

# Lung Cancer FRONTIERS

The Forum for Early Diagnosis and Treatment of Lung Cancer



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## Lung Cancer in the Military: An Opportunity for Change

By *Barbara G. Campling, MD, FRCPC*

Lung cancer is by far the leading cause of cancer death in men and women, and the majority of cases are caused by smoking. Until recently, the prevalence and intensity of smoking was much higher in active duty military personnel and veterans than in civilians.<sup>1,2</sup> Thus, it is not surprising that the incidence of lung cancer was 76% higher among male veterans who obtained healthcare through the Veterans Health Administration (VHA) than males included in the Surveillance, Epidemiology, and End Results (SEER) program cancer registry.<sup>3</sup> Furthermore, the lung cancer survival rate is lower in Veterans Affairs (VA) patients,<sup>4</sup> making lung cancer an urgent health priority in military and veteran populations.

Active service military personnel and retirees with more than 20 years of service may obtain healthcare through the Military Health System (MHS), an enterprise within the Department of Defense (DOD). In addition, eligible veterans of military service may obtain healthcare through the VHA, although not all choose to do so.<sup>3</sup> Veterans who obtain healthcare through the VHA have had a higher prevalence of smoking than those using other sources of healthcare.<sup>2</sup>

### Role of the military in the tobacco epidemic

*“You ask me what it is we need to win this war.  
I answer tobacco as much as bullets.”*

— General John Pershing, World War I

The purpose of **Lung Cancer Frontiers** is to acquire and disseminate new knowledge about lung cancer and how it can be most quickly and effectively diagnosed and treated.

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## Lung Cancer in the Military: An Opportunity for Change

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In the early 1900s, pipe and cigar smoking were increasing in popularity, but cigarettes were uncommon. Two factors changed this completely. First there was the invention of the cigarette-rolling machine in 1880 that enabled mass production of cigarettes. Then there was World War I. Cigarettes became “the universal emblem of the camaraderie of mortal combat.”<sup>5</sup> In the war effort, cigarette factory employees worked double or even triple shifts. When servicemen returned home, they brought their addiction to nicotine with them and proceeded to transmit the habit to friends and family.

With the outbreak of World War II, cigarettes were declared essential wartime supplies. Millions of deployed servicemen smoked an average of 30 cigarettes a day.<sup>5</sup> Provision of cigarettes to servicemen continued throughout the Korean and Vietnam wars. It was not until 1976 that the government stopped distributing cigarettes in military rations. In the past, tobacco was sold at discounted prices in DOD and VA facilities.

### Smoking and smoking cessation in the military

Until recently, the prevalence of smoking was higher in military veterans than in the general population,<sup>1</sup> and those who did smoke tended to smoke more heavily.<sup>2</sup> The 1987-88 National Health Interview Survey showed that 74.2% of veterans had smoked at some time in their lives, compared to 48% of the general population.<sup>1</sup> Among subjects who had not initiated smoking by age 18 years, veterans were more likely than non-veterans to become smokers, suggesting that military service itself may be a risk factor for cigarette smoking.<sup>1</sup>

Tobacco use takes a heavy toll on both active duty military personnel and veterans. In 2007, the DOD and Department of Veterans Affairs (DVA) sought guidance from the Institute of Medicine (IOM) in combating tobacco use. The IOM formulated a number of recommendations, calling for a “tobacco free military.”<sup>6</sup> Their recommendations included:

- Set a date by which the military will be tobacco-free, with mandatory compliance;
- Raise the priority given to tobacco control within the DOD and VA;

- Eliminate the sale of tobacco at all military installations; and
- Implement comprehensive tobacco-control programs and report progress publicly.

