case in-vitro. It has been theorized that perhaps the reason that blockage of serotonin reuptake is effective is that this proliferation system is an autocrine growth regulatory mechanism and that inhibiting the reuptake of serotonin leads to decreases in its availability upon continued nicotinic agonist stimulus. Thus, while over a very short time SSRI's might increase the
autocrine stimulus, in the long haul they will decrease it (150). Two other drugs that may inhibit the development of SCLC are calcium channel blockers and drugs that raise the level of cAMP in the body. Calcium channel blockers as mentioned above, are used most commonly in the treatment of hypertension, angina, congestive heart failure and heart arrhythmia’s (suprventricular tachycardia). Blockage of calcium channels in this signal transduction system would block the release of neuropeptides, specifically serotonin, which has been shown to be an autocrine growth factor for SCLC's. As mentioned above, the most commonly known drugs to increase cAMP in cells are the methylxanthine drugs. Among these are theophylline, theombrine and caffeine, which are found in tea, cocoa and coffee, respectively. Theophylline is also prescribed as treatment for asthma. The postulated mechanism of action of these drugs is the inhibition of the enzyme phosphodiesterase. This enzyme is responsible for the breakdown of cAMP into AMP. Blockage of this enzyme would theoretically cause a rise in cAMP and would lead to activation of PKA. In some cell lines, as mentioned above, PKA has been shown to block the activation of Raf-1 by phosphorylation rendering it unable to activate the MAP kinase cascade, whose activation has been discovered to lead to SCLC proliferation. Activation of PKA by the methylxanthine drugs may however increase the risk of development of SCLC. This is due to the fact that in some cell lines PKA phosphorylates and activates Raf (specifically B-Raf), which in turn activates the MAP kinase cascade. In yet other instances, PKA can activate the protein Rap-1, which can activate the MAP kinase cascade irrespective of Ras or Raf, the classical upstream activators of MAP kinases, all of which would lead to increases in cell proliferation of SCLC or its precursor cells.
One other class of drugs that might increase the risk for SCLC development are the MAO-A inhibitors. MAO inhibitors are also prescribed in the treatment of depression, panic disorder and obsessive compulsive disorders to name a few. These drugs, as mentioned previously, act to inhibit the function of the oxidase that degrades serotonin, norepinephrine and tyramine. It is proposed that the MAO-A inhibitors will allow greater levels of serotonin to accumulate in the neurosecretory vesicles of the developing SCLC and thus allow for greater autocrine stimulation.

One other drug that might have chemopreventive activity in SCLC are the cyclooxygenase inhibitors. The cyclooxygenase inhibitors as mentioned above act to prevent the conversion of arachidonic acid into prostaglandins / thromboxanes. These drugs, specifically aspirin and NSAIDs are the most commonly taken drugs in the United States. Therapeutically they are taken to reduce pain and inflammation as well as in the prevention of heart attacks and strokes. Certain prostaglandins, specifically PGE2 as mentioned above, have been shown to not only inhibit apoptosis and proper immune responses to tumors, but also stimulate cell growth, cell immortality, cell invasion, cell metastasis, carcinogen activation and angiogenesis. Interestingly, the effects on angiogenesis have been purportedly linked to the COX-2 enzyme not only in tumors, but also in endothelial / stromal cells. Given the fact that COX-2 has been reported to be sparsely found, if at all in small cell carcinoma cells of the lung in humans, the potential chemopreventive activity of cyclooxygenase inhibitors would most likely be in the prevention of angiogenesis for developing SCLC's. As previously explained, evidence suggests that tumor cells can only develop to the size of 2mm^3 in diameter before their
metabolic capacity exceeds that of the normal surrounding vascular system. Without an influx of new blood vessels to supply nutrients and oxygen and take away waste products all new cell growth / mass will die off. Coupled with the notion that angiogenesis has been linked to tumor metastasis, it is possible that cyclooxygenase inhibitors could block the progression of a growing tumor at which time it might be removed by the immune system or lay in a sense dormant.

1.11.3) Chemopreventive Possibilities for Squamous Cell Carcinoma of the Lung

Using the above findings, a potential model for squamous cell lung cancer growth signaling can be created (Figure 6, see Appendix A). Based on the speculated and known players within the squamous cell lung cancer growth pathways, a number of potential chemopreventives can be used to target areas within the pathways where inhibition of growth may take place. Among these are the cyclooxygenase inhibitors, calcium channel blockers, drugs that increase cAMP and HMG-CoA reductase inhibitors. As mentioned previously, cyclooxygenase inhibitors act to prevent the conversion of arachidonic acid into prostaglandins / thromboxanes and are used therapeutically to reduce pain and inflammation as well as in the prevention of heart attacks and strokes. One set of products of the arachidonic acid cascade, prostaglandins, as mentioned above, have been implicated in tumor growth, metastasis, angiogenesis, carcinogen activation, immune suppression and inhibition of apoptosis. Given that COX-2 has been found to be elevated in squamous cell carcinomas, possibly the inhibition of COX-2 end-product formation will reduce the development of this histologic type of cancer. As mentioned above, the
most commonly known drugs to increase cAMP in cells are the methylxanthine drugs. Among these are theophylline, theobromine and caffeine, which are found in tea, cocoa and coffee respectively. Theophylline is also prescribed as treatment for asthma. The postulated mechanism of action of these drugs is the inhibition of the enzyme phosphodiesterase. This enzyme is responsible for the breakdown of cAMP into AMP. Blockage of this enzyme would theoretically cause a rise in cAMP and would lead to activation of PKA. In some cell lines, PKA has been shown to block the activation of Raf-1 by phosphorylation rendering it unable to activate the MAP kinase cascade, whose activation has been discovered to lead to SCLC proliferation. Activation of PKA by the methylxanthine drugs may however increase the risk of development of squamous cell lung carcinoma. This is due to the fact that in some cell lines PKA phosphorylates and activates Raf (specifically B-Raf), which in turn activates the MAP kinase cascade. In yet other instances, PKA can activate the protein Rap-1, which can activate the MAP kinase cascade irrespective of Ras or Raf, the classical upstream activators of MAP kinases, all of which would lead to increases in cell proliferation of squamous cell lung cancer or its precursor cells. Calcium channel blockers as discussed above, are used most commonly in the treatment of hypertension, angina, congestive heart failure and heart arrhythmia’s (suprventricular tachycardia). Blockage of calcium channels in the squamous cell lung cancer growth pathway might potentially block the activation of PKC. Blockage of PKC in this cell type might not inhibit growth of cells, but might inhibit the ability of the tumor to metastasize. Yet another drug that may inhibit squamous cell carcinoma formation are the HMG-CoA reductase inhibitors. As mentioned above, these cholesterol lowering drugs are thought to inhibit the
farnesylation and hence the insertion of Ras into the cell membrane, thus inhibiting its action. Blockage of Ras would lead to at least partial inhibition of the MAP kinase cascade, which is purported to be part of the growth signaling cascade of squamous carcinoma cells.

1.11.4) Chemopreventive Possibilities for Large Cell Carcinoma of the Lung

While little is known about the possible signal transduction pathways within the large cell carcinoma tumors of the lung, speculation on possible chemoprevention of this type of cancer can also be made. This is based on the assumption that large cell carcinomas are similar to adenocarcinomas and squamous cell carcinomas of the lung, though having de-differentiated to a state where their morphology appears different. Based on this assumption, one could begin to surmise that the chemoprevention agents theorized above for adenocarcinoma and squamous cell carcinoma of the lung, might also work on large cell lung carcinoma. Key among these would be the NSAIDs which inhibit the cyclooxygenase cascade, a pathway thought to be important for the tumorigenicity of both tumor types (adenocarcinoma and squamous cell carcinoma) from which large cell carcinoma may arise.
1.12) Epidemiologic Studies of NSAID use and Cancer in Humans

As mentioned previously, a number of epidemiologic studies have been done examining the effects of the NSAIDs on certain cancer types. A description and the results of those studies are listed below.

Studies of NSAID use and Prostate Cancer

Two case control studies have been published in which NSAID use was examined in relation to occurrence of prostate cancer alone. In the first study, daily use of either aspirin or ibuprofen was found to decrease the risk of development of prostate cancer by 66% (OR= 0.34, 95% CI= 0.23-0.58). The study also reported a 65% decreased risk of prostate cancer in males reporting to take prescription NSAIDs (OR= 0.35, 95% CI= 0.15-0.84) (168). In the second study, regular use of aspirin and NSAIDs (one or more per week) was found to lower the risk of developing prostate cancer, however not at significant levels (OR = 0.88, 95% CI= 0.64 – 1.20) (169).

Studies of NSAID use and Bladder Cancer

A few case-control studies have been done to investigate the effects of NSAID use on the risk of development of bladder cancer. One such study showed that regular analgesic users (use consisting of 2 or more times a week for a month or longer) overall were at decreased risk of bladder cancer compared to irregular (never used 2 or
more times a week for a month or longer) or non-users (used less than 20 times in a person's lifetime) (OR= 0.81, 95% CI= 0.68-0.96). The study also showed that acetaminophen use was not correlated to bladder cancer incidence while use of phenacetin was associated with an increased risk of bladder cancer. Among NSAIDs reducing cancer, the acetic acids were shown to decrease the risk of bladder cancer the most (OR= 0.54, 95% CI= 0.31-0.94) while aspirin, other salicylates and oxicam showed the weakest effects (OR’s = 0.88, 0.81 and 0.92 respectively with none achieving significance with the 95% CI) (170).

The effect of NSAIDs on bladder cancer was also looked at in two other studies that examined the risk of a broad array of cancers as well as bladder cancer. The results of these studies can be found below in the paragraph examining studies of NSAID use in relation to a number of cancers.

