

# Premalignant and Malignant Cells in Sputum From Lung Cancer Patients

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**BACKGROUND:** The objective of this study was to assess the frequency of premalignant and malignant cells in sputum from patients with lung cancer and to measure the dependence of these cells on cancer stage, histologic type, tumor size, and tumor location. **METHODS:** This analysis included 444 patients with lung cancer. First, all patients were asked to produce sputum spontaneously; then, they underwent sputum induction. Slide preparations of the sputa were screened for the presence of abnormal cells. **RESULTS:** Of all patients with lung cancer who had produced adequate specimens, 74.6% had sputum that was positive for premalignant or worse cells, whereas 48.7% had sputum that was positive for malignant cells alone. Surprisingly, the presence of premalignant or worse cells in sputum depended only moderately on disease stage (82.9% of stage IV cancers vs 65.9% of stage I cancers), tumor size (78.6% of tumors >2 cm vs 64.7% of tumors ≤2 cm), and location (83.3% of central lesions vs 68% of peripheral lesions) and was found to be independent of histologic tumor type (78.4% of squamous cell carcinomas vs 71.5% of adenocarcinomas, 74.5% of small cell carcinomas, and 75% of large cell carcinomas). **CONCLUSIONS:** The findings of the current study suggested the important potential of sputum cytology for lung cancer detection and risk assessment across all stages, histologic types, tumor sizes, and locations. However, the high sensitivities in this study were achieved with a level of scrutiny not feasible in the laboratory routine. The diagnostic potential of sputum cytology may be exploited better through the standardization and automation of sputum preparation and analysis. *Cancer (Cancer Cytopathol) 2009;117:000-000. © 2009 American Cancer Society.*

**KEY WORDS:** atypia, dysplasia, induced sputum, lung cancer, lung neoplasms/diagnosis, premalignant, incidence, sensitivity and specificity, spontaneous sputum, sputum cytology.

**Lung** cancer is a disease with a poor prognosis. Of >215,000 patients with newly diagnosed lung cancer predicted for the United States in 2008, only approximately 15% are expected to survive for 5 years.<sup>1</sup> Screening has proven crucial in reducing the mortality rate for malignancies of certain other organs, such as cervix, prostate, breast, and colon. For lung cancer, however, large screening trials in the 1970s and

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1980s using chest x-rays and sputum cytology failed to establish a significant decrease in patient mortality.<sup>2-4</sup> A renewed optimism for lung cancer screening arose with the introduction of x-ray computed tomography (CT) into the radiologic routine. A screening study of >31,000 high-risk patients using low-dose CT has reported a 10-year survival rate of 92% for those patients who underwent surgical resection within 1 month after detection.<sup>5</sup> Other studies on CT screening have reported lesser reductions in lung cancer-specific mortality or even no reduction at all,<sup>6-8</sup> and the debate concerning whether mass CT screening should be implemented is ongoing. Although CT currently is unsurpassed for localizing peripheral pulmonary lesions, it has several limitations that seem prohibitive in a mass screening setting. One important limitation is the poor discrimination between benign lesions and malignant lesions; 90% to 95% of all pulmonary nodules detected by CT reportedly are benign.<sup>9</sup> Even after stringent selection, from 20% to 30% of patients who are followed-up with invasive diagnostic procedures turn out to have nonmalignant lesions.<sup>9</sup> Thus, smoking history and age alone are not sufficient selection criteria for CT imaging of nonsymptomatic patients, and there remains a need for screening methodologies that more appropriately define a patient's lung cancer risk before CT imaging. But what is the best triage method to complement CT?

The worldwide search for lung cancer biomarkers has focused on an ever increasing number of molecular elements of carcinogenesis at the genetic, epigenetic, and protein levels. Although some of these studies have reported high sensitivities or specificities, most of them lack reproducibility, and none of the proposed biomarkers have been validated in large-scale clinical trials.<sup>10</sup> This may explain the continuing interest in sputum cytology, with which the cellular features of all stages of lung carcinogenesis have been studied and categorized for decades. Sputum cytology is the cancer detection method with the highest specificity; a review of 16 published studies on sputum cytology involving >28,000 patients reported an average specificity of 99%.<sup>11</sup> However, there is much less agreement for the sensitivity of sputum cytology, which ranged in the same review from 42% to 97% with an aver-

