EARLY LUNG CANCER DETECTION IN URANIUM MINERS WITH ABNORMAL SPUTUM CYTOLOGY

Technical Progress Report
for 7/01/96 through 6/30/99

Geno Saccomanno, Ph.D., M.D., Pathologist
Principal Investigator

St. Mary's Hospital & Medical Center
Saccomanno Research Institute
Grand Junction, Colorado

June, 2000

Prepared for
The United States Department of Energy
Grant number DE-FG02-90ER60939

DISCLAIMER

The primary goal of this grant, DE-FG02-90ER60939, has been to monitor the health effects of radon exposure and/or cigarette smoking on uranium workers from the Colorado Plateau. This is the final report for the preceding three years of this grant. The activities described in this report were supported by the Department of Energy under the grant, "*Early Lung cancer Detection in Uranium Miner with Abnormal Sputum Cytology*". Dr. Geno Saccomanno continued to be the Principal Investigator for this ongoing lung cancer screening project and epidemiological study.

During the three-year grant period, 1,889 sputum cytology sample collection kits were mailed to an average of 260 study participants per year for collection, processing and microscopic evaluation of sputum specimen slides. Five hundred fifty-two of those kits were sent during the July 1998 - June 2000 period. Six hundred sixty-three sputum cytology reports were posted during the final period of the grant (Table I). About 30 new or reactivated miners were added to the study each year and replaced those lost from the study population due to either death or attrition. Twenty-three new cases of lung cancer were diagnosed from this cohort in the first two years of this grant. The total number of confirmed tumor registry cases who have been enrolled in this study is 580, and more than 200 cases are pending, awaiting confirmation from tissue specimens and medical records.

The Geno Saccomanno Uranium Workers' Archive continues to be used as a primary information resource by the U. S. Department of Justice, families of former uranium miners, and their attorneys or agents. Documentation of smoking histories, radon exposure and the confirmation of primary lung carcinomas and lung disease through the evaluation, both cytology and pathology, of uranium miner sputum samples from this study provide the necessary data to prove eligibility for monetary compensation under the Radon Exposure Compensation Act. To date, about 2,100 requests for documentation have been processed with more than 300 received within the last two years. Thirty-two of these requests were full record searches, with 26 of those being for the Department of Justice.

An ongoing collaboration with Dr. Steven Belinsky, Lovelace Respiratory Research Institute (LRRI) in Albuquerque, NM, examined tissue samples from miners and non-miners from the Geno Saccomanno Archive. Two hundred thirty-six cases of lung adenocarcinoma and 84 squamous cell carcinoma cases were sent to Dr. Belinsky to be analyzed for aberrant methylation of DNA. The results of this study have been described in a manuscript titled, "Predicting lung cancer by detecting aberrant promoter methylation in sputum". This paper is currently being reviewed for publication in a peer-reviewed journal. The manuscript has been appended to this report, and is summarized below.

Lung carcinoma, the leading cause of tumor-related death, is a key example of a cancer where morality could be greatly reduced through the development of sensitive molecular markers detectable at the earliest stages of disease. By increasing the sensitivity of a PCR approach to detect methylated DNA

sequences, we now demonstrate that aberrant methylation of the p16 and/or O^6-methylguanine-DNA methyltransferase (MGMT) promoters can be detected in DNA from sputum in 100% of patients with squamous cell lung carcinoma up to 3 years prior to clinical diagnosis. Moreover, the prevalence of these markers in sputum from cancer-free, high-risk subjects approximates lifetime risk for lung cancer.

Sputum samples and matched squamous cell carcinomas (SCCs) were obtained from 21 people previously enrolled in a Lung Cancer Surveillance Study conducted through St. Mary's Hospital, Grand Junction, CO and from 91 cancer-free, former uranium miners from Grants, New Mexico, also participating in a cancer surveillance study. All subjects had a history of smoking, and approximately 50% were exposed to radon through uranium mining.