Studies of NSAID Use and Melanoma

One study on the effects of NSAID usage in relation to development of malignant melanoma has been performed to date. The study was done as a case-control study involving women only. In women using NSAIDs regularly (one or more pills per day for more than one year), it was found that there was a decreased risk of their developing malignant melanoma (OR= 0.45, 95% CI= 0.22-0.92) (171).
A number of published studies have examined the relationship between NSAID use and more than one cancer type. In the first of such studies, performed by Rosenberg, a hospital based case control study was done examining the relationship between regular use of NSAIDs (4 days a week for at least 3 months at least a year and a half before admission to the hospital) and a number of common cancers. The study found a reduced risk of breast, lung, ovarian, colon, rectal and bladder cancer incidence in both cancer and non-cancer controls. However all of these findings, other than colon and rectal, were non-significant at the level of alpha=.05. Additionally, virtually no reduction in risk was found for malignant melanoma, leukemia/lymphoma or endometrial cancer (OR’s = 1.0 with no significance at alpha=.05) (172). In yet another hospital based case-control study by Rosenberg et al., the relationship between NSAID use and digestive cancers (other than colon cancer) was examined. This study showed a decreased risk in stomach cancer in continued regular users of NSAIDs (4 or more times a week for at least 3 months, initiated at least 1 year before admission that continued to admission). This reduction of risk was 70% and was significant at the alpha=.05 level (OR= 0.3, 95% CI= 0.1-0.6). Reduced risks of pancreatic, esophageal, gallbladder and liver cancers were also seen however these did not remain significant at the alpha=.05 level. Similar results were seen in regular users with over a 5 year duration of use (173). Another study of NSAID use and the incidence rate of a number of cancer types was a case control study, performed using a UK national prescription database. Due to the nature of the study, only prescription NSAID use was viewed in relation to the cancers examined. This
study showed an association between prescription NSAID intake (7 or more prescriptions 13 to 36 months before diagnosis date) and reduced risk of esophageal, stomach and colon cancer that continued to be significant at the alpha = .05 level. The study also showed association with reduced risk of rectal and lung cancer however these were non-significant at the alpha = .05 level. The study also showed increased incidences of a few cancers in relation to NSAIDs such as pancreatic, bladder, breast and prostate, however, only prostate and pancreatic cancers showed significance at the alpha=.05 level and barely so (174). In yet another report, a population based case-control study was done by Blot et al. to examine the association between NSAID use and subtypes of esophageal and stomach carcinomas. In the current users of NSAIDs (once a week for 6 months or more beginning at a reference period 1 year earlier), risk of esophageal carcinomas (squamous and adenocarcinomas) was found to be reduced (OR= 0.43, 95% CI= 0.21-0.89 and OR= 0.37, 95% CI= 0.19-0.73 respectively). The risk of gastric adenocarcinoma not involving the cardia of the stomach was also found to be decreased significantly (OR= 0.34, 95% CI= 0.20-0.59). Risk of development of adenocarcinoma of the cardia of the stomach was found to be decreased by 25%, however this decline in risk was not found to be statistically significant at the alpha = .05 level (175).

Two prospective studies evaluating the efficacy of aspirin use on a number of cancer outcomes have also been performed. The first of these, performed by Thun et al. reported on the effect of regular use of aspirin (16 times a month or more for more than 1 year) in the Cancer Prevention Study II. Persons enrolled in the study filled out a baseline questionnaire and were then assessed for vital status every two years
for a six year period. Overall the study was able to show a significant decreased risk in only colon cancer (OR= 0.63, 95% CI= 0.44-.089) (176). Second, a similar type of study using NHANES I data was performed by Everson and Schreinemachers. There study covered a follow-up period of over 12 years and measured the effects of persons using aspirin (at least once in the previous 30 days before baseline interview) in reference to their developing cancer in the next 12 years. After adjusting for gender and age, it was shown that a decreased risk of lung cancer (OR= 0.68, 95% CI= 0.49 - 0.94), breast cancer (OR= 0.70, 95% CI= 0.50 – 0.96) and digestive cancers (OR= 0.79, 95% CI= 0.62 – 1.00) was found. A number of other cancers were examined but were found to be non-significant when evaluated at the alpha=.05 level (177).

Studies of NSAID use and Ovarian Cancer

To date, 4 studies have been done to assess the association between NSAIDs and the risk of development of ovarian cancer. A population based case control study performed in the Northeastern United States, was reported by Greenberg et al. They studied regular use (once a week for 6 months or more) of the NSAIDs, aspirin, ibuprofen, prescribed analgesics and paracetamol on the inhibition of ovarian cancer development. Aspirin was shown to decrease the risk of ovarian cancer by 25%, but this was not significant at the alpha =.05 level. Regular use of ibuprofen and prescribed analgesics were shown to not have any effect at all on ovarian cancer incidence either. Paracetamol(also known as acetaminophen) however, was shown to significantly decrease a woman’s risk of developing ovarian cancer (OR= 0.50, 95% CI= 0.31-0.86). This
protective effect was further increased by increasing number of tablets taken per week and increasing duration of use. Use of paracetamol (more than once a week for over 6 months) was also shown to significantly reduce serous histological grades of ovarian tumors and poorly differentiated histological grades of ovarian tumors (OR= 0.53, 95% CI= 0.28-0.98 and OR= 0.38, 95% CI= 0.17-0.83 respectively) (178). Another case-control study, a hospital based study, done in Italy, measured the association of regular aspirin intake (once a month or more for 6 months or longer) in relation to ovarian cancer incidence. This study did not find association between the two (179). In the third case control study, a hospital based case-control study performed by Swede et al., the association between regular usage of aspirin and acetaminophen (not an NSAID) (once a week for 6 months or more prior to onset of disease) in relation to development of ovarian cancer was ascertained. Their results indicated that regular use of aspirin was not associated with a decreased risk of ovarian cancer (OR= 1.0, 95% CI= 0.73-1.39). In contrast, regular use of acetaminophen was shown to decrease the risk of developing ovarian cancer (OR= 0.56, 95% CI= 0.34-0.86). (Note: acetaminophen has limited if any systemic COX-2 or prostaglandin inhibitory properties) (180). Lastly, a hospital based case-control study was done by Rosenberg et al. Their results showed no association between acetaminophen or NSAIDs, used once a week or more for 6 months or longer at least one year before admission, in relation to development of ovarian cancer (OR= 1.0, 95% CI= 0.6-1.5 and OR= 0.7, 95% CI= 0.5-1.0 respectively). When use of NSAIDs was restricted to persons using 4 days or more per week for 6 months or longer at least one year before admission, acetaminophen use and NSAID use remained nonsignificant in terms of reducing the risk of ovarian cancer. However, when the NSAID group
was broken up into aspirin and non-aspirin groups, non-aspirin NSAIDs were shown to decrease the risk of ovarian cancer (OR= 0.5, 95% CI= 0.3-0.9). NSAIDs as a whole, taken 4 or more days a week for over 6 months, 1 year before hospital admission, were also shown to decrease the risk of development of ovarian cancer if used over 5 plus years in duration (OR=0.5, 95% CI= 0.3-0.9) (181).

Studies of NSAID use and Breast Cancer

A great deal of work has been done studying the relationship between NSAID intake and risk of developing breast cancer. To date at least 6 cohort studies and 8 case-control studies have been performed. The results of these studies were analyzed by Kkuder and Mutgi in a meta-analysis. The results of this meta-analysis showed that when combined, the case-controls showed a reduced risk of breast cancer development as did the cohort studies (OR= 0.83, 95% CI= 0.79-0.88 and OR= 0.78, 95% CI= 0.62-0.99, respectively). Further, the results of this meta-analysis showed that when combining the case-control and cohort studies together, using a random effects model to account for lack of homogeneity using Cochrans Q statistic, there was a reduction in risk of developing breast cancer in persons taking NSAIDs (OR= 0.82, 95% CI= 0.75-0.89). A dose-dependent response was not able to be calculated from the accrued studies. The meta-analysis was also not able to examine whether differences in reduction of risk between aspirin and non-aspirin NSAIDs existed, something that was seen in two case-control studies in which non-aspirin NSAIDs were reported to have greater protective effects than aspirin itself (182).
Studies of NSAID use and Colon Cancer

A great deal of research has been done epidemiologically that implicates regular NSAID use as a protective agent against the development of colon cancer. To date, at least 23 studies (both prospective and retrospective) have been performed, of which 21 show evidence of a chemopreventive effect of NSAIDs on colon cancer. Generally, it is accepted that regular use of aspirin or other NSAIDs decreases one's risk of developing colon cancer by 40-50% (183, 184).

1.13) COX-2, Prostacyclins and Lung Cancer

The use of COX-2 inhibitors may also have adverse effects towards chemoprevention of lung cancer. One of the possible mechanisms by which this might occur is through the inhibition of prostacyclin (PGI2) synthesis. PGI2 is a downstream mediator of the arachidonic acid metabolic cascade. In normal lung tissue PGI2 is found in high levels. However, in non-small cell lung tumors PGI2 is found in very low levels. This, along with evidence for a role of PGI2 in inhibiting tumor metastasis has suggested that PGI2 has properties that suppress lung cancer formation. Evidence for this effect has been borne out in a murine mouse model (185). Whether or not PGI2 levels play a role in human lung cancer and whether COX-2 use increases the development towards a cancerous state in humans is unknown.
CHAPTER 2

MATERIALS AND METHODS

2.1) Study Objectives

The main goal of this study was to test whether certain classes of drugs affected a person's risk for development of lung cancer. Most notable among these compounds were the NSAID compounds for which the case and control questionnaires were initially designed to study.

2.2) Type of Study

A case-control study was undertaken in order to examine the relationship of certain variables to the odds of developing lung cancer. This type of study was done in order to minimize cost and time spent on the study as opposed to doing a lengthy and costly prospective cohort study. To create a functional model for development of lung cancer, and to ascertain odds ratios for the variables to be studied in relation to lung cancer, the method of logistic regression was employed using SAS version 8 software (186).
2.3) Ascertainment of Cases and Controls

Lung cancer cases for the study were obtained from the Arthur G. James Cancer Hospital in Columbus, Ohio from 1995 to 2001. Cases were collected from a pool of lung cancer patients whom were set to undergo surgery in an attempt to resect the lung cancer from the lung. Information on cases were procured from a combination of presurgical histories taken by an anesthesiologist and from corresponding patient discharge records. In the anesthesiologist histories detailed information on NSAID use, lung cancer cell type, previous medical history and previous cigarette smoking activity were available. From patient discharge records information on other medications being taken could be obtained as well as a confirmation of the information found in the anesthesiologists charts. Thus, all cases obtained from the Arthur G. James Cancer Hospital were persons diagnosed with lung cancer who opted for surgical resection.

Controls for the study were gathered from two different sources depending on gender. Female and male controls for the study were obtained from females attending breast cancer screenings (n=11,160) and males attending prostate cancer screenings (n=929) at the Arthur G. James Cancer Hospital in Columbus, Ohio. At the screening, self administered questionnaires were filled out by each patient. All three questionnaires (cases, male controls and female controls) can be seen in Appendix B.
2.4) Variables to be Studied

The variables to be studied for cases and controls are:

Age – in years

Race (White, African-American, Other)

Gender (Male, Female)

Use of aspirin (# of pills taken per week, # years duration used)

Use of acetaminophen (# of pills taken per week, # years duration used)

Use of ibuprofen (# of pills taken per week, # years duration used)

Use of indomethacin (# of pills taken per week, # years duration used)

Use of Prescription NSAIDs (# of pills taken per week, # years duration used)

Number of cigarettes smoked per day

Number of years smoking cigarettes (used with number of cigarettes smoked per day to create packyears variable)

Previous Cancers (if yes then type of cancer and when)

FOR CASES ONLY:

Histologic type of lung cancer
2.5) Matching

Cases and controls were matched by gender and cigarette packyears smoking history in a 1:1 ratio. This was done in an attempt to eliminate the confounding presence of these known effectors in the study, especially the effects of cigarette smoking, the number one risk factor for lung cancer.