age of 66%. Factors that influence sputum sensitivity include the number and frequency of diagnostic cells in sputum, the specimen-collection method, the specimen-preparation technique, and the experience level of the individual who is screening the specimen for abnormal cells. Also, it appears plausible that a small tumor might not shed large numbers of cancer cells into sputum, thus contributing to the variance in the sensitivity of sputum cytology. However, sputum cytology is not limited to the detection of cancer cells alone. The majority of lung cancers are preceded by the occurrence of premalignant (mainly squamous dysplastic) lesions, which typically develop in multiple sites and accumulate over many years. Consequently, dysplastic cells are present in sputum in substantially greater numbers than cancer cells.<sup>12</sup> The strong relation between squamous dysplasia and increased risk of either having lung cancer or developing lung cancer within a few years has been established in several studies using sputum cytology and bronchoscopy.<sup>13-20</sup> Hence, dysplastic cells identified in sputum may be a useful biomarker for triaging patients to CT or bronchoscopy.

The main objective of this study was to assess how frequently premalignant (dysplastic) and malignant cells occur in sputa from patients with lung cancer and whether using the presence of dysplastic cells as a threshold for further diagnostic follow-up would increase the sensitivity of sputum cytology for the detection of lung cancer. We also were interested in evaluating whether the presence of dysplastic or worse cells in sputum depends on disease stage, histologic type, tumor size, and tumor location. The data also allowed us to make a comparison of spontaneous and induced collection of sputum with regard to specimen adequacy.

## MATERIALS AND METHODS

This study was an analysis of previously unpublished data collected during a phase 3 clinical trial on the performance of uridine 5'-triphosphate (UTP) inhalation that tested its ability to improve the quality of expectorated sputum with regard to the cytologic diagnosis of lung cancer. The trial was sponsored by Inspire Pharmaceuticals (Durham, NC). The double-blind, placebo-controlled, double-arm study was performed at 58 study centers within the United States, 4 centers in Canada, and 1 center in Costa Rica. All participating investigators received institutional

review board (IRB) approval (or approval by an IRB equivalent outside the United States) before patient enrollment. Written consent was obtained from each patient. Sputum specimens were collected between April 2001 and December 2003. The outcome of the original, early terminated trial did not indicate a statistically significant diagnostic difference between inhalation of UTP and the placebo (saline) with regard to lung cancer detection, and the drug was not commercialized. Nevertheless, given the magnitude and clinical rigor of the original trial, we were interested in further analyzing the data that were collected during the trial to answer several additional questions.

### Patients

In total, 704 patients who were at very high risk for lung cancer were selected for the trial. Included were men and nonpregnant, nonlactating women aged  $\geq 18$  years who had a forced expiratory volume in 1 second (FEV<sub>1</sub>),  $\geq 40\%$  of the predicted normal value for their age with conditions suspicious for primary lung cancer based on chest radiograph(s), CT scan(s), positron emission tomography scan(s), symptoms, risk profile, or history suggestive of malignancy. Patients who had a pre-established cancer diagnosis and/or who had received previous treatment for malignancy were excluded from enrollment. The study protocol provided that, within 120 days after sputum collection, each patient would be followed-up for confirmation of whether malignancy was present based on histopathologic or cytopathologic diagnosis.

### Study Protocol

Each enrolled patient was asked to produce a spontaneous sputum specimen and then underwent sputum induction to produce a second sputum specimen. Induction was performed with either placebo (4 mL 0.9% sodium chloride solution; weight/volume) or a single dose of 20 mg or 60 mg UTP (4 mL of either 5 mg/mL or 15 mg/mL INS316 solution; Inspire Pharmaceuticals) using a PARI LC-D disposable jet nebulizer powered by a PARI compressor (PARI Respiratory Equipment, Monterey, Calif). The sputum specimens were weighed, fixed with Saccomanno solution, and shipped to a central laboratory, where a small volume from each specimen was removed for the

**Table 1.** Diagnostic Scoring System for Slides Prepared From Sputum Specimens Used by the Cytopathologists

Category	Cytopathologic Diagnosis
1	Nondiagnostic (insufficient pulmonary material)
2	Nondiagnostic (distorted, poorly preserved or stained, other)
3	Benign
4	Atypical cells present, probably benign
5*	Atypical cells present, suspicious for malignancy
6	Malignant cells present (with cell type specification)

\*Category 5 ("atypical cells present, suspicious for malignancy") refers mostly to the presence of squamous dysplastic cells.

preparation of 2 pick-and-smear slides. The remainder of each specimen was homogenized in a blender and spun down, and the cell pellet was resuspended in 30 mL of 95% ethanol. From this fixed cell suspension, portions were removed for the preparation of 2 Megafunnel-Cytospin slides (ThermoShandon, Runcorn, England) and 2 cell-block slides. The rest of the cell suspension was then stored in 95% ethanol at room temperature, and was not used for diagnosis. To determine specimen adequacy, alveolar-macrophage counts were performed in the central laboratory on a standardized area of the Cytospin slides.