Beginning in the mid-1990s, the VHA has undergone an “extreme makeover” that now serves as a model for other health care systems.<sup>7</sup> An integral part of the makeover was the computerized patient record system (CPRS), which has facilitated a change in tobacco control within the VHA.<sup>8</sup> All veterans who receive care through the VHA are now routinely screened for tobacco use through clinical reminders embedded in the CPRS. Healthcare providers are expected to follow practice guidelines for smoking cessation, and compliance is tied to performance incentives.<sup>8</sup>

The DOD and VHA have made remarkable progress. In 2011, the prevalence of smoking in active duty military personnel was down to 24%.<sup>9</sup> While still somewhat higher than in the general population (19%),<sup>10</sup> smoking prevalence in active service personnel has declined tremendously from 51% in 1980 and 32% in 2005.<sup>6</sup> Smoking rates were highest in the Marine Corps (30.8%) and lowest in the Air Force (16.7%). Despite this recent progress, the newest cohort of veterans from Iraq and Afghanistan continue to perceive smoking as a normal part of military life.<sup>11</sup>

A recent survey of nearly 8 million VA enrollees showed that 20% were current smokers,<sup>12</sup> similar to the smoking prevalence of 19% in the general population.<sup>10</sup> The proportion of former smokers (48%) was still higher than in the general population (21.1%),<sup>10</sup> but significantly lower than in past surveys of veterans.<sup>1</sup>

Military deployment is associated with increased smoking initiation, and it is more strongly associated with smoking recidivism, as shown in the Millennium Cohort, a longitudinal study undertaken by the DOD.<sup>13</sup> Participants were recruited from a stratified, random sample of the more than 2 million adults serving in the military in the year 2000. Baseline surveys were administered prior to the conflicts in Iraq and Afghanistan, and follow up surveys were conducted from June 2004 to January 2006. At baseline, among the 48,304 participants, most (59.2%) had never smoked, 25.9% were past smokers, and 14.9% were current smokers. During

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the study, 23.9% of participants were deployed. Smoking prevalence increased by 57% among those who were deployed and by 44% among those who were not deployed. Among participants who were former smokers at baseline, the rates of recidivism were higher among those who were deployed once (39.4%) or more than once (40.3%) than among those who were not deployed (28.7%). Furthermore, quit rates were lower among deployed personnel (7.6%) than among those who were not deployed (10.3%).

### Lung cancer in the military

Smoking-related lung cancers usually develop after decades of tobacco exposure. Thus, lung cancer is much more common in veterans than active duty personnel. Exposure to other carcinogens, such as asbestos, radiation, combustion products, and possibly Agent Orange, may increase the risk of lung cancer beyond that caused by smoking alone. In a study of male veterans using the VA medical system from 1970-1982, Harris et al. found that the incidence of lung cancer was 76% higher in this group than in males included in the SEER cancer registry.<sup>3</sup>

In a population-based study of lung cancer cases in Pennsylvania from 1995-1999, we found that the overall survival of patients treated in VA healthcare facilities was inferior to that of patients treated in the rest of the state.<sup>4</sup> Because women constituted less than 1% of veterans with lung cancer at that time, the analysis was restricted to males. The median survival was 6.3 months for VA patients compared with 7.9 months for civilians. The 5-year survival rate was 12% for VA patients and 15% for patients in the rest of the state. The proportion of African American patients was much higher in the VA population, so we questioned whether the survival difference may have been due to a racial disparity. However, we found that there was no difference in the survival of black and white patients within the VA system, and there was no difference in survival of black patients within or outside the VA. The disparity was entirely within the white population.

While the prevalence of smoking has changed significantly since these studies were done, it is likely that disparities in incidence and survival for lung cancer still persist in the VA. It is unclear whether these disparities are due to differences in comorbidities, socioeconomic status, or systematic differences in the diagnosis, staging, or treatment of lung cancer in the VA.

### Lung cancer screening in active duty, retired military, and veteran populations

The VHA has an outstanding track record in cancer screening. Rates of screening for colorectal,<sup>14</sup> breast,<sup>15</sup> and cervical cancers<sup>16</sup> are much higher than in the private sector. Veterans who are eligible for cancer screening can easily be identified and compliance rates are remarkably high due to the system of clinical reminders embedded in the CPRS.