2.6) NSAID Classification Scheme

NSAID use of persons in the study are classified initially into one of nine mutually exclusive groups. These groups were coded in a hierarchical manner so that if a person was coded into the first group, they were not eligible to be coded into another group. The groups were ordered based on COX-2 blocking activity of the drug (prescription NSAID / Indomethacin > ibuprofen > aspirin > acetaminophen (no COX-2 blocking activity) and by number of times a week used (daily > non-daily). Below is the order in which persons could be coded into one of the NSAID / Acetaminophen categories:

1) prescription NSAID / Indomethacin use daily for 2 or more years (6 or more times/week)
2) ibuprofen use daily for 2 or more years (6 or more times/week)
3) aspirin use daily for 2 or more years (6 or more times/week)
4) acetaminophen use daily for 2 or more years (6 or more times/week)
5) prescription NSAID / Indomethacin use less than daily for 2 or more years (1-5 times/week)
6) ibuprofen use less than daily for 2 or more years (1-5 times/week)
7) aspirin use less than daily for 2 or more years (1-5 times/week)
8) acetaminophen use less than daily for 2 or more years (1-5 times/week)

Persons using neither acetaminophen nor NSAIDs at least once per week or using them for less than two years were classified as nonusers.

2.7) Coding of Other Variables

Variables in the study were coded on a 0,1 basis with a two notable exceptions. These would be the variables age and packyears of cigarette smoking, which were measured as continuous variables.

2.8) Calculation of Needed Controls for Study

A number of drugs were investigated in this study, the most important being the effect of NSAIDs on lung cancer. In order to determine whether these drugs have enhancing or inhibitory effects on lung cancer development, a proper number of individuals had to be examined.
To determine the number of study subjects needed the following formula was used:

\[ n = \frac{\left[\frac{1}{1+c} \times (p(avg) \times q(avg)) \times (Z_{\alpha/2} + Z_{\beta/2})^2\right]}{[(p(case) - p(control))^2]} \]

where:

- \(c\) = controls / case
- \(p(avg)\) = combined probability of exposure for cases and controls
- \(p(case)\) = probability of exposure for a case
- \(p(control)\) = probability of exposure for a control
- \(\alpha\) = chance of rejecting Ho when it is correct
- \(\beta\) = chance of accepting Ho when it is incorrect (note: \(1-\beta = \text{power}\))

When using this formula, a lot of information must be known such as the \(p(case)\), \(p(control)\), \(\alpha\) and \(\beta\). However, the level of \(c\), \(\alpha\) and \(\beta\) can be controlled by the researcher and thus only \(p(case)\) and \(p(control)\) must be found out. To find \(p(case)\) all one must do is find \(p(control)\). The problem is in finding the \(p(control)\) which correlates to the exposure seen to that element in the population. Unfortunately, studies of the exposure to individuals to certain pharmaceuticals are not often done. As a way to come up with this data, one can look at previous case-control studies and view the data for the controls.
Assuming the study is accurate and without control bias, the control data should mimic that which is seen in the overall population at large. After accumulating this data, the p(case) can then be calculated using the following formula:

\[ p(\text{case}) = \frac{(p(\text{control})) \times (R)}{(1 + p(\text{control})) \times (R-1)} \]

where:

\( R = \) relative risk we are looking for

Conversely, if the p(case) was known, a p(control) based on R could also be found in order to make the calculation.

In using these formulas one is in essence finding the number of cases and controls needed for the relative risk that we are looking for to be significant at the levels of \( \alpha \) and \( \beta \) chosen (187).

Given that we are looking for a 50% reduction in the odds of using NSAIDs in lung cancer cases compared to the chosen controls, and that the frequency of NSAID use in the cases was found to be approximately 30%, the number of cases and controls that we would need to find a significant effect using a 1:1 matching design at the alpha=0.05 level and with 80% power was 62 cases and 62 controls or 124 persons. Using the same parameters to find a 25% reduction in the odds of using NSAIDs in lung cancer cases compared to the chosen controls required (30% use in controls vs 22.5% use in cases) 272 cases and 272 controls or 544 persons were needed.
Making matters simpler was the fact that we obtained more than 400 lung cancer cases and over 900 male controls and 11,000 female controls. Thus, our goal was to gather as many cases and controls as possible into our study in a 2:1 (controls / case) match if possible and a 1:1 match otherwise.
CHAPTER 3

RESULTS

3.1) Eligibility of Cases and Controls

Of the 469 lung cancer cases obtained, 384 were deemed eligible to enter the study (no previous cancer other than basal cell skin carcinoma within the last 10 years). These 384 cases were composed of 239 males and 145 females. The rate-limiting factor for a control match for our cases was the pool of male controls that were available, of which we had information on 929 males. From the pool of 929 male controls who filled out a questionnaire, 861 were eligible for study (no previous cancer other than basal cell skin carcinoma within the last 10 years). From this available pool of 861 male controls, only 213 had smoked 20 or more years (used as a benchmark for heavy smoker status) and only 430 had ever smoked 1 packyear or more. The 213 heavy smoking male controls was less than the number of male cases by 28 persons. Since the only way to get a 2:1 (control:case) ratio would be to use a number of light smokers and some non-smokers, the decision was made to match up the cases and controls on a 1:1 basis. From the pool of 11,160 female controls who filled out a questionnaire, 9780 were eligible for study (no previous cancer other than basal cell skin carcinoma within the last 10 years). From this available pool of 9780 women 145 women were chosen to create a 1:1 match.
3.2) Selection of Male and Female Controls

Male controls were chosen by taking the 239 males with the largest packyears exposure to cigarettes in our available pool of male controls which produced a range of smokers from 16.5 – 116.25 packyears of exposure. Since there were over 1000 females reporting over a 20 packyear exposure to cigarettes, there were no problems in obtaining enough control females, 145, to create a 1:1 match. Female controls were chosen by taking the closest 145 females to the mean packyears computed for the female cases, which was approximately 46 packyears. Thus, females ranging from 43-49 packyears of exposure to cigarettes were chosen as controls. This method allowed us to obtain exactly 145 female controls.

3.3) Descriptive Statistics of Cases and Controls Studied

A description of the age, cigarette packyears of smoking and race of the cases and chosen controls can be seen in Table 3 (See Appendix C). As seen in this table, the mean age of the controls ended up being approximately 5 years younger on average than the cases (58.69 years vs 63.72 years). The mean number of packyears smoking cigarettes was also slightly different for the two groups with the controls having less of the exposure (42.15 packyears vs 50.39 packyears). The frequency of the different race categories was fairly similar between the cases and controls. As seen, there were only 2 cases and 3 controls overall that fell into the ‘other’ race category. Thus, persons from
the African-American category and the Other categories were combined into one category designated the non-white category.

### 3.4) Descriptive Statistics of Cases and Controls Studied by Gender

A breakdown and comparison of the case and control groups by gender can be seen in Table 4 (See Appendix C). As seen, both the control group of males and control group of females have a mean age 5 years lower than that of their case counterparts. In a comparison of packyears exposure to cigarettes among the two groups it is readily apparent that the female cases and controls are very similar in terms of mean packyears. Male controls however are shown to have a mean packyears of exposure 13 years less than the male cases. Race distribution for both groups (white vs non-white) were similar for both groups and genders. Thus, it is evident that the overall lower packyears of exposure among all controls compared to cases is due almost exclusively to the male controls while both control groups are younger overall than the cases by approximately five years.

### 3.5) Histologic Breakdown of Cases Studied

Given that the distribution of histologic types of lung cancers for adenocarcinoma, squamous cell, small cell and large cell carcinomas of the lung have been shown to be approximately 35%, 30%, 15% and 10% respectively, an expected number of each lung cancer subtype was calculated. The expected number of each of the histological
subtypes (adenocarcinoma, squamous cell carcinoma, small cell carcinoma and large cell carcinoma) from our 384 eligible lung cancer cases was 134, 115, 58 and 38, respectively.

The histologic breakdown of the 384 lung cancer cases studied can be seen in Table 5 (See Appendix C). As seen there were 120 adenocarcinomas, 108 squamous cell carcinomas, 9 small cell carcinomas and 38 large cell carcinomas seen amongst these cases. The expected counts among these four groups as mentioned above were 134, 115, 58 and 38, respectively. Thus the differences between the observed and expected counts for adenocarcinoma, squamous cell and large cell carcinomas were very low. However, the 9 observed cases of small cell carcinoma was well below the expected count of 58. This indicates a sampling bias away from small cell carcinoma. In addition, 5% of our cases had lung cancers of unknown cell types and another 12% had lung cancers known only as non-small cell lung cancer. From this it can be inferred that the number of cases of adenocarcinoma, squamous and large cell carcinomas seen amongst the cases are probably slightly undercounted.

3.6.1) Effects of NSAIDs on Lung Cancer (All persons)

Data on NSAID use (prescription NSAID/Indomethacin, ibuprofen or aspirin) and acetaminophen use for all cases and controls can be seen in Table 6 (See Appendix C). The odds ratios adjusted for age, race and packyears of cigarettes were 0.52 (95% CI= 0.38 – 0.72) for persons using NSAIDs and 1.25 (95% CI=0.68 – 2.29) for persons
using acetaminophen, a non-COX-2 blocking analgesic. For persons using NSAIDs less than daily (1-5 times/week) the odds ratio was 0.54 (95% CI= 0.36 – 0.82) and for persons using NSAIDs on a daily basis (6 or more times/week) the odds ratio was 0.51 (95% CI= 0.35 – 0.75). The test for trend (NSAID daily > NSAID non-daily > non-user) resulted in a p-value < .001.

NSAID use for all persons in the study was further broken down into categories of the type of NSAID used, prescription/indomethacin, ibuprofen and aspirin. These latter two groups were further broken down into less than daily and daily use. The adjusted OR’s for those taking prescription compounds was found to be 0.64 (95% CI= 0.36 – 1.12), those taking ibuprofen was 0.42 (95% CI= 0.24 – 0.75) and for those taking aspirin was 0.53 (95% CI= 0.36 – 0.77). For persons taking ibuprofen less than daily or daily and for persons taking aspirin less than daily or daily, the adjusted OR’s were 0.55 (95% CI= 0.26 – 1.16), 0.31 (95% CI= 0.14 – 0.71), 0.57 (95% CI= 0.33 – 0.96) and 0.50 (95% CI= 0.31 – 0.79) respectively. The test for trend (ibuprofen use < aspirin use < nonuser) resulted in a p-value of <.001.

3.6.2) Effects of NSAIDs on Lung Cancer (Male)

The data for cases and controls, stratified by gender can be seen in Tables 7 and 8 (males and females, respectively) (See Appendix C). In examining the data for males only, in much the same manner as the data for all persons combined, NSAID use was shown to protect against lung cancer after adjusting for age, smoking history and
race (adjusted OR = 0.58, 95% CI= 0.38 – 0.87). Stratifying NSAID use into non-daily and daily use resulted in odds ratios of 0.73 (95% CI= 0.42 – 1.25) and 0.48 (95% CI= 0.29 – 0.79) respectively. The test for trend (daily NSAID use > non-daily NSAID use > non-user) was significant with a p-value of .004. Also much like the data for all persons combined, acetaminophen, was shown not to affect lung cancer risk with an adjusted OR= 1.76 (95% CI = 0.72 – 4.32).