The pick-and-smear slides, the Megafunnel-Cytospin slides, and the cell-block slides from each patient were evaluated microscopically in the majority of cases by 3 (and in a few cases, by 2 or 4) cytopathologists (Y.S.E., W.J.F., P.K.G., and M.B.Z.) in an independent, blinded fashion. Each diagnosis was assigned to 1 of the numbered categories listed in Table 1. Categories 1 and 2 described slides that were not suitable for cytologic evaluation. Categories 3 and 4 described findings that were normal or that had no relevance to malignancy or premalignancy. Category 5 was comprised of slides with mostly squamous dysplastic cells of various degrees of severity, or, in some cases, cells that were suspicious for malignancy, but did not permit a definitive diagnosis because of compromised preservation. Category 6 was used exclusively for the cases in which cancer cells were detected.

### Data Analysis

#### Selected patients for analysis

For our analysis, we selected those patients from the original cohort who were diagnosed with lung cancer

**Table 2.** Classification of Abnormal Sputum Diagnoses With Relevance to Lung Cancer as Used in the Data Analysis

Classification of Abnormal Specimens for Analysis	Classification Criteria
Premalignant or worse	At least 1 pick-and-smear slide, Cytospin slide, or sectioned cell block was categorized as "5" or "6" by any of the cytopathologists
Cancer	At least 1 pick-and-smear slide, Cytospin slide, or cell block slide was categorized as "6" by any of the cytopathologists

within the 120-day follow-up period and whose clinical data were complete.

### Specimen adequacy criteria

We considered sputum specimens to be adequate if they met the following criteria: at least 50 alveolar macrophages were present per slide and, in addition, at least 1 slide was categorized as Category 3 or 4 by at least 1 of the cytopathologists. Categories 5 and 6 detected by at least 1 cytopathologist were considered adequate independent of the presence of macrophages. All other specimens were considered inadequate for cytopathologic analysis.

### Incidence of premalignant cells and cancer cells in sputa from patients with lung cancer

All cytopathologically abnormal specimens from patients with confirmed lung cancer were categorized as shown in Table 2. The incidence of sputa diagnosed as "pre-malignant or worse" or "cancer" was analyzed separately with regard to cancer stage, histologic type, tumor size, and tumor location (central vs peripheral). Peripheral tumors were defined as those located distal to the subsegmental bronchi, and all other tumors were defined as central. The analyses were performed separately for spontaneous sputa and induced sputa as well as for spontaneous sputa and induced sputa combined.

### Statistical Analysis

Data for this analysis were supplied by Inspire Pharmaceuticals, Inc as Statistical Analysis System (SAS) datasets.

**Table 3.** Numbers of Patients Who Were Able to Expectorate Sputa Spontaneously and After Induction and the Numbers of Patients Whose Sputa Were Adequate for Cytopathologic Evaluation

Ability to Expectorate/Specimen Adequacy	No.	%
<b>Patients attempting to expectorate</b>	444	100
Patients able to produce sputum specimens		
Spontaneous	352	78.9
Induced	425	94.1
Either spontaneous or induced	426	95
<b>Patients with adequate sputum specimens*</b>		
Spontaneous	248	70.5
Induced	390	91.8
Either spontaneous or induced	398	93.4

\* The percentages in the 3 lower rows represent the proportion of patients who delivered adequate specimens among all those patients who were able to expectorate a specimen. Adequacy criteria were  $\geq 50$  macrophages and/or the presence of abnormal cells on the slide.

We used SAS statistical software (version 9.1.3; SAS Institute Inc, Cary, NC) for the additional analysis of data.

## RESULTS

Among the 704 enrolled patients, 446 were diagnosed clinically with lung cancer within the 120-day follow-up period. Of these, 444 patients were included in the current analysis (2 patients were excluded because of incomplete clinical data). Our analysis indicated that 70.5% of all spontaneous sputa and 91.8% of all induced sputa complied with the adequacy criteria (Table 3). The difference in the adequacy rate between the spontaneous sputa and induced sputa was statistically significant.