In the past, multiple randomized trials of lung cancer screening with chest x-rays showed no reduction in mortality from this disease.<sup>17</sup> However, chest x-rays are not sufficiently sensitive to detect most early stage lung cancers. Renewed interest in screening high-risk subjects for lung cancer came with the demonstration that low-dose spiral CT scans could detect early stage, curable cancers.<sup>18</sup> However, CT screening was not widely accepted until recently. A dramatic change occurred with the publication of the National Lung Screening Trial (NLST), which showed a 20% reduction in lung cancer specific mortality in high-risk subjects screened with low-dose spiral CT scans compared with chest x-rays.<sup>19,20</sup>

In November 2012, lung cancer screening was implemented at the Walter Reed National Military Medical Center. The VHA is now preparing to implement screening as a demonstration project at 8 VA hospitals. Screening for lung cancer involves much more than just a CT scan. It is a process requiring a multidisciplinary team, including primary care providers, radiologists, pulmonary physicians, thoracic surgeons, and oncologists. The electronic medical record could be used to identify and recruit patients at risk for lung cancer. In addition, detailed information about smoking history, carcinogen exposure, and deployment history could be collected prospectively on subjects who undergo screening. This would enable the refinement of risk prediction models for lung cancer. All lung cancer screening programs should also be closely coupled with smoking cessation programs.

The rate of positive screening tests with low-dose CT scanning in the NLST was 24.2%.<sup>19</sup> The vast majority of these were false positive results, some of which required further radiologic and invasive investigations. The cost of these procedures and the risk to patients who are not found to have lung cancer are important considerations when implementing a lung cancer screening program. The

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mortality reduction from lung cancer screening is highest in those who are in the highest risk groups.<sup>21</sup> Because of their past prevalence and intensity of smoking, military personnel and veterans are at higher risk for lung cancer than civilians and thus may stand to benefit more from screening.

### Summary

The military in the United States and elsewhere has played a role in igniting the worldwide tobacco epidemic. The DOD and DVA have implemented initiatives to help to extinguish this epidemic. As a result of these efforts, the prevalence of smoking in military and veteran populations has declined dramatically in recent years. However, lung cancer incidence reflects cumulative smoking habits over many decades. Consequently, the incidence of lung cancer will remain high in military and veteran populations for years to come.

Lung cancer screening with low-dose CT scans presents an opportunity to improve survival of veterans who are at highest risk for lung cancer, many of whom became addicted to tobacco while in military service. With their remarkable track record in screening for other cancers, the MHS and the VHA could lead the way in reducing lung cancer mortality and set an example for the private sector.

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### Disclosures

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## Selections from the Peer-Reviewed Literature

By Hiram Rivas-Perez, MD and Patrick Nana-Sinkam, MD



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### A blood-based proteomic classifier for molecular characterization of pulmonary nodules

Li XJ, Hayward C, Fong PY, Dominguez M, Hunsucker SW, Lee LW, McLean M, Law S, Butler H, Schirm M, Gingras O, Lamontagne J, Allard R, Chelsky D, Price ND, Lam S, Massion PP, Pass H, Rom WN, Vachani A, Fang KC, Hood L, Kearney P. *Sci Transl Med* 2013; 5:207ra142.

**ABSTRACT:** Each year, millions of pulmonary nodules are discovered by computed tomography and subsequently biopsied. Because most of these nodules are benign, many patients undergo unnecessary and costly invasive procedures. We present a 13-protein blood-based classifier that differentiates malignant and benign nodules with high confidence, thereby providing a diagnostic tool to avoid invasive biopsy on benign nodules. Using a systems biology strategy, we identified 371 protein candidates and developed a multiple reaction monitoring (MRM) assay for each. The MRM assays were applied in a three-site discovery study ( $n = 143$ ) on plasma samples from patients with benign and stage IA lung cancer matched for nodule size, age, gender, and clinical site, producing a 13-protein classifier. The classifier was validated on an independent set of plasma samples ( $n =$

104), exhibiting a negative predictive value (NPV) of 90%. Validation performance on samples from a non-discovery clinical site showed an NPV of 94%, indicating the general effectiveness of the classifier. A pathway analysis demonstrated that the classifier proteins are likely modulated by a few transcription regulators (NF2L2, AHR, MYC, and FOS) that are associated with lung cancer, lung inflammation, and oxidative stress networks. The classifier score was independent of patient nodule size, smoking history, and age, which are risk factors used for clinical evaluation of pulmonary nodules. Thus, this molecular test provides a potential complementary tool to help physicians in lung cancer diagnosis.