When male NSAID use is broken down into prescription, ibuprofen and aspirin groups similar trends are seen as compared to all persons with adjusted OR’s of 0.80 (95% CI= 0.37 – 1.76), 0.58 (95% CI= 0.27 – 1.25) and 0.52 (95% CI= 0.32 – 0.84) respectively. The test for trend (ibuprofen use > aspirin use > non-user) also agreed with the data seen for all persons with a p-value of .015.

3.6.3) Effects of NSAIDs on Lung Cancer (Female)

The data for females, much like that for males and all persons combined shows a decreased odds of lung cancer for persons using NSAIDs after adjusting for age, smoking history and race (adjusted OR= 0.44, 95% CI= 0.26 – 0.74). Stratifying the use of NSAIDs into non-daily and daily use results in adjusted OR’s of 0.34 (95% CI= 0.17 – 0.66) and 0.55 (95% CI= 0.30 – 1.02), respectively. The test for trend (NSAID use daily > NSAID use non-daily > non-user) was significant at p=.019. Acetaminophen as in males and in all persons was shown not to affect the odds of having lung cancer (adjusted OR= 0.88, 95% CI= 0.37 - 2.09).
Additionally, the affect of female NSAID use, broken down into prescription/indomethacin, ibuprofen and aspirin groups separately shows adjusted OR’s of 0.42 (95% CI= 0.18 – 0.99), 0.32 (95% CI= 0.13 – 0.78) and 0.52 (95% CI= 0.28 – 0.98) respectively. The test for trend (ibuprofen use > aspirin use > non-user) is shown to be significant with a p-value of .011.

3.6.4) Effects of NSAIDs on Lung Cancer (Adenocarcinoma)

Data for cases and controls with cases substratified by lung cancer cell type (adenocarcinoma, squamous cell carcinoma and large cell carcinoma) can be seen in Tables 9, 10 and 11 respectively. For these groups, adjustments for smoking, age, race and gender were made. For the adenocarcinoma subgroup, NSAID use was shown to also protect against lung cancer however non-significantly (adjusted OR = 0.68, 95% CI= 0.43 – 1.07). When broken up into non-daily and daily use the adjusted OR’s remained similar for both groups (0.67, 95% CI = 0.37 – 1.23 and 0.69, 95% CI= 0.40 – 1.18 respectively). The test for trend (Daily NSAID use > non-daily NSAID use > non-user) was nearly significant with an associated p-value of 0.134. Acetaminophen use was shown to not affect lung cancer risk with an adjusted OR of 1.74 (95% CI = 0.79 – 3.80).

Substratifying the NSAID compounds into prescription NSAIDs / indomethacin, ibuprofen and aspirin groups revealed adjusted OR’s of 1.01 (95% CI = 0.51 – 2.23), 0.74 (95% CI = 0.35 – 1.54) and 0.57 (0.32 – 1.01) respectively. The test for trend (ibuprofen use > aspirin use > non-user) was nearly significant with a p-value of .093.
3.6.5) Effects of NSAIDs on Lung Cancer (Squamous Cell Carcinoma)

For squamous cell carcinoma the adjusted OR for NSAID users is 0.56 (95% CI= 0.34 – 0.93). When the NSAID category was substratified into non-daily and daily use the OR’s remained low at 0.50 (95% CI= 0.25 – 1.01) and 0.61 (95% CI= 0.34 – 1.09) respectively. The test for trend (NSAID daily > NSAID non-daily > non-user) was nearly significant with a p-value of .057. For persons using acetaminophen, the affect on lung cancer was nonsignificant (OR= 0.80, 95% CI= 0.29 – 2.43).

Substratifying the NSAID by compound into prescription / indomethacin, ibuprofen and aspirin groups results in adjusted OR’s of 0.48 (95% CI = 0.18 – 1.27), 0.32 (95% CI= 0.12 – 0.90) and 0.64 (95% CI= 0.36 – 1.12) respectively. The test for trend (ibuprofen > aspirin > non-user) was significant with a p-value of .036.

3.6.6) Effects of NSAIDs on Lung Cancer (Large Cell Carcinoma)

For the large cell carcinoma subgroup, NSAID use was shown once again to be associated with decreased odds of lung cancer (adjusted OR = 0.32, 95% CI= 0.14 – 0.72). When the NSAID use group was broken down into non-daily and daily use, adjusted OR’s of 0.19 (95% CI= 0.04 – 0.83) and 0.40 (95% CI= 0.16 – 1.03) were found respectively. The test for trend (daily NSAID > non-daily NSAID > non-user) was significant with a p-value of .022. Acetaminophen use was not associated with a significant effect on lung cancer (adjusted OR = 0.59, 95% CI= 0.13 – 2.73).
Substratifying the NSAID group into prescription NSAID/indomethacin, ibuprofen and aspirin groups resulted in adjusted OR’s of 0.21 (95% CI= 0.03 – 1.59), 0.17 (95% CI= 0.02 – 1.29) and 0.41 (95% CI= 0.16 – 1.04) respectively. A test for trend (ibuprofen > aspirin > non-user) was significant with a p-value of .030.
As indicated in Table 6, NSAID use was associated with a 48% decreased odds of lung cancer in all persons combined. This decrease in risk was shown to exist across stratification by gender and by lung cancer cell type (adenocarcinoma, squamous cell and large cell carcinoma), indicating a stable effect of NSAIDs on lung cancer. This effect was shown to be dose dependent and more protective for persons using NSAIDs daily than person taking NSAIDs non-daily. Likewise, ibuprofen was shown to be slightly more effective than aspirin in terms of decreasing lung cancer risk. Use of acetaminophen, a non-COX-2 blocking analgesic was shown to have no significant effect on lung cancer development. These results, that the COX-2 blocking NSAIDs decreased the risk of lung cancer in heavy smokers and acetaminophen, a non-COX-2 blocking agent did not, point to a possible mechanism by which the NSAIDs may bring about decreased risk of lung cancer; via blockade of the COX-2 enzyme.

Given the purported animal model evidence suggesting a key role for COX-2 in the mitogenic signaling pathway for adenocarcinoma and increased COX-2
expression in adenocarcinomas as compared to other lung cancer cell types, it was puzzling why equal or weaker effects of NSAID use in this group seemed to exist as compared to squamous cell carcinoma and large cell carcinoma. One might have expected that NSAID use would have its greatest effect in the adenocarcinoma group as compared to the other groups. While no conclusion can be reached to explain this, a number of possibilities exist. One such possibility stems from the fact that NSAIDs might be working on other more important pathways in tumorigenesis. Given the abundance of mechanisms by which NSAIDs have been purported to inhibit the development of tumors (decreases in activation of carcinogens, increases in apoptosis, decreases in tumor angiogenesis, decreases in tumor metastasis, blockade of immune suppression and inhibition of telomerase) it is quite possible that one or more of these effects is the mechanism by which NSAIDs decrease the risk of lung tumor development. This would potentially overshadow or mask any effects that mitogenic blockade might bring about.

Another potential explanation for this result is that the acetaminophen user group in the adenocarcinoma cases was larger than any other group. Had this group been coded as non-users rather than being left out of the reference category the results of the squamous and large cell groups would have been less significant (OR’s a little bit closer to 1) and the adenocarcinoma results would have been a little more significant (OR a bit farther from 1). The differences resulting from this can be seen in the Tables 12,13 and 14 (Adenocarcinoma, Squamous Cell Carcinoma and Large Cell Carcinoma respectively, See Appendix D). As seen, the squamous cell carcinoma and large cell carcinoma
groups are affected little if any by this method. However, the adenocarcinoma lung cancer group achieves significance in the NSAID user group and OR’s for all groups go down more than in the other two lung histological cell types.

4.2.1) Selection Bias

Selection bias occurs when there is a difference in exposures for persons included in a study and for persons who were eligible to be included in a study but were not. This type of bias can be introduced into either the case group, the control group or both groups (188).

One potential selection bias in the study, as mentioned previously, was in the sampling of cases. This became evident when of the 384 lung cancer cases studied, only 8 were found to be of the small cell carcinoma subtype. Previous calculations had shown that approximately 58 small cell lung cancer cases were expected of our 384 total lung cancer cases. Thus, only 14% of the expected number of small cell carcinoma cases were actually observed. The reasons for this most likely are due to the fact that case data was obtained from anesthesiology records taken prior to surgery for resection of the lung cancer. Small cell carcinoma is the most radiosensitive and chemosensitive histological subtype of lung cancer, stemming largely from the fact that it is the lung cancer with the fastest doubling time. Additionally, small cell carcinomas of the lung are frequently found to have metastasized upon diagnosis. Thus, surgery for this type of tumor is often
not part of the preferred treatment regimen (189). As a result, the lack of small cell carcinomas making up our case sample is not entirely surprising.

The possibility exists then that our lung cancer group may not entirely reflect that which is seen in the true lung cancer population overall. This would be the case if persons with small cell lung cancer have different levels of exposure overall to NSAIDs than do the rest of our cases. Due to this type of sampling bias, it is thus made extremely difficult to translate the findings of this study to small cell lung carcinomas and overall lung cancer as a group, which would include small cell carcinoma as approximately 15% of its makeup. Rather, the only statements that can be made with any validity are those focusing on non-small cell lung carcinomas.

Likewise, the possibility for bias also existed due to the method used to obtain the female controls used in our study from the group of 10,000 + eligible women. As seen, the SD for the female controls for the variable cigarette packyears of smoking is extremely low compared to female cases. This is due to the fact that in an attempt to match up the female cases and controls on the packyears variable, only a cluster of eligible control females with exposures of between 43 and 49 packyears were chosen as controls. In doing this, both groups (female cases and controls) had similar mean packyears exposure to cigarettes, however the case females had exposures over a much wider range (0-240 packyears). Thus, our method of choosing smoking controls left open a possibility of biasing the control group, as it created the possibility for a homogenous control group. This would have affected the variability in a number of exposures of
interest, most notably in the main variable of interest, use of NSAIDs. As a check against this type of selection bias, female characteristics of NSAID use and mean age were quantified for the control group chosen, and for all eligible control females with reported smoking exposures greater than or equal to 10 packyears. Comparison of these two groups revealed that the two groups were the same and that a biasing of the female controls that were chosen did not occur.

4.2.2) Information Bias

Information bias occurs when errors in measurement of exposures or diseases take place (188). A number of potential information biases existed in this study.