The relation between the frequency of detecting premalignant cells and cancer cells in sputa from patients with lung cancer who subsequently were diagnosed within the 120-day follow-up period is expressed in Table 4. Of the patients who delivered at least 1 adequate sputum specimen, 48.7% were cytopathologically positive for cancer cells (Category 6), and 74.6% were positive for premalignant or worse cells (Categories 5 and 6). A breakdown between spontaneous sputa and induced sputa is provided in Table 4; the differences between the 2 collection methods were statistically significant. In addition, our analysis indicated a 53.1% increase in sensitivity when the criterion for a positive sputum diagnosis was tied to the presence of premalignant or worse cells (Categories 5 and 6) instead of cancer cells alone (Category 6).

**Table 4.** Incidence of Abnormal Sputum Diagnoses for Lung Cancer in Relation to Lung Cancer Stage, Type, Size, and Location\*

Follow-Up Diagnosis	Sputum Cytology											
	Spontaneous Sputum				Induced Sputum				Either Spontaneous or Induced Sputum			
	Premalignant or Worse		Cancer		Premalignant or Worse		Cancer		Premalignant or Worse		Cancer	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<b>TNM stage</b>												
I	30	53.6	14	25	49	62	27	34.2	54	65.9	32	39
II	12	41.4	8	27.6	29	67.4	15	34.9	30	69.8	17	39.5
IIIA	19	54.3	12	34.3	39	66.1	24	40.7	45	75	30	50
IIIB	22	66.7	12	36.4	43	78.2	32	58.2	44	78.6	36	64.3
IV	32	66.7	19	39.6	62	82.7	36	48	63	82.9	39	51.3
Unknown stage	33	70.2	19	40.4	56	70.9	35	44.3	61	75.3	40	49.4
Total	148	59.7	84	33.9	278	71.3	169	43.3	297	74.6	194	48.7
<b>Cancer type</b>												
Adenocarcinoma	50	60.2	30	36.1	89	66.4	55	41	98	71.5	66	48.2
Squamous cell carcinoma	43	62.3	24	34.8	85	78	61	56	87	78.4	63	56.8
Small cell carcinoma	15	55.6	10	37	33	71.7	15	32.6	35	74.5	20	42.6
Large cell carcinoma	6	40	3	20	20	74.1	8	29.6	21	75	10	35.7
Mixed type	2	28.6	2	28.6	7	70	3	30	8	80	4	40
Other	32	68.1	15	31.9	44	68.8	27	42.2	48	73.9	31	47.7
Total	148	59.7	84	33.9	278	71.3	169	43.3	297	74.6	194	48.7
<b>Tumor size, cm</b>												
≤2	40	51.3	21	26.9	72	63.2	34	29.8	75	64.7	41	35.3
>2	107	63.7	62	36.9	204	74.5	133	48.5	220	78.6	151	53.9
<b>Tumor location</b>												
Central	68	65.4	39	37.5	134	80.7	80	48.2	140	83.3	92	54.8
Peripheral	78	54.9	45	31.7	142	64	89	40.1	155	68	102	44.7

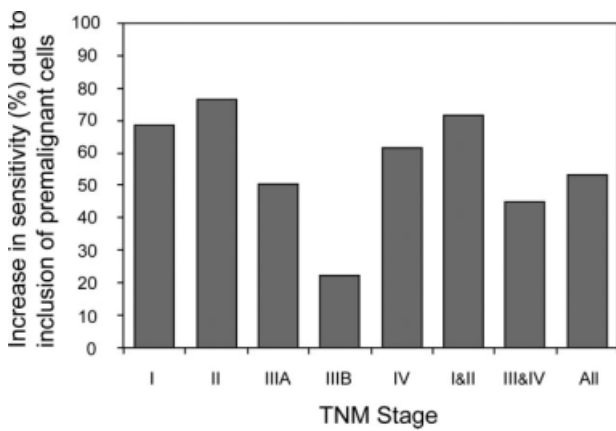
\* Information regarding tumor size and location was not available for all patients.

With regard to cancer staging, we were particularly interested in assessing whether early stage cancers could be detected by sputum cytology. The incidence of specimens diagnosed as “pre-malignant or worse” and “cancer” generally was higher in late-stage cancers than in early stage cancers. However, the differences were statistically insignificant (<10% between stage I and the average of all stages). When the criterion for a positive sputum diagnosis was tied to the presence of pre-malignant or worse cells (Categories 5 and 6) instead of cancer cells alone (Category 6), the sensitivity increased, and this difference was more pronounced for early stage cancers (the increase was 69% for stage I cancer and 76% increase for stage II cancer compared with an average increase of 44.8% stage IIIA, IIIB, and IV cancers) (Fig. 1).