Aberrant methylation of *p16* and/or *MGMT* is detected in the sputum of all tested lung cancer patients at the time of diagnosis. Sputum was collected from 10 of the 21 individuals enrolled in the St. Mary's Hospital Lung Cancer Surveillance Study at the time of diagnosis of their SCC; however, only four samples were diagnostic of cancer by cytological criteria. In marked contrast to cytology findings, one or both gene promoters tested were abnormally methylated in all of these sputum samples. Abnormal *p16* gene methylation was present in sputum from all eight patients whose tumors were also positive for this marker, but not in sputum from the two individuals whose tumors were negative for this change. Four of the six patients with abnormal methylation of *MGMT* in their tumors also had this change detected in their sputum, including the two patients whose tumors lacked the *p16* change. Methylation of *p16* was present in sputum of the two individuals whose sputum was negative for *MGMT*. Aberrant *MGMT* methylation was not detected in sputum or tumor from three cases; in one case, *MGMT* methylation was detected in sputum, but not in the tumor.

Detection of *p16* and *MGMT* promoter methylation precedes clinical cancer. For the other 11 of 21 original individuals with SCC, sputum samples were obtained well before the diagnosis of SCC at times ranging from 5 to 35 months. In only one patient was the sputum sample thought to have unequivocal signs of cancer by cytological criteria. However, abnormal methylation of the *p16* promoter region was detected in DNA from sputum of all 11 subjects with the longest time to tumor diagnosis being 35 months. Where multiple sputum samples were available either as replicate specimens or as temporal samples, methylation of *p16* was always present. A 90% concordance was also observed between *p16* methylation in the primary SCC and paired sputum samples. Sputum from seven of 11 cases also showed methylation of the *MGMT* gene. A 78% concordance was noted between *MGMT* methylation in the primary SCC

and the paired sputum sample. For the two discordant samples, *MGMT* methylation was detected in the tumor but not in the single sputum sample obtained 34 or 35 months prior to cancer.

Methylation of *p16* and *MGMT* in cancer-free, high-risk subjects. Sputum samples were obtained from cancer-free subjects who are at very high risk for lung cancer development because of their exposure to tobacco, radon, or both. The frequency for detecting aberrant methylation of the *p16* or *MGMT* genes was similar across all exposure groups. Both *p16* and *MGMT* methylation was detected in only four of 123 cancer-free subjects (3%) as opposed to 10 of 21 (48%) of the patients with SCC.

The much lower incidence ($p<.001$) of detecting both sputum markers in cancer-free subjects than in the 21 individuals having proven lung cancer emphasizes two facts. First, hypermethylation changes in sputum DNA do not simply reflect exposure to risk factors for lung cancer but rather track with either a very high-risk status or actual presence of cancer. Second, the average incidence of ~25% for detection of either sputum marker in the cancer-free individuals is approximately equal to the known risk of lung cancer development for the populations studied. This indicates that these hypermethylation markers may potentially identify those patients at high risk who are most likely to actually get the disease. Findings from this study strongly support implementing longitudinal studies in subjects at high-risk for developing lung cancer, as well as the need for development of additional hypermethylation markers for lung and other common human cancers. Although detecting methylation of *p16* and *MGMT* methylation in sputum most likely confers a higher risk for lung cancer, the time to tumor is quite variable, presumably due to the necessity for acquiring additional genetic alterations that promote tumor progression. Thus, longitudinal studies with these methylation markers should facilitate the development of more accurate risk models to incorporate time to tumor and the relationship to multiplicity of biomarkers in the sputum.

Another collaboration involving uranium miners from the Saccomanno abnormal sputum cytology study, looked for an accumulation of Lead 210 in the skull as a biomarker of radon exposure. The Saccomanno Research Institute arranged for uranium miners with the appropriate radon exposure history to be tested by Dr. Guilmette at LRRI in Albuquerque, New Mexico. This study is ongoing. Current results have been reported at several scientific meetings, including annual meetings of the Health Physics Society and most recently, the 10th Congress of the International Radiation Protection Association. A manuscript that will be published in the Proceedings of the meetings is attached to this report, and is summarized below.