First, the possibility of bias due to the manner in which the data was collected existed. This arose from the fact that data from cases and controls were collected in two different manners. The control data were collected from self reports via questionnaire while the case data was collected from anesthesiology reports and discharge records from the hospital. There is little doubt that the information in the anesthesiology records was anything but thorough on the use of NSAIDs given the fact that use of these compounds can have an effect on clotting factors within the body, which can lead to complications during surgery if not accounted for. However, at times in the anesthesiology records, data on duration of use of the NSAID or any other drug taken, there was little if any information. Similarly, at other times in the discharge records there were NSAIDs or acetaminophen listed as patient medication that had not been listed in the
anesthesiology records. Both of the situations lead to the potential for study bias because duration of NSAID use was not known. In order to account for these and create as fair a study as possible, measures were taken to ensure that if bias were to occur, that it would push the odds ratio towards the null hypothesis (that is increase the OR towards 1 or above). To do this, all cases were assumed to have used the NSAID or acetaminophen containing compounds for more than 2 years. This in essence would act to elevate the number of NSAID and acetaminophen users among the cases. Given the fact that lung cancer itself and surgery to remove a lung tumor is a painful process one would assume that a number of persons using NSAIDs among the cases would be short time users. That is, there is an extremely strong likelihood that a number of these cases who were coded as NSAID users of 2 years of more were really persons having only recently begun using the drugs to control symptoms of pain. Thus, if anything, the OR’s reported in this study are elevated from their true values.

Another possible biasing of information in the study was recall bias by both the controls and the cases. This would result in inaccurate reporting of information. Guarding against recall bias is extremely difficult. One of the few ways to protect a study from it are to have a number of proxy indicators that might detect faulty recall. In our instance, the most important measure of the study was that of NSAID intake. NSAIDs are anti-inflammatory drugs taken to alleviate painful symptoms of a number of conditions. Acetaminophen is another type of drug used to treat similar conditions. Thus, one would expect that if a recall bias existed it would affect both types of drugs in a similar manner. The fact that one class of pain relief drug, the NSAIDs, were
associated with a decreased risk of lung cancer and another class of pain relief drug, acetaminophen containing compounds, were not, gives the suggestion that an information bias of this type did not affect the study.

4.2.3) Confounding

Confounding is a term used to convey a variable of interest, X, that systematically varies to the disease of interest in relation to another independent variable, Y, (the confounder). This relationship then can distort the “real” effects of the variable, X, and cause one to not truly observe the effects of that variable, X, but rather the effects of the confounder, Y, and can lead one to make false conclusions. The effects of a confounding variable can be minimized or negated in a study by using proper statistical analysis techniques (188).

The potential for bias due to confounding variables in this study existed. The most likely potential confounders were age and packyears of cigarette smoking, variables which have known effects on lung cancer incidence and possibly use of NSAIDs. In the case of these two variables, increasing age and increased exposure to cigarette carcinogens via smoking are known to increase the risk of developing lung cancer. Increasing age can logically also be expected to increase the chances that a person uses NSAIDs on a frequent basis. This would be due to the fact that as one gets older the chances increase of having arthritis for which NSAIDs are a popular treatment option; the chances increase for risk of stroke or MI, for which aspirin is recommended as
prophylactic treatment; and the chances of minor aches and pains increases as the body becomes less flexible and more prone to injury, for which NSAIDs are a popular treatment option. Increases in NSAID use for cigarette smokers as compared to non-smokers have also been reported (173). Thus, selections of cases and controls with differing distributions across these two variables could lead to faulty findings if not controlled for. To attempt to control for this initially, controls with large packyears of exposure to cigarettes were chosen. This to an extent minimized the differences between the case and control groups as far as packyear exposure to cigarettes was concerned. However even after doing this, the cases were older by approximately 5 years compared to the controls and had a larger packyears exposure to cigarettes in the males. To further control for this, the logistic regression method was employed, in an attempt to eliminate their possible confounding effects on the study.

Another variable that was controlled for was gender. This was done by matching up cases and controls on a one to one basis in the same step where controls were matched up for cigarette packyear exposure. This allowed for an abolition of effect of gender on analysis of all persons. However, when stratification was done on NSAID use for different histological subtypes of cancer, gender had to be controlled for using logistic regression techniques. Racial differences between the two groups (cases vs controls) were also controlled for. Race and gender were mainly controlled for their potential to bias the lung cancer histological subtype analyses, for which African Americans and females have been reported to have increased risks for development of adenocarcinoma of the lung compared to other tumor types, as discussed previously.
4.3.1) Internal Validity

Internal validity is a term used to recognize whether a study was done properly or not. It encompasses whether cases and controls were properly selected, if exposures were adequately assessed, whether outcomes / disease of interest were adequately assessed and if the methods used to determine whether an association between exposure and disease were properly performed (188).

Given the data that was available and the methods that were used to obtain it, I believe that the study was done as well as possible. As discussed above a number of selection, information and confounding biases had the potential to affect the study. In each of these cases extreme caution and careful planning were done to examine if bias existed, to rid the bias if possible and if bias was not able to be eliminated, to bias the results toward the null hypothesis of no effect. It is my belief that if this study were to be done again and in such a manner as to not run into some of the data problems encountered in this study, the findings, if different at all from the ones determined in this study, would show an even greater protective effect of NSAIDs on lung cancer. However, one must realize that no one epidemiologic study can prove anything by itself. The chance of random error always has the potential to be present in any study and lead one to make false conclusions.
4.3.2) External Validity

External validity is a measure that determines if a study of a given sample can be extrapolated to the larger population as a whole. Thus, it questions whether the findings of this study can be generalized to the larger population for which it is meant to impact. One key prerequisite for a study to have external validity is that it must have internal validity (188).

As mentioned above, no one study by itself can prove anything. Numerous studies on this subject will have to be done in order to see if the results stand up to the scrutiny of further testing. As this is one of the first studies to be done to ascertain the effects of NSAID use on lung cancer, only time and future study will tell if the study and its findings do indeed have internal validity.

Assuming that the study is indeed internally valid, as mentioned previously, our study sample of cases does not mirror that of the population in terms of histology. This is due to the lack of small cell carcinoma lung cancer cases in our sample. Thus, the external validity of this study is likely limited to persons with non-small cell carcinoma of the lung. Since non-small cell lung cancers comprise approximately 85% of the lung cancer cases in the general population, our study results, should they be valid, have implications for a majority of the population. This population is likely limited to cigarette smokers who accounted for (362/384) 94% of our case population and 100% of
our control population. Thus, as to whether these findings might also be important for the 5-10% of the population of non-smokers who contract lung cancer remains in doubt.

4.4) Future Direction

While this study did indeed support the notion that NSAIDs inhibit lung cancer development, it was not able to examine some of the questions that we wished to explore. Most notably were the effects of beta-blockers, beta-agonists, theophylline containing drugs and TCAs on adenocarcinoma and SSRIs, calcium channel blockers and NSAIDs on small cell carcinoma. Additionally the effects of BMI, alcohol use, ERT and HMG-CoA blocker use was not able to be examined by histological subtype of cancer and lung cancers as a group overall. The failure to look at these variables stemmed from a lack of data amongst the controls on these variables of interest, a lack of small cell carcinoma cases amongst the obtained cases and lack of information for a number of cases on the variables of interest. In the future, different data sources and methods of obtaining the data will need to be used if these unanswered hypotheses are to be examined. In addition, future studies on NSAID use and the risk of lung cancer will need to be performed in an attempt to evaluate the internal validity and external validity for the findings above. It will only be after repeated studies that the finding that NSAID use can decrease the risk of lung cancer in smokers will be accepted and used to create interventions and public policy.
LIST OF REFERENCES


APPENDIX A:

TABLES AND FIGURES FOR CHAPTER 1
Table 1: Systemic Carcinogens in Tobacco Smoke. These tobacco carcinogens have been identified as those for which “sufficient evidence for carcinogenicity” exist either in humans or laboratory animals by the International Agency for Research on Cancer (IARC). (Table from Hecht 1999 (2)).

Table 2: Pulmonary Carcinogens in Tobacco Smoke. These tobacco carcinogens are those from Table 1 for which evidence of pulmonary carcinogenesis exists in laboratory animals, as demonstrated by the IARC. (Table from Hecht 1999 (2)).
Figure 1: A Model of Lung Cancer Induction. As seen, lung cancer can be brought about by activated carcinogens that form DNA adducts. If these DNA adducts are not repaired and key genes are affected such as tumor suppressor genes or oncogenes, lung cancer can result. (Table from Hecht 1999 (2)).

Figure 2: A Model of the Activation of the Cigarette Carcinogens NNK and B[a]P. The pathways to activation and DNA adduct formation for the two carcinogens NNK and B[a]P are shown above. NNK and B[a]P are the two cigarette carcinogens most identified with lung cancer formation. Activation of the two carcinogens can come about through reactions with P-450 enzyme family members, as well as through the actions of the cyclooxygenase enzymes. While B[a]P is more linked to squamous cell carcinoma formation, NNK is more linked with adenocarcinoma formation and possibly small cell carcinoma formation. (Table from Hecht 1999 (2)).
Figure 3: An Overview of the Cyclooxygenase Cascade. Pictured above is the pathway from which arachidonic acid (AA) is converted into mediators of inflammation, specifically prostaglandins, thromboxanes and leukotrienes. As seen, the non-steroidal anti-inflammatory drugs are able to block this cascade by inhibiting the conversion of AA into downstream products via their inhibition of the cyclooxygenase enzymes. (Table from Bertolini et al. 2001 (188)).
Figure 4: A Signal Transduction Model of Adenocarcinoma of the Lung. Using information from studies by Schuller et al. and others, a potential model for mitogenic signaling by adenocarcinoma cells can be generated. From this model a number of chemoprevention strategies can be seen to have merit, most notably the use of NSAIDs and Beta-blockers.
Figure 5: A Signal Transduction Model of Small Cell Carcinoma of the Lung. Using information from studies by Schuller et al. and others, a potential model for mitogenic signaling by adenocarcinoma cells can be generated. From this model a number of chemoprevention strategies can be seen to have merit, most notably the use of Selective Serotonin Reuptake Inhibitors (SSRI’s). Likewise, the use of NSAIDs, which might inhibit angiogenic formation to these tumors, may also prove to inhibit their development.
Figure 6: A Signal Transduction Model of Squamous Cell Carcinoma of the Lung. Using information from studies outlined in Section 1.10.3, a potential model for metastatic and angiogenic signaling by squamous cell carcinomas can be generated. From this model a number of chemoprevention strategies can be seen to have merit, most notably the use of NSAIDs, which might inhibit mitotic, angiogenic and metastatic signaling in these tumors, thus inhibiting their development.
APPENDIX B:

QUESTIONNAIRES USED FOR STUDY
QUESTIONNAIRE FOR CASES
(FILLED OUT DURING CHART REVIEWS)

EPIDEMIOLOGIC STUDY
OF LUNG CANCER AMONG SMOKERS

Date of Interview ________________ Interviewer ________________

DEMOGRAPHIC INFORMATION

Patient Number ________________ Date of Birth ____________
Sequence # ____________ Sex ____________
Marital Status ________ Race ________
Age ________ Education ________
County and State of Birth ________________________________

Weren you born in the United States _____________________________
If not, what country _____________________________
When did you come to the United States _____________________________

CHART DATA:

Current Diagnosis ____________________________ Age at Diagnosis ________
Histology ____________________________ Cell Type ____________________________
Stage at Diagnosis ____________________________ Present Stage ________
Concurrent Second Primary ____________________________ Date of Diagnosis ________
Histology ____________________________

MEDICAL HISTORY

How tall are you ________
How much do you weigh today ________ Weight before Illness ________
What was your weight at 20 years old ________
What is the most you have ever weighed ________ when ________

Have you ever had surgery such as your to remove your appendix or gall bladder, if so where and when and at what age ________

Has your doctor told you that you have any of the following conditions.