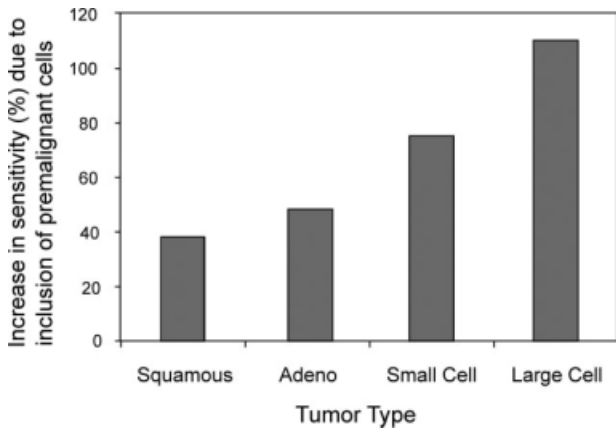
When evaluating the data from the perspective of cancer type, the results for the most common cancer types, squamous cell carcinoma and adenocarcinoma, were of

special interest. Cancer cells (Category 6) as well as pre-malignant or worse cells (Categories 5 and 6) were identified more often in sputa from patients with squamous cell carcinomas than in sputa from patients with adenocarcinomas (Table 4). The differences, however, were relatively small (8.6% and 6.9%, respectively) and were statistically insignificant. The increase in sensitivity for the diagnostic category “pre-malignant or worse” over “cancer” was similar for adenocarcinomas and squamous cell carcinomas (48.5% and 38.1%, respectively); however, the differences in sensitivity between the 2 histologic types remained statistically insignificant. For small cell carcinomas and large cell carcinomas, the gain in sensitivity was considerably higher (75% and 110%, respectively) (Fig. 2).

When comparing tumor size, larger tumors (>2 cm) were associated more frequently with sputum that was positive for cancer cells (Category 6) and for the diagnosis “pre-malignant or worse” (Categories 5 and 6)



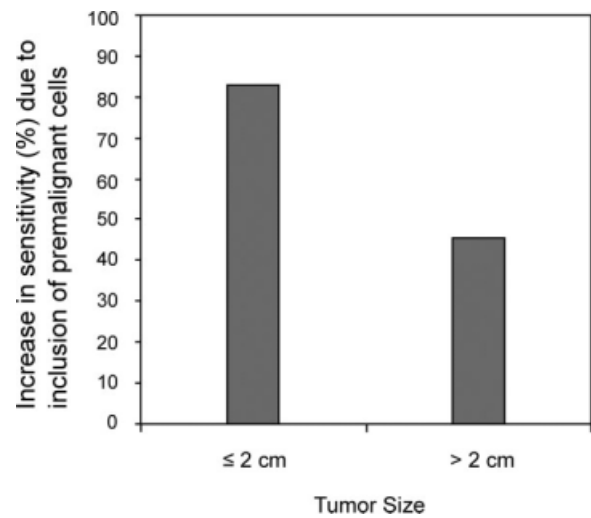
**FIGURE 1.** An increase in sensitivity for abnormal cells indicative of lung cancer resulted from setting the diagnostic threshold for positive sputum cytology to “pre-malignant or worse” with regard to TNM stage.



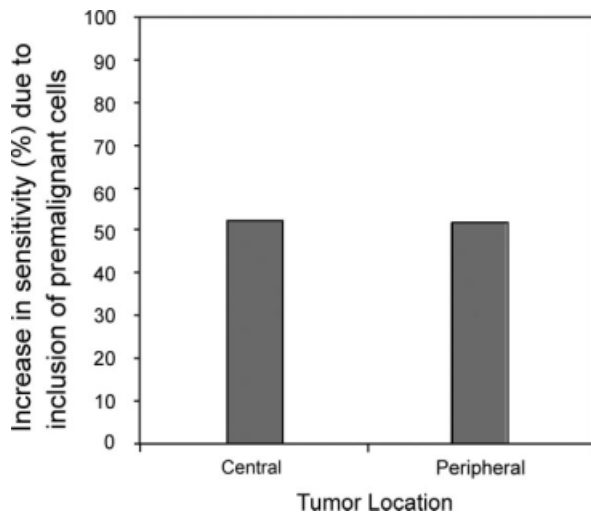
**FIGURE 2.** An increase in sensitivity for abnormal cells indicative of lung cancer resulted from setting the diagnostic threshold for positive sputum cytology to “pre-malignant or worse” with regard to the histologic tumor type. Adeno indicates adenocarcinoma.