**EDITORIAL COMMENT:** With the advent of lung cancer screening, both clinicians and scientists will be faced with the task of developing diagnostic biomarkers that complement imaging, thus reducing expensive and unnecessary diagnostic studies. In the current study, the authors present a 13-protein blood-based classifier that may be used to differentiate malignant from benign pulmonary nodules.

In order to identify initial protein biomarker candidates, the authors searched the literature and analyzed tissue samples from resected adenocarcinomas, squamous cell carcinomas, small cell

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carcinomas, large cell carcinomas, and adjacent normal lung tissue for proteins over-expressed or over-secreted by lung cancer tumor cells. They identified 388 initial protein candidates. Of these, 371 proteins could be measured by MRM.

To develop a diagnostic biomarker, or classifier, the 371 proteins were applied to a test set consisting of plasma samples from 143 subjects with lung nodules. Subject characteristics were: age  $\geq 40$ , any smoking status, any comorbid conditions, no prior malignancy within 5 years except skin cancer, nodules  $\geq 4$  mm and  $\leq 30$  mm in size, histopathological diagnosis of non-small cell lung cancer (NSCLC) or a benign process including inflammatory (eg, granulomatous, infectious) and non-inflammatory processes (eg, hamartoma). All lung cancers had clinical stage  $\leq T1A$  or 1B, N0 or N1, and M0. Seventy-two subjects had lung cancer (adenocarcinoma = 41), while 71 subjects had a benign nodule, most commonly a granuloma ( $n = 49$ ). Subjects were matched for age, gender, nodule size and site.

Ultimately a 13-protein biomarker, or classifier, was derived based on the proteins' presence in  $\geq 50\%$  of the plasma samples. The assay was robust and their logistic regression coefficients were stable. The classifier generated a score that ranged from 0-1 based on the expression of the various proteins in the classifier. Using a lung cancer prevalence of 15% that was predicted to be present in a population that had these risk characteristics, and a threshold classifier value of 0.60, the classifier had a NPV of  $95 \pm 2\%$  and a specificity of  $66 \pm 11\%$ .

The protein panel classifier was then validated in a separate cohort consisting of 52 lung cancer and 53 benign samples, with the same inclusion criteria used in the test set. As the utility of the test was felt to be in ruling out malignant disease, performance of the classifier was expressed in terms of its NPV and specificity.

Again, assuming a lung cancer prevalence of 15% in a patient population with these characteristics, the 13-protein classifier had a NPV of  $90 \pm 5\%$  and specificity of  $44 \pm 13\%$ . Correlation between age, smoking history, nodule size, and the classifier was weak, indicating that the classifier provided significant clinical information independent of these known clinical risk factors.

The 13-protein classifier was subsequently submitted to Ingenuity Systems pathway analysis to determine what regulated the proteins in the classifier. Four nuclear transcription regulators were found to be associated with the 13 classifier proteins: the proto-oncogene c-Fos, nuclear factor erythroid 2-related factor 2, aryl hydrocarbon receptor, and myc proto-oncogene protein.

This retrospective study in patients with NSCLC identified a classifier made up of 13 proteins that had a high NPV for lung cancer in a high-risk patient population with lung nodules identified by CT. These findings were independent of nodule size, subject age, and smoking history, thus providing a tool that is complementary to known clinical risk factors for lung cancer. However, the classifier was not integrated with the clinical risk factors to provide a single, comprehensive risk score. Lastly, the authors identified a relationship between the 13-protein classifier and transcription regulators associated with other cancers and other inflammatory process.

The diagnostic approach to pulmonary nodules detected by chest x-ray and CT scans is a complicated one. Physicians must integrate nodule characteristics, patient age, smoking history, and history of extrathoracic malignancy to determine whether or not to observe a lung nodule or proceed with further testing. Occasionally, patients undergo unnecessary testing and delays in diagnosis. These issues are of particular concern in the context of lung cancer screening by low-dose chest CT scanning in which false positive rates tend to be very high.