1) Heart Attack (requiring hospitalization) ________
2) Stroke (MD Diagnosed) ________
3) High Blood Pressure (non Pregnancy related)
   Req. Treatment with Diet or medication ________
4) Chronic Bronchitis (chronic cough) ________
5) Emphysema ________
6) Asthma ________
7) Tuberculosis ________
8) Diabetes (non pregnancy related) ________
9) Osteoarthritis
10) Rheumatoid Arthritis
11) Overactive Thyroid
12) Underactive Thyroid
13) Cancer site

Have you ever received radiation (X-Rays, radioisotopes, cobalt) as a medical treatment
If yes, for what condition
How old were you at that time
To what part of your body was the radiation directed

FAMILY CANCER HISTORY

Have any of your relatives ever been diagnosed with cancer, if so would you please provide the following information:
(1) mother (2) father (3) sister (4) brother (5) daughter (6) son (7) grandmother (8) grandfather

<table>
<thead>
<tr>
<th>Relative</th>
<th>Site of Cancer</th>
<th>Age at Diagnosis</th>
<th>Stage at Diagnosis</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OCCUPATIONAL HISTORY

What company or type of industry do you work for
What has been your main occupation
What is your spouse’s occupation
How many years have you worked at this occupation

Other jobs held

OCCUPATIONAL EXPOSURES

Have you been exposed to any of these substances for at least 8 hours a week for at least one year.

Printing Inks
Sulfur Dioxide
Mineral Oils, cutting oils
Benzene, Acetone or other solvents
Uranium, Plutonium
X-Rays
Paint
Glues
Cotton dust
Fiberglass
Building insulation
Wood dust
Coal dust
Metal dust
Asbestos
Petroleum Products

Diesel Exhaust
Textile dyes
Natural Gas Fumes
Gasoline Fumes
Coal Oven emissions
Gasoline Exhaust
Plasctcs
Rubber and Rubber Products
Pesticides, Herbicides and weed Killers
Arsenic
Nickel
Chromium
Microwaves, Radar, radio frequencies
Dry Cleaning Chemicals
Asphalt, Tar and Pitch
Other
QUESTIONNAIRE FOR CASES (cont.)

For any exposure listed please provide the following information to the best of your ability.

Exposure to __________________ Where __________________ Years exposed __________
Exposure to __________________ Where __________________ Years exposed __________
Exposure to __________________ Where __________________ Years exposed __________
Exposure to __________________ Where __________________ Years exposed __________

Other Exposures __________________________________________________________

TOBACCO HISTORY

Have you ever smoked cigarettes _____
How long were you a smoker _____
At what age did you begin smoking _____
How many years have you smoked _____
How many cigarettes per day did you smoke? _____

Have you ever smoked cigars or a pipe and if so for how long? _____

For Quitters (those who have been smoke free for at least one year).

If you have quit smoking, how long have you been smoke free? __________

Health related reasons for quitting smoking.

1) Angina __________
2) Hypertension __________
3) Myocardial Infarction __________
4) Stroke __________
5) Other cardiovascular problems __________
6) Poor circulation in extremities __________

ALCOHOL HISTORY

Do you consume alcoholic beverages at least once a week? _____
Indicate the average number of drinks per week that apply.

Beer __________
Wine __________
Liqueur __________

NUTRITION QUESTIONS

How many meals per week do you eat that contain fish or fish products? __________

What do you use are your primary cooking oils?

Canola Oil __________
Corn Oil __________
Soybean Oil __________
Safflower Oil __________
Olive Oil __________
Peanut Oil __________
Vegetable Oil __________
Other __________

How many eggs per week do you consume? __________
How many times per week do you eat red meat? __________

BEVERAGES WITH CAFFEINE

Coffee (cups per day) _____
Tea (cups per day) _____
Soda (cans per day) _____
## QUESTIONNAIRE FOR CASES (cont.)

**MEDICATION HISTORY**

Please list any medications you might have taken for the following conditions and if so what medication was prescribed?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Date of Dx.</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina (chest pain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Have any of the following been prescribed for you by a physician and, if so, how long have you taken them.

**Antihypertensive Medications**

<table>
<thead>
<tr>
<th>Category</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuretics</td>
<td>Lasix</td>
</tr>
<tr>
<td></td>
<td>Maxide-Dyazide</td>
</tr>
<tr>
<td></td>
<td>Hydrodiuril</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>Cardizem</td>
</tr>
<tr>
<td></td>
<td>Norvasc</td>
</tr>
<tr>
<td></td>
<td>Verapamill</td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>Captopril</td>
</tr>
<tr>
<td></td>
<td>Vasotec</td>
</tr>
<tr>
<td></td>
<td>Zestir/Prinivil</td>
</tr>
<tr>
<td>Beta Blockers</td>
<td>Inderal</td>
</tr>
<tr>
<td></td>
<td>Tenormin</td>
</tr>
<tr>
<td></td>
<td>Lopressor</td>
</tr>
<tr>
<td>Glaucoma Drugs</td>
<td>Timolol</td>
</tr>
<tr>
<td>Antidepressants</td>
<td></td>
</tr>
<tr>
<td>5 HT Uptake Drugs</td>
<td></td>
</tr>
</tbody>
</table>

**Bronchodilators & Decongestants**

<table>
<thead>
<tr>
<th>Category</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic Drugs</td>
<td></td>
</tr>
<tr>
<td>Xanthine Drugs</td>
<td></td>
</tr>
</tbody>
</table>
Use of Non-steroidal anti-inflammatory drugs.
Indicate which of the following pain relievers (NSAIDS) that you take or have taken regularly, how many and for what period of time.

<table>
<thead>
<tr>
<th>Pain Reliever</th>
<th>No. Pills per week</th>
<th>No. of years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin (or pain relievers containing aspirin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaminophen (Tylenol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen (Advil, Motrin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin (Indocin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib (Celebrex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rofecoxib (Vioxx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any other drug for pain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
QUESTIONNAIRE FOR MALE CONTROLS

OHIO STATE UNIVERSITY
PROSTATE SCREENING PROJECT QUESTIONNAIRE

RECORD #:__
Race:_________ Marital Status: S M W D
Total years of education: ________
What was your weight at age 21? ________ lbs.
What is your height _______ and weight _______ today?
Occupation(s) No. years employed _________

Have you ever been diagnosed with cancer?
NO □ YES □

Do you have a family history of prostate cancer?
NO □ YES □

Family history of other cancer?
NO □ YES □

Mark the medications that you are now taking or you have ever taken regularly:
□ Aspirin (or pain relievers that contain aspirin)
□ Acetaminophen (Tylenol)
□ Indomethacin (Indocid)
□ Ibuprofen (Advil, Motrin)
□ Any other drug (prescription or non-prescription) for pain/inflammation relief:
□ I never use any of these medications
□ I use some of these medications less than once per week

Have you ever used tobacco?
NO □ YES □ Current using □
Type of tobacco used (mark all that apply):
Cigarettes □
Pipe □
Cigar □
Chew □
Age began ________
Total years ________
Average No. cigarettes/times per day ________

Have you ever consumed alcoholic beverages?
NO □ YES □
Indicate the average number of drinks per week for all that apply:
Beer ________
Wine ________
Liquor ________

How many years has this been your pattern?

What is your fluid intake of the following beverages:
Glasses (8 oz) per day
Water ________
Whole milk ________
Low fat or skim milk ________
Orange, fruit juices ________
Coffee, tea ________
Soft Drinks ________

How many servings (handfuls) of nuts and seeds do you eat per week?

How many carrots do you eat per week?

Age at puberty (first sexual climax) ________

Age at first intercourse ________

Number of children ________

Average number of sexual climaxes per week...
...at age 21 ________
...during past year ________

Have you ever had:
□ Benign prostate hypertrophy NO □ YES □
□ Prostate infection
□ Bladder infection
□ Gonorrhea or syphilis
□ Herpes (penis or scrotum)
□ Kidney/ureter stones
□ Vasectomy

Male pattern baldness? NO □ YES □
Age at onset ________

INFORMED CONSENT: I understand that some information I provide on this form may be used to study new risk factors for prostate cancer. I also understand that my response to these questions is voluntary and would be greatly appreciated. The information will be kept completely confidential and my name will not be linked to that information.

SIGNATURE ______________________ DATE: __ / __
QUESTIONNAIRE FOR FEMALE CONTROLS

FAMILY HISTORY

Family history of breast cancer?
No □ Yes □

How many?

Mother □

Sister(s) □

Daughter(s) □

Grandmother(s) □

Aunt(s) □

Other: □

Maternal □

Paternal □

Age(s) at diagnosis:

Family history of other cancer?
No □ Yes □

If yes, list relation and type of cancer

GYNECOLOGICAL HISTORY

Date of last menstrual period: / / 

Age at first menstrual period:

Have you ever had a full-term pregnancy?
No □ Yes □

Age at first full-term pregnancy:

Total number of live-born children:

Total number of children breast-fed:

Are you pregnant now? No □ Yes □

Did you take DES (Diethylstilbestrol) during pregnancy?
No □ Yes □ Unknown □

If yes, during your first or second trimester?

If yes, unknown

Did your mother take DES during her pregnancy with you?
No □ Yes □ Unknown □

Have you had a hysterectomy?
No □ Yes □

Age at hysterectomy:

Type: □ Uterine only □ Ovarian only

□ Uterine and ovarian □ Not sure

Have you gone through menopause?
No □ Yes □ CURRENTLY □ INDUCED □

Age at menopause:

Are you using or have you ever used birth control pills?
No □ Yes □

Currently Using □

Age began:

Total years:

Height: ft. in.

Current weight: lbs.

Weight, age 18: lbs.

INFORMED CONSENT: Mammography is only one component of a complete breast evaluation. Monthly breast self-examination, regular physical examination, and regular mammography provides the most thorough evaluation available today. I give my consent for mammography. If my mammogram is abnormal, further consultation and evaluation by my physician will be required. I understand that I may be contacted to confirm that I have completed the recommended follow-up. Information that I provide on this form may be used to identify new risk factors for breast cancer. I understand that my response to all of these questions is voluntary and would be greatly appreciated. If my information is used for research purposes, my name will not be linked to that information.