Epidemiological studies of lung cancer incidence among uranium miners from various populations around the world have shown a significant variability in the relative risk per unit exposure – a range of a factor of 30. A significant fraction of the uncertainty associated with these risk coefficients may be due to differences in the methods and quality of data used in calculating cumulative exposures, in Working Level Months (WLM), for the various miner populations. We hypothesize that *in vivo* measurement of ^{210}Pb, a long-lived radon decay product retained in bone, will provide a better measure of the exposure of individual miners to radon and progeny during their mining careers. To accomplish these *in vivo* measurements, the Lovelace Respiratory Research Institute (LRRI) *In Vivo* Bioassay Facility (IVBF) was modified to optimize a counting geometry for measuring ^{210}Pb in the skull. Six phoswich detectors (12.7 cm diameter) were positioned about the head of a reclining subject (one posterior, one anterior, and four on the sides of the head), and photon emission from the skull was measured using anticoincidence multichannel analysis electronics. The recorded WLM exposures for each uranium miner are being compared with a WLM exposure calculated using a Pb biokinetic model coupled to the ICRP Publication 66 respiratory tract dosimetry model. To date, 189 miners from the Grants, NM mining district have been measured. An additional 61 miners from the Colorado Plateau region (recruited out of the Saccomanno Research Institute miners database) have been measured. The miners from the Grants and Colorado Plateau regions belong to two different epidemiological study populations whose risk factors, as calculated by the BEIR IV Committee (in excess relative risk per WLM) differ by factors of 3.6 or 6.8, depending on the risk model used. One of the objectives of this study is to determine whether the ^{210}Pb *in vivo* measurements performed on miners from these two populations are similar or different when compared to the recorded WLMs on which the epidemiological studies have been historically based.

The grant subcontract, an epidemiological study, with Dr. Frank Gilliland "*Mortality in non-smoking Uranium Miners Study*" continued to focus on the assessment of the mortality rate among the non-smoking cohort of former uranium miners for whom sputum cytology was available in the Geno Saccomanno Uranium Workers Archive. The former miners worked in Colorado, New Mexico, Utah and/or Wyoming mines. From the original 17,800 miner cohort, 4,400 individuals were identified along with their date of birth, Social Security number, and working level months (WLM). Many of these potential subjects had no verifiable record of underground mining and missing or conflicting smoking histories and were eliminated from the study. The final cohort consisted of 2216 non-smoking, former underground uranium miners. Most, 95.7 %, of the former miners were male, of whom 76.3 % were non-Hispanic white, with 13.3 % American Indian, 6.0% Hispanic and 4.4 % unknown. The subjects were first matched against the National Death Index (1979 - 1995) and the Social Security Administrations pre-1979 database on benefits, then the New Mexico Tumor Registry, New Mexico Department of Motor

Vehicle records and St Mary's Hospital Tumor Registry to determine the vital status information of this cohort. 342 deaths were determined and 331 death certificates were obtained. Two certified nosologists coded the cause of death and any differences were resolved by consensus between nosologists. The distribution of death is presented in the accompanying paper. We identified 60 lung cancer cases, 54 of which were included in the preliminary analysis.

Primary cohort analyses was conducted by calculating standardized mortality ratios using ethnic-specific New Mexico rates (age- and calendar year-specific). New Mexico rates were chosen because many cohort members were from New Mexico and ethnic-specific rates with low levels of ethnic misclassification were available for Hispanics and American Indians. The true dates of cohort entry were complied from the date of the first sputum sample sent to Dr. Saccomanno.

Analyses that assess the role of radon-progeny in lung cancer mortality require careful exposure estimates. Radon-progeny exposure estimates for the cohort have been developed at St. Mary's Hospital. With the help of Dr. Victor Archer (University of Utah), a number of sources were used to construct exposure estimates including company records and Public Health Service survey records. Unfortunately, exposure estimates were available for only 20.2 % of the 2216 cohort members. Further cohort analyses await exposure estimates for the remaining 1769 (79.8 %) members.