The current study offers a novel complementary assay that may be applied during the risk assessment of patients with solitary lung nodules. An acceptable screening tool for lung cancer needs to have an acceptable sensitivity, specificity, positive predictive value, or NPV, depending on whether the goal is to rule-in or rule-out lung cancer. A NPV close to 100% is associated with a greater probability that a person with a negative test result is truly free of disease. The 13-protein classifier had an acceptable NPV ( $95 \pm 2\%$ ) for malignancy.

In addition, the 13 proteins in the classifier appear to be regulated by nuclear transcription factors important in cancer. This correlation supports the concept that the classifier is measuring processes relevant to tumor biology. However, the transcription regulators are also associated with oxidative

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stress. Thus, it is unclear whether the 13-protein classifier will provide robust accuracy in the presence of infection or an inflammatory process.

One of the limitations of this study was its retrospective design. The authors commented on this limitation and recognize the need for a large, prospective study to further validate the findings. In addition, while the area under the ROC curve was 0.82 in the discovery cohort, it was only 0.60 in the validation cohort. This suggests that additional refinement and prospective validation of this biomarker will be required. Lastly, the prognostic information provided by the 13-protein classifier was independent of smoking history, age, and nodule size. A classifier that may be integrated into a risk calculation for solitary nodules would be of particular interest to the practicing clinician.

### A prognostic DNA methylation signature for stage I non-small cell lung cancer

**Sandoval J, Mendez-Gonzalez J, Nadal E, Chen G, Carmona FJ, Sayols S, Moran S, Heyn H, Vizoso M, Gomez A, Sanchez-Cespedes M, Assenov Y, Müller F, Bock C, Taron M, Mora J, Muscarella LA, Liloglou T, Davies M, Pollan M, Pajares MJ, Torre W, Montuenga LM, Brambilla E, Field JK, Roz L, Lo Iacono M, Scagliotti GV, Rosell R, Beer DG, Esteller M. *J Clin Oncol* 2013; 31:4040-7.**

**PURPOSE:** Non-small-cell lung cancer (NSCLC) is a tumor in which only small improvements in clinical outcome have been achieved. The issue is critical for stage I patients for whom there are no available biomarkers that indicate which high-risk patients should receive adjuvant chemotherapy. We aimed to find DNA methylation markers that could be helpful in this regard.

**PATIENTS AND METHODS:** A DNA methylation microarray that analyzes 450,000 CpG sites was used to study tumoral DNA obtained from 444 patients with NSCLC that included 237 stage I tumors. The prognostic DNA methylation markers were validated by a single-methylation pyrosequencing assay in an independent cohort of 143 patients with stage I NSCLC.

**RESULTS:** Unsupervised clustering of the 10,000 most variable DNA methylation sites in the discovery cohort identified patients with high-risk stage I NSCLC who had

shorter relapse-free survival (RFS; hazard ratio [HR], 2.35; 95% CI, 1.29 to 4.28;  $P = .004$ ). The study in the validation cohort of the significant methylated sites from the discovery cohort found that hypermethylation of five genes was significantly associated with shorter RFS in stage I NSCLC: *HIST1H4F*, *PCDHGB6*, *NPBWRI*, *ALXI*, and *HOXA9*. A signature based on the number of hypermethylated events distinguished patients with high- and low-risk stage I NSCLC (HR, 3.24; 95% CI, 1.61 to 6.54;  $P = .001$ ).

**CONCLUSION:** The DNA methylation signature of NSCLC predicts the outcome of stage I patients and can be practically determined by user-friendly polymerase chain reaction assays. The analysis of the best DNA methylation biomarkers improved prognostic accuracy beyond standard staging.

**EDITORIAL COMMENT:** Clinical stage IA and stage IB NSCLC have a 5-year survival of 50% and 43%, respectively (*J Thorac Oncol* 2007; 2:706-14). Currently, there are no available prognostic biomarkers for stage I NSCLC that predict high risk of recurrence after surgery with curative intent. Such biomarkers could be of value in identifying high-risk populations that would benefit from adjuvant chemotherapy. The primary objective of the current study was to identify DNA methylation markers that predict RFS of stage I lung cancer patients.