Signature

Date

Signature

Date

Project Director
APPENDIX C:

TABLES OF RESULTS
Table 3: Descriptive Characteristics of the 384 Lung Cancer Cases and the 384 Gender Matched Control Smokers in the Study. As seen, the case and control groups were made up of 239 males and 145 females each. Overall, the control groups were slightly younger and exhibited slightly less cigarette exposure as measured by packyears. This was largely due to the greater number of smokers with over 60 packyears of smoking history in the case group as compared to the control group. The racial makeup of the two groups, as seen, was almost identical.
<table>
<thead>
<tr>
<th>Factor</th>
<th>Cases (n=384)</th>
<th>Controls (n=384)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=239)</td>
<td>Female (n=145)</td>
</tr>
<tr>
<td></td>
<td>Male (n=239)</td>
<td>Female (n=145)</td>
</tr>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>4 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>40-49</td>
<td>22 (9)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>50-59</td>
<td>57 (24)</td>
<td>32 (22)</td>
</tr>
<tr>
<td>60-69</td>
<td>72 (33)</td>
<td>51 (35)</td>
</tr>
<tr>
<td>70-79</td>
<td>69 (28)</td>
<td>39 (27)</td>
</tr>
<tr>
<td>&lt;=80</td>
<td>6 (4)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Packyears smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>23 (10)</td>
<td>18 (12)</td>
</tr>
<tr>
<td>20-39</td>
<td>60 (25)</td>
<td>38 (26)</td>
</tr>
<tr>
<td>40-59</td>
<td>68 (28)</td>
<td>47 (32)</td>
</tr>
<tr>
<td>60-79</td>
<td>38 (16)</td>
<td>29 (20)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>50 (21)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>209 (87)</td>
<td>134 (92)</td>
</tr>
<tr>
<td>Non-white</td>
<td>28 (12)</td>
<td>11 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Characteristics of the Males and Females making up the case and control groups. As seen below, both control groups (male and female) were slightly younger than their respective control groups. Further, the female case and control groups show identical mean packyear exposure to cigarettes, while the male controls show a smaller mean packyear exposure than the male cases. Further, the female controls show a tight clustering of packyear exposure (range 43-49) as compared to cases and male controls. This is due to the nature in which these female controls were selected. As in the previous table, it is evident that racial differences are small if any between the cases and controls.
<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous Cell</td>
<td>108</td>
<td>28.13%</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>120</td>
<td>31.25%</td>
</tr>
<tr>
<td>Small Cell</td>
<td>9</td>
<td>2.34%</td>
</tr>
<tr>
<td>Large Cell</td>
<td>38</td>
<td>9.90%</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>14</td>
<td>3.65%</td>
</tr>
<tr>
<td>Bronchoalveolar</td>
<td>8</td>
<td>2.08%</td>
</tr>
<tr>
<td>Mixed Cell</td>
<td>16</td>
<td>4.17%</td>
</tr>
<tr>
<td>Unknown Cell Type</td>
<td>20</td>
<td>5.21%</td>
</tr>
<tr>
<td>NSCLC only</td>
<td>47</td>
<td>12.24%</td>
</tr>
<tr>
<td>(no other indicator)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>2</td>
<td>0.52%</td>
</tr>
<tr>
<td>Spindle Cell</td>
<td>2</td>
<td>0.52%</td>
</tr>
<tr>
<td>Total Lung Cancers</td>
<td>384</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 5: Frequency of Histologic Subtypes of Lung Cancer Studied: As seen below, adenocarcinomas and squamous cell carcinomas of the lung accounted for approximately sixty percent of the lung cancers studied. Of note is the fact that small cell carcinomas of the lung accounted for only two percent of the cancers studied. This low frequency indicates a bias of the sample towards non-small cell lung carcinomas and away from small cell carcinomas. Normally, a frequency of 15-20 percent small cell carcinomas would be expected. This bias in number of small cell cases is most likely attributable to the data source from which information was obtained, anesthesiology reports. This is most likely due to the fact that small cell lung cancers are generally not treated via surgical means.
Table 6: The Effects of NSAIDs on Lung Cancer Development in all Persons Studied:

As seen below, use of NSAIDs shows a statistically significant, protective effect against lung cancers overall in all persons studied. After adjusting for age and smoking history, it is shown that in persons using NSAIDs, there is a 48% decreased risk of developing lung cancer. Additionally, the pain relieving compound acetaminophen is shown not to have affected the development of lung cancer in a statistically significant fashion. Given the fact that acetaminophen has no affect on cyclooxygenase activity, the hypothesis that NSAIDs inhibit cancer formation through cyclooxygenase inhibition may indeed have merit. When the NSAID group is further broken down into ibuprofen and aspirin classes, a slight dose response can be seen in which persons using NSAID compounds on a more regular basis (six or more times per week), are protected to a greater extent than those persons using less than six times per week. Likewise, it is also seen that the compound ibuprofen, which has greater COX-2 blocking activity than aspirin, shows a greater protective effect among its users than the aspirin users.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>233</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>118</td>
<td>172</td>
<td>0.56</td>
<td>0.42 – 0.76</td>
<td>0.52</td>
<td>0.38 – 0.72</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>53</td>
<td>77</td>
<td>0.56</td>
<td>0.38 – 0.84</td>
<td>0.54</td>
<td>0.36 – 0.82</td>
<td>.003</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>65</td>
<td>95</td>
<td>0.56</td>
<td>0.39 – 0.81</td>
<td>0.51</td>
<td>0.35 – 0.75</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>33</td>
<td>21</td>
<td>1.29</td>
<td>0.72 – 2.30</td>
<td>1.25</td>
<td>0.68 – 2.29</td>
<td>.471</td>
</tr>
</tbody>
</table>

p-value of test for trend (NSAID >= 6/ week > NSAID < 6/week < Non-user ) = <.001

**adjusted for age, packyears of smoking and race**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser (Ref)</td>
<td>233</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>25</td>
<td>33</td>
<td>0.62</td>
<td>0.36 – 1.08</td>
<td>0.64</td>
<td>0.36 – 1.12</td>
<td>.119</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>22</td>
<td>44</td>
<td>0.41</td>
<td>0.24 – 0.71</td>
<td>0.42</td>
<td>0.24 – 0.75</td>
<td>.003</td>
</tr>
<tr>
<td>Ibuprofen less than 6 times/week</td>
<td>13</td>
<td>23</td>
<td>0.46</td>
<td>0.23 – 0.94</td>
<td>0.55</td>
<td>0.26 – 1.16</td>
<td>.116</td>
</tr>
<tr>
<td>Ibuprofen 6 or more times/week</td>
<td>9</td>
<td>21</td>
<td>0.35</td>
<td>0.16 – 0.79</td>
<td>0.31</td>
<td>0.14 – 0.71</td>
<td>.006</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>71</td>
<td>95</td>
<td>0.61</td>
<td>0.43 – 0.88</td>
<td>0.53</td>
<td>0.36 – 0.77</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Aspirin less than 6 times/week</td>
<td>31</td>
<td>39</td>
<td>0.65</td>
<td>0.39 – 1.08</td>
<td>0.57</td>
<td>0.33 – 0.96</td>
<td>.036</td>
</tr>
<tr>
<td>Aspirin 6 or more times/week</td>
<td>40</td>
<td>56</td>
<td>0.59</td>
<td>0.38 – 0.92</td>
<td>0.50</td>
<td>0.31 – 0.79</td>
<td>.003</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>33</td>
<td>21</td>
<td>1.29</td>
<td>0.72 – 2.30</td>
<td>1.25</td>
<td>0.68 – 2.29</td>
<td>.471</td>
</tr>
</tbody>
</table>

p-value of test for trend (Ibuprofen user > Aspirin user > Non-user ) = <.001

**adjusted for age, packyears of smoking and race**
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>151</td>
<td>131</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>69</td>
<td>99</td>
<td>0.60</td>
<td>0.41 – 0.89</td>
<td>0.58</td>
<td>0.38 – 0.87</td>
<td>.008</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>33</td>
<td>39</td>
<td>0.73</td>
<td>0.44 – 1.24</td>
<td>0.73</td>
<td>0.42 – 1.25</td>
<td>.251</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>36</td>
<td>60</td>
<td>0.52</td>
<td>0.33 – 0.84</td>
<td>0.48</td>
<td>0.29 – 0.79</td>
<td>.004</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>19</td>
<td>9</td>
<td>1.83</td>
<td>0.80 – 4.18</td>
<td>1.76</td>
<td>0.72 – 4.32</td>
<td>.217</td>
</tr>
</tbody>
</table>

p-value of test for trend (NSAID >= 6/ week > NSAID < 6/week < Non-user ) = .004

**adjusted for age, packyears of smoking and race

<table>
<thead>
<tr>
<th>Group</th>
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<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser (Ref)</td>
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<td>131</td>
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<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>14</td>
<td>16</td>
<td>0.76</td>
<td>0.36 – 1.61</td>
<td>0.80</td>
<td>0.37 – 1.76</td>
<td>.583</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>13</td>
<td>20</td>
<td>0.56</td>
<td>0.27 – 1.18</td>
<td>0.58</td>
<td>0.27 – 1.25</td>
<td>.164</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>42</td>
<td>63</td>
<td>0.58</td>
<td>0.37 – 0.91</td>
<td>0.52</td>
<td>0.32 – 0.84</td>
<td>.008</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>19</td>
<td>9</td>
<td>1.83</td>
<td>0.80 – 4.18</td>
<td>1.76</td>
<td>0.72 – 4.32</td>
<td>.217</td>
</tr>
</tbody>
</table>

p-value of test for trend (Ibuprofen user > Aspirin user > Non-user ) = .015

**adjusted for age, packyears of smoking and race

Table 7: The Effects of NSAIDs on Lung Cancer Development in all Males Studied: As seen below, use of NSAIDs shows a statistically significant, protective effect against lung cancers overall in all males studied. After adjusting for age and smoking history, it is shown that in persons using NSAIDs there is a 42% decreased risk of developing lung cancer. This is very similar to the results shown in Table 6 above in which a 48% decreased risk was shown for males and females combined. Additionally, the pain relieving compound acetaminophen is shown, as above, not to have affected the development of lung cancer in a statistically significant fashion. Further, the use of NSAIDs six or more times a week is shown to have a significant protective effect against lung cancer development, while persons using less than six or more times per week are no longer shown to be protected in a statistically significant manner, thus possibly indicated a dose response.
Table 8: The Effects of NSAIDs on Lung Cancer Development in all Females Studied:
As seen below, use of NSAIDs shows a statistically significant, protective effect against lung cancers overall in all females studied. After adjusting for age and smoking history, it is shown that in persons using NSAIDs, there is a 56% decreased risk of developing lung cancer. This is very similar to the results seen in Tables 6 and 7 (all persons and all males respectively), which showed protective effects of similar magnitude. Additionally, the pain relieving compound acetaminophen is shown not to have affected the development of lung cancer in a statistically significant fashion. When the NSAID group is further broken down into ibuprofen and aspirin classes, the compound ibuprofen, which has greater COX-2 blocking activity than aspirin, shows a greater protective effect among its users than the aspirin users.
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>64</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>43</td>
<td>172</td>
<td>0.75</td>
<td>0.48 – 1.16</td>
<td>0.68</td>
<td>0.43 – 1.07</td>
<td>.097</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>18</td>
<td>77</td>
<td>0.70</td>
<td>0.39 – 1.25</td>
<td>0.67</td>
<td>0.37 – 1.23</td>
<td>.197</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>25</td>
<td>95</td>
<td>0.79</td>
<td>0.47 – 1.33</td>
<td>0.69</td>
<td>0.40 – 1.18</td>
<td>.175</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>13</td>
<td>21</td>
<td>1.85</td>
<td>0.88 – 3.90</td>
<td>1.74</td>
<td>0.79 – 3.80</td>
<td>.168</td>
</tr>
</tbody>
</table>