A nested case-control study within the cohort was conducted. Because most of the cases were male, we restricted the case-control study to males. We chose three controls from the cohort for each lung cancer death. Controls were selected from the cohort members alive at the time of the case's death. Therefore, controls became cases if they died from lung cancer at a later time. Cases and controls were similar in ethnicity. After much additional effort by the St. Mary's Hospital staff, exposure estimates were available for ~96 % of cases and controls.

A paper describing this study, "Radon Progeny Exposure and Lung Cancer Risk Among Non-smoking Uranium Miners" has been accepted for publication in the peer-reviewed journal, *Health Physics*, and is included at the end of this report. The abstract (below) summarizes the results of the analyses.

Studies of miners provide the basis for public health efforts to reduce residential radon progeny exposure. Because of the preponderance of households do not have members who smoke indoors, studies of non-smoking miners contribute essential data for risk assessment for residential radon progeny exposure. We studied a cohort of 2216 never-smokers who were underground uranium miners employed in the western U.S. from 1956 to the early 1990s and who participated in a screening program for lung cancer conducted by Saccomanno and colleagues. After determining the vital status and cause of death in the cohort, we conducted a nested case-control study of 55 lung cancer deaths in males and three age-matched controls for each case. The relative risk of lung cancer was

29.2 (95 % CI 5.1, 167.2) for miners with greater than 1450 WLM compared with those exposed to less than 80 WLM. Temporal factors affected risk, including average dose rate, which was inversely associated with lung cancer risk, and the length of time since last exposure, which was directly associated with decreased risk. As in studies of non-smokers and smokers combined, the exposure response relationship in never-smokers was consistent with a decreased slope at higher WLM, which resulted, in part, from an inverse dose rate effect.

Recent Publications:

Belinsky SA, Nikula KJ, Palmisano WA, **Saccomanno G**, Michels R, Gabrielson E, Baylin SB and Herman JG. 1998 Aberrant Methylation of p16 ^{INK4a} is an Early Event in Lung Cancer and a Potential Biomarker for Early Diagnosis. *Proc. Natl. Aca. Sci.* 95: 11891-11896.

Bailey-Wilson JE, Wiest JS, Anderson M and **Saccomanno G**. 1998 *Biology of Lung Cancer*: Genetics of Lung Cancer. ed. Kane and Bunn, Marcel Dekker, Inc., New York. pp.53-98.

Kennedy TC, Proudfoot SP, Piantadosi S, Wu L, **Saccomanno G**, Petty TL and Tockman MS. 1999 Efficacy of two Sputum Collection Techniques in Patients with Air Flow Obstruction. *Acta Cytol.* 43: 630-636.

Gilliland FD, Hunt WC, Archer VE and **Saccomanno G**. 2000 Radon Progeny Exposure and Lung Cancer Risk Among Non-smoking Uranium Miners. *Health Physics* in press.

Guilmette RA, Leggett RW, Laurer GR, Snipes MB, Hoover MD, Lambert WE, Coons TA and Gilliland FD. 2000 Assessment of Exposure of Uranium Miners to Radon Progeny Using *In Vivo* Measurement of ^{210}PB in Bone. Presented at Int'l. Radiaiton Protection Association, Xth Conference, Hiroshima, Japan.

Palmisano WA, Divine KK, **Saccomano G**, Gilliland FD, Baylin SB, Herman JG and Belinsky SA. Predicting Lung Cancer by Detecting Aberrant Promoter Methylation in Sputum.

Table I

Early Lung Cancer Detection Study
DOE grant: DE-FG02-90ER60939
Statistics July 1 1998 – May 31, 2000

Basic Sputum Study of Uranium Miners

Sputum Kits:	
Sent	552
Returned	459
Reports Sent	663
Aerosol Samples	15
Study Participants:	
Current	250
New Enrollees	17
Enrollment Kits Sent	33
Tumor Registry Miners:	
Total Number	580
Alive with confirmed Ca	25
Pending Tumor Registry	259
New Doctors in Study	37

Radiation Exposure Compensation Act Searches:	
US Department of Justice	26
Attorneys, Other searches	6
WLM only	316