A discovery cohort of 444 lung cancers (72.5% adenocarcinomas, 27.5% squamous cell carcinomas) was analyzed for DNA methylation markers. The majority of the cases were stage I ( $n = 380$ ), and the median clinical follow-up was 7.2 years. DNA methylation status was determined using a 450,000 CpG methylation microarray. Comparison of DNA methylation results of the NSCLCs to normal lung tissue identified 10,000 promoter CpGs with the most variable CpG levels. Hierarchical clustering based on methylation status distinguished two main types of tumor DNA methylation. One group (Group A) consisted of 70 patients, while the other group (Group B) included 374 patients. Analyzing the subset of the 444 patients that had undergone resection and had not received adjuvant therapy (198 patients), those subjects who had a Group A methylation profile had a significantly shorter RFS (~2.5 vs 8 years,  $P < 0.001$ ). In univariate and multivariate analyses of stage, histology, smoking history, age, and sex, Group A patients still had a shorter RFS (HR  $\geq 2.40$ ,  $P < 0.001$ ).

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Another profile of the same 10,000 promoter CpGs was plotted in an unsupervised manner using only patients with stage I NSCLC (237 patients) derived from the 444 patients. Hierarchical clustering of their DNA methylation status distinguished two groups of patients: Group 1 included 63 patients and Group 2 contained 174 subjects. Again, studying the subset of patients that had not received adjuvant therapy, the Group 1 methylation pattern identified patients with a shorter RFS (Group 1 = 5 years vs Group 2 = 11 years; HR = 2.35 [1.29-4.28];  $P < 0.03$ ).

Ranking the 10,000 CpG sites to identify a simplified panel found 150 CpG sites were significantly enriched in Group A vs Group B ( $t$  test,  $P < 0.001$ ) and in Group 1 vs Group 2 ( $t$  test,  $P < 0.001$ ). Testing the methylation status in the 147 stage I patient subset identified 54 CpGs associated with shorter RFS, further simplifying the panel.

The top 10 genes associated with the 54 CpG sites were tested for their ability to predict RFS in a separate validation cohort of 143 stage I cancers. Five of the 10 genes were significantly associated with recurrence ( $P < 0.005$ ). Shorter RFS was seen with hypermethylation of the following genes: histone cluster 1, H4f (*HIST1H4F*; HR, 3.55;  $P < 0.001$ ), protocadherin gamma subfamily B6 (*PCDHGB6*; HR, 2.95;  $P = 0.002$ ), neuropeptide B/W receptor 1 (*NPBWR1*; HR, 2.71;  $P = 0.004$ ), ALX homeobox protein 1 (*ALX1*; HR, 2.29;  $P = 0.015$ ), and homeoboxA9 (*HOXA9*; HR, 2.03;  $P = 0.027$ ). There was an 18% recurrence rate by 3 years in patients with 0-1 of these methylated genes (95% CI, 16.1% to 19.5%) compared to a 48% recurrence rate (95% CI, 39.8% to 56.4%) in those with >2 methylated genes.

The investigators found that DNA methylation signature status carries prognostic information and that this biomarker may provide a new tool for determining risk of recurrence in patients with resected stage I NSCLC. This biomarker requires further validation, but it could potentially be used to guide appropriate surveillance and adjuvant therapy. It would be interesting to study the effects of chemotherapy in patients who present with tumors with the methylation characteristics of Group A with shorter RFS. It is worth noting that of the 5 genes reported to predict shorter RFS, hypermethylation of *HOXA9* showed no statistically significant association with shorter RFS. This is important because *HOXA9* is a gene associated with tumorigenesis in lung cancer.

One of the limitations of the study is that is unclear what type of procedure (lobectomy versus pneumonectomy) was performed on patients with shorter RFS. The type of surgical intervention may contribute to recurrence rates in patients with stage I disease. Another limitation is that the results of DNA methylation patterns were reported only for patients with a poor prognosis. Further studies examining patterns of hypermethylation that predict positive outcomes could be conducted with the goal of using a combination of these biomarkers to determine prognosis. The results could assist in selection of alternative or adjuvant therapies for stage I disease. The DNA methylation pattern could also be used to provide insight into molecular mechanisms of tumor progression.