**adjusted for age, packyears of smoking, gender and race**

Table 9: The Effects of NSAIDs on the Development of Adenocarcinoma of the Lung:
As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs does not show a statistically significant, protective effect against adenocarcinomas of the lung. However, the use of NSAIDs in this group almost achieves statistical significance as showing a protective effect (p=.097). As in the previous Tables (6,7,8) above, the pain relieving compound acetaminophen is shown not to have affected the development of lung cancer in a statistically significant fashion.
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>65</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>36</td>
<td>172</td>
<td>0.62</td>
<td>0.39 – 0.97</td>
<td>0.56</td>
<td>0.34 – 0.93</td>
<td>.025</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>13</td>
<td>77</td>
<td>0.50</td>
<td>0.26 – 0.95</td>
<td>0.50</td>
<td>0.25 – 1.01</td>
<td>.053</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>23</td>
<td>95</td>
<td>0.71</td>
<td>0.42 – 1.22</td>
<td>0.61</td>
<td>0.34 – 1.09</td>
<td>.095</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>7</td>
<td>21</td>
<td>0.98</td>
<td>0.40 – 2.41</td>
<td>0.80</td>
<td>0.29 – 2.43</td>
<td>.673</td>
</tr>
</tbody>
</table>

p-value of test for trend (NSAID >= 6/ week > NSAID < 6/week < Non-user ) = .057
**adjusted for age, packyears of smoking, gender and race

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>Nonuser (Ref)</td>
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<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>6</td>
<td>33</td>
<td>0.53</td>
<td>0.21 – 1.33</td>
<td>0.48</td>
<td>0.18 – 1.27</td>
<td>.140</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>5</td>
<td>44</td>
<td>0.33</td>
<td>0.13 – 0.88</td>
<td>0.32</td>
<td>0.12 – 0.90</td>
<td>.030</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>25</td>
<td>95</td>
<td>0.77</td>
<td>0.46 – 1.30</td>
<td>0.64</td>
<td>0.36 – 1.12</td>
<td>.120</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>7</td>
<td>21</td>
<td>0.98</td>
<td>0.40 – 2.41</td>
<td>0.80</td>
<td>0.29 – 2.43</td>
<td>.673</td>
</tr>
</tbody>
</table>

p-value of test for trend (Ibuprofen user > Aspirin user > Non-user ) = .036
**adjusted for age, packyears of smoking, gender and race

Table 10: The Effects of NSAIDs on the Development of Squamous Cell Carcinoma of the Lung: As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs shows a statistically significant, protective effect against squamous cell carcinomas of the lung. Additionally, as in the previous Tables (6,7,8,9) above, the pain relieving compound acetaminophen is shown not to have affected the development of lung cancer in a statistically significant fashion. When the NSAID group is further broken down into ibuprofen and aspirin classes, the compound ibuprofen, which has greater COX-2 blocking activity than aspirin, shows a greater protective effect among its users than the aspirin users.
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
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<td>191</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>8</td>
<td>172</td>
<td>0.32</td>
<td>0.14 – 0.71</td>
<td>0.32</td>
<td>0.14 – 0.72</td>
<td>.006</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>2</td>
<td>77</td>
<td>0.18</td>
<td>0.04 – 0.76</td>
<td>0.19</td>
<td>0.04 – 0.83</td>
<td>.027</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>6</td>
<td>95</td>
<td>0.43</td>
<td>0.17 – 1.07</td>
<td>0.40</td>
<td>0.16 – 1.03</td>
<td>.058</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>2</td>
<td>21</td>
<td>0.65</td>
<td>0.14 – 2.92</td>
<td>0.59</td>
<td>0.13 – 2.73</td>
<td>.500</td>
</tr>
</tbody>
</table>

p-value of test for trend (NSAID >= 6/week > NSAID < 6/week < Non-user) = .022
**adjusted for age, packyears of smoking, gender and race

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser (Ref)</td>
<td>28</td>
<td>191</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>1</td>
<td>33</td>
<td>0.21</td>
<td>0.03 – 1.57</td>
<td>0.21</td>
<td>0.03 – 1.59</td>
<td>.130</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>1</td>
<td>44</td>
<td>0.16</td>
<td>0.02 – 1.17</td>
<td>0.17</td>
<td>0.02 – 1.29</td>
<td>.086</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>6</td>
<td>95</td>
<td>0.43</td>
<td>0.17 – 1.07</td>
<td>0.41</td>
<td>0.16 – 1.04</td>
<td>.060</td>
</tr>
<tr>
<td>Acetaminophen use</td>
<td>2</td>
<td>21</td>
<td>0.65</td>
<td>0.14 – 2.92</td>
<td>0.59</td>
<td>0.13 – 2.73</td>
<td>.500</td>
</tr>
</tbody>
</table>

p-value of test for trend (Ibuprofen user > Aspirin user > Non-user) = .030
**adjusted for age, packyears of smoking, gender and race

Table 11: The Effects of NSAIDs on the Development of Large Cell Carcinoma of the Lung: As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs shows a statistically significant, protective effect against large cell carcinomas of the lung. Additionally, as in the previous Tables (6,7,8,9,10) above, the pain relieving compound acetaminophen is shown not to have significantly affected the development of lung cancer in a statistically significant fashion.
APPENDIX D:

TABLES OF RESULTS IF ACETAMINOPHEN USERS CODED AS NON-NSAID USERS
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
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<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>43</td>
<td>172</td>
<td>0.69</td>
<td>0.63</td>
<td>0.41–0.98</td>
<td>.041</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>18</td>
<td>77</td>
<td>0.64</td>
<td>0.62</td>
<td>0.34–1.13</td>
<td>.116</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>25</td>
<td>95</td>
<td>0.72</td>
<td>0.64</td>
<td>0.38–1.09</td>
<td>.098</td>
</tr>
<tr>
<td>Nonuser (Ref)</td>
<td>77</td>
<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>12</td>
<td>33</td>
<td>1.00</td>
<td>0.99</td>
<td>0.48–2.05</td>
<td>.971</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>11</td>
<td>44</td>
<td>0.69</td>
<td>0.66</td>
<td>0.32–1.37</td>
<td>.265</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>20</td>
<td>95</td>
<td>0.58</td>
<td>0.52</td>
<td>0.30–0.91</td>
<td>.022</td>
</tr>
</tbody>
</table>

Table 12: The Effects of NSAIDs on the Development of Adenocarcinoma of the Lung When Acetaminophen Users are Coded as Nonusers: As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs now shows a statistically significant, protective effect against adenocarcinomas of the lung. Additionally the NSAID aspirin, by itself shows a significant protective effect against adenocarcinoma. The compound ibuprofen also shows a protective effect, however this is not significant at the alpha = .05 level. These results contrast with Table 9 in which NSAIDs did not show an effect against this histologic type of cancer. As discussed, this previous finding may have been due to the fact that there were many users of acetaminophen amongst the cases with adenocarcinoma that blurred the true NSAID effect.
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>72</td>
<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>36</td>
<td>172</td>
<td>0.62</td>
<td>0.57</td>
<td>0.35 – 0.93</td>
<td>.025</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>13</td>
<td>77</td>
<td>0.50</td>
<td>0.50</td>
<td>0.25 – 1.00</td>
<td>.051</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>23</td>
<td>95</td>
<td>0.71</td>
<td>0.62</td>
<td>0.35 – 1.10</td>
<td>.107</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
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<th>Controls</th>
<th>Crude OR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser (Ref)</td>
<td>72</td>
<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>6</td>
<td>33</td>
<td>0.54</td>
<td>0.55</td>
<td>0.21 – 1.45</td>
<td>.225</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>5</td>
<td>44</td>
<td>0.33</td>
<td>0.33</td>
<td>0.12 – 0.93</td>
<td>.036</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>25</td>
<td>95</td>
<td>0.77</td>
<td>0.67</td>
<td>0.38 – 1.18</td>
<td>.165</td>
</tr>
</tbody>
</table>

Table 13: The Effects of NSAIDs on the Development of Squamous Cell Carcinoma of the Lung When Acetaminophen Users are Coded as Nonusers: As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs as in the previous coding scheme shows a statistically significant, protective effect against squamous cell carcinomas of the lung. When the NSAID group is further broken down into ibuprofen and aspirin classes, the compound ibuprofen, which has greater COX-2 blocking activity than aspirin, shows a greater protective effect among its users than the aspirin users. These results show virtually no change as compared to the results gathered using the previous coding scheme (See Table 10). Thus, it can be inferred that acetaminophen use in this group had no effect on the results.
<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser</td>
<td>30</td>
<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSAID use</td>
<td>8</td>
<td>172</td>
<td>0.33</td>
<td>0.32</td>
<td>0.14 – 0.72</td>
<td>.006</td>
</tr>
<tr>
<td>NSAID 1-5 times/week</td>
<td>2</td>
<td>77</td>
<td>0.18</td>
<td>0.19</td>
<td>0.04 – 0.83</td>
<td>.027</td>
</tr>
<tr>
<td>NSAID 6 or more times/week</td>
<td>6</td>
<td>95</td>
<td>0.44</td>
<td>0.41</td>
<td>0.16 – 1.04</td>
<td>.059</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Controls</th>
<th>Crude OR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonuser (Ref)</td>
<td>30</td>
<td>212</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prescription NSAID use</td>
<td>1</td>
<td>33</td>
<td>0.21</td>
<td>0.21</td>
<td>0.03 – 1.62</td>
<td>.134</td>
</tr>
<tr>
<td>Ibuprofen use</td>
<td>1</td>
<td>44</td>
<td>0.16</td>
<td>0.17</td>
<td>0.02 – 1.26</td>
<td>.082</td>
</tr>
<tr>
<td>Aspirin Use</td>
<td>6</td>
<td>95</td>
<td>0.45</td>
<td>0.42</td>
<td>0.17 – 1.04</td>
<td>.062</td>
</tr>
</tbody>
</table>

Table 14: The Effects of NSAIDs on the Development of Large Cell Carcinoma of the Lung When Acetaminophen Users are Coded as Nonusers: As seen below, after adjusting for age, gender and smoking history, the use of NSAIDs, as in the previous coding scheme, shows a statistically significant, protective effect against large cell carcinomas of the lung. These results show virtually no change as compared to the results gathered using the previous coding scheme. Thus, it can be inferred that acetaminophen use in this group had no effect on the results.