### A genomics-based classification of human lung tumors

**The Clinical Lung Cancer Genome Project and Network Genomic Medicine. *Sci Transl Med* 2013;5:209ra153.**

**ABSTRACT:** We characterized genome alterations in 1255 clinically annotated lung tumors of all histological subgroups to identify genetically defined and clinically relevant subtypes. More than 55% of all cases had at least one oncogenic genome alteration potentially amenable to specific therapeutic intervention, including several personalized treatment approaches that are already in clinical evaluation. Marked differences in the pattern of genomic alterations existed between and within histological subtypes, thus challenging the original histomorphological diagnosis. Immunohistochemical studies confirmed many of these reassigned subtypes. The reassignment eliminated almost all cases of large cell carcinomas, some of which had therapeutically relevant alterations. Prospective testing of our genomics-based diagnostic algorithm in 5145 lung cancer patients enabled a genome-based diagnosis in 3863 (75%) patients, confirmed the feasibility of rational reassignments of large cell lung cancer, and led to improvement in overall survival in patients with *EGFR*-mutant or *ALK*-rearranged cancers. Thus, our findings provide support for broad implementation of genome-based diagnosis of lung cancer.

**EDITORIAL COMMENT:** In the last decade, we have transitioned into the era of personalized genomics of cancer. This transition has come with the realization that cancers harbor distinct genetic signatures that may be leveraged for prognosis and therapeutic intervention. In the case of lung

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cancer, the discovery of mutations in genes such as *EGFR* has led to the development of targeted therapeutics and improved outcomes in select subpopulations of patients; however, there are likely to be additional genetic aberrancies that could provide insight into molecular sub-classification of lung tumors and help identify novel, actionable targets.

This study is one of the largest to link genomic alterations in lung cancer to both clinical and histological subtypes. The primary goal was to identify genomic subtypes of lung cancer that may be used for personalizing therapeutic decisions. It was based on two cohorts of lung tumors. The first was a retrospective set of lung tumors (N = 1255) undergoing high throughput genetic analysis (originating in 2008) derived from the Clinical Lung Cancer Genome Project (CLCGP), while the second was a prospective set (N = 3863) undergoing selected molecular testing within the 18-hospital Network Genomic Medicine (NGM).

Samples from the CLCGP were subjected to copy number analysis (N = 1032), mutation detection (N = 1127), fluorescence in situ hybridization (FISH) (*ALK*, *RET*, *ROS1*) for rearrangement frequency, gene expression analysis (array, RNAseq), and whole-exome sequencing. The primary goals were to determine frequencies of genetic alterations across subtypes and identify histology-specific signatures. Among 5145 cases from the NGM, 3863 tumors were deemed adequate for molecular alteration testing (*ALK* rearrangement, *BRAF* mutation, *EGFR* mutation, *HER2/neu* amplification, *KRAS* mutation, *PIK3CA* mutation, *DDR2* mutation, and *FGFR1* amplification).

The investigators first conducted a genetic analysis on 1255 lung tumor specimens from the CLCGP cohort. An evaluation for somatic copy number alterations (SCNAs) in 1032 samples revealed distinct and overlapping variations, many of which have been previously described in lung tumors. In particular, squamous cell carcinomas (SQs) harbored distinct amplifications in chromosomal region 3q, which contains the *SOX2* gene. Some amplified chromosomal regions in adenocarcinoma (AD) included 5p, 7p (*EGFR*), 8q (*MYC*), and 11q (*CCND1*). In small cell lung cancer, 1p (*MYCL1*), 2p (*MYCN*), and 5p,8p (*FGFR1*) were some of the amplifications noted. On the other hand, large cell carcinomas (LCs) did not have distinct SCNAs, but rather had SCNAs observed in other histological subtypes.

Mutations in *TP53* (53.6%), *KRAS* (16.1%), and *STK11* (9.8%) were the most common. Other common mutations included *EGFR* (7.2%), *KEAP1* (6.6%), and *NFE2L2* (4.5%). In addition, select mutations had histological specificity, such as *NFE2L2* in SQs. Perhaps the most important finding was that 55% of lung tumors had a genetic alteration that could be targeted for therapy.

Interestingly, while the common mutations were not associated with survival, the investigators identified combinations of mutations (eg, *TP53* and *RBI1*) that may be of prognostic significance. By integrating both SCNAs and mutations, the investigators were able to identify histologically distinct genetic signatures. Furthermore, they were able to identify both co-occurring and mutually exclusive alterations that were histology-specific. For example, *EGFR* amplifications correlated with mutations in AD but not in SQ. Furthermore, *ERBB2* mutations were mutually exclusive from mutations in *BRAF*, *HRAS*, *KRAS*, *NRAS*, or *STK11*. A statistical model using histology-specific genomic alterations was fairly accurate in classifying histological subtypes. However, LC did not appear to carry a distinct pattern of genomic alteration, but rather shared characteristics with other subtypes. This was further validated by gene expression array in an independent cohort of 261 lung tumors.

The second phase of this study sought to evaluate 5145 cases of lung cancer in the NGM for key genomic alterations in *ALK*, *BRAF*, *DDR2*, *EGFR*, *ERBB2*, *FGFR1*, *KRAS*, and *PIK3CA*. Ultimately, genomic testing could only be conducted in 3863 cases. Genetic mutations were reported back to the patient's physician and could be used to guide therapeutic decision-making. For example, 76% of patients with advanced-stage disease and *EGFR* mutations were subsequently treated with an *EGFR* inhibitor, while 50% of patients with *ALK* translocations received crizotinib. Patients receiving *EGFR* inhibitors had a median overall survival of 31.6 months, compared to 9.6 months for those not receiving targeted therapy ( $P < 0.001$ ). Additionally, among individuals with *ALK* rearrangements, those who received crizotinib (50%) survived longer than those who did not receive crizotinib. In addition, within the NGM cohort, those who underwent genotyping had a better outcome than those whose genotyping could not be done, and genotyping impact on outcome was independent of histology or stage.

## Selections from the Peer-Reviewed Literature

*continued from page 9*

In summary, this study represents one of the largest multi-institutional studies to examine lung cancer tissues for genetic alterations. While patients with lung cancer continue to have poor 5-year survival rates, there is emerging evidence that targeted therapies will have a significant impact on patient outcomes. By testing two large cohorts of lung cancer patients, the authors provide further validation of the molecular heterogeneity that exists within lung cancer and establish the most common genetic alterations, including both copy number alterations and mutations present in subtypes of lung cancer. Importantly, the authors noted that LC does not harbor a distinct signature but instead overlaps with other subtypes. Often considered an aggressive subtype of lung cancer, LC does not have any actionable targets. This study may represent the first step in identifying such molecular targets. Lastly, this large study further supports the concept of using genetic analysis of lung tumors to guide therapeutic decisions.

The discovery of mutations in *EGFR* and *ALK* and subsequent use of targeted therapeutic agents in lung cancer has revolutionized how clinicians approach lung cancer in

selected patients. In the prospective cohort in this study, the investigators examined nearly 4000 lung cancer cases for 8 of the most common mutations, rearrangements, and amplifications. Most importantly, the findings were reported and likely used to guide therapy. The observation that individuals across both academic and community hospitals with selected mutations (eg, *EGFR* and *ALK*) who received targeted therapy had better outcomes is an important finding. It solidifies the importance of genetic testing in lung cancer. There are likely to be multiple other genetic alterations (and combinations) that may one day guide therapy. It is daunting to consider the presence of multiple alterations and the potential molecular permutations that may exist within histologically similar tumors. However, understanding the molecular heterogeneity of lung cancer is one of several important steps in ultimately improving outcomes.

### Disclosures

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### 14th Annual Targeted Therapies of the Treatment of Lung Cancer Meeting

**February 19-22, 2014**

Santa Monica, CA

Information: [iascl.org](http://iascl.org)

### European Lung Cancer Conference

**March 26-29, 2014**

Geneva, Switzerland

Information: [iascl.org](http://iascl.org)

### Latin American Lung Cancer Conference

**August 21-23, 2014**

Lima, Peru

Information: [iascl.org](http://iascl.org)

### Chicago Multidisciplinary Symposium in Thoracic Oncology

**October 30-November 1, 2014**

Chicago, IL

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