compared with smaller tumors ( $\leq 2$  cm) (Table 4). The differences were statistically significant (18.9% for cancer cells and 13.9% for premalignant or worse cells). Setting the criteria for positive sputum cytology to the diagnostic category “pre-malignant or worse” increased the sensitivity for patients who had smaller tumors considerably more than for patients who had larger tumors (82.9% vs 45.7%, respectively) (Fig. 3).

Similar results were obtained for tumor location; central tumors were associated more frequently with sputum that was positive for cancer cells and for cells from



**FIGURE 3.** An increase in sensitivity for abnormal cells indicative of lung cancer resulted from setting the diagnostic threshold for positive sputum cytology to “pre-malignant or worse” with regard to tumor size.



**FIGURE 4.** An increase in sensitivity for abnormal cells indicative of lung cancer resulted from setting the diagnostic threshold for positive sputum cytology to “pre-malignant or worse” with regard to tumor location.

the “pre-malignant or worse” category than peripheral tumors (10.1% vs 15.3%, respectively) (Table 4). The differences were statistically significant for the category “pre-malignant or worse” but statistically insignificant for the category “cancer.” By using the category “pre-malignant or worse” as the criterion for positive sputum cytology, the sensitivity increased by 52%, independent of the tumor location (Fig. 4).

## DISCUSSION

In the current study, nearly 50% of all patients with lung cancer who produced adequate sputum specimens were sputum-positive for cancer cells; whereas nearly 75% were sputum-positive for cells that, at the least, were in a premalignant state. In a screening setting, three-quarters of the cancer patients in this cohort would have been either detected as having cancer or would have been identified to be at very high risk of cancer, with potential referral for tumor localization and follow-up diagnostics. Both cancerous and precancerous sputum diagnoses require diagnostic follow-up, and the grade of abnormality (detected by sputum cytology) might be useful in guiding the selection of imaging modalities and test intervals. The results of this study emphasize the significant diagnostic potential of sputum cytology. Unfortunately, this potential is not fully exploited in sputum cytology as currently performed in clinical laboratories, because the level of scrutiny of most research studies cannot be applied in routine settings. For example, it is not feasible to have each specimen screened by 3 or 4 cytopathologists, as it was in our study. Indeed, the average sensitivity in our study was only 38% and 20% (for premalignant or worse cells and for cancer cells, respectively). Nevertheless, the finding that high sensitivities have been achieved in several studies (5 of the 16 studies on sputum cytology that were reviewed in a recent publication reported sensitivities >80%<sup>11</sup>) suggests that sputum in fact may contain sufficient numbers of diagnostic cells. The challenge is to achieve consistently high sensitivities in the laboratory routine. Optimizing and standardizing the protocols for specimen collection and preparation likely would improve the performance of sputum cytology. For instance, it has been demonstrated that the sensitivity depends on the number of sputum specimens analyzed per patient: Böcking et al reported an increase from 68% to 85% when 3 specimens instead of 1 were screened.<sup>21</sup> Furthermore, the employment of state-of-the-art cell selection techniques can be used to significantly improve the specimen quality by increasing the ratio of epithelial cells relative the unwanted cell types. Magnetic-assisted cell sorting (MACS), for example, reportedly achieves 36-fold enrichment of epithelial cells in sputum from patients with cancer.<sup>22</sup> Automated systems for MACS already are in use for some clinical applications and may prove effective in routine sputum processing. Automation also may be the key

to substantially improving the microscopic screening process and has been used successfully for Papanicolaou-smear screening in clinical laboratories for over a decade.<sup>23,24</sup> Several attempts have been undertaken to use automated and semiautomated systems for the detection of cancer cells and dysplastic cells in sputum based on morphologic features of abnormal cell clusters,<sup>25</sup> ploidy measurements,<sup>26-30</sup> or the detection of genetic abnormalities with fluorescence in situ hybridization.<sup>31</sup> The majority of these studies reported very high sensitivities; however, we await the confirmation of the results from large-scale clinical trials. Recent technical developments have made it possible to combine flow cytometry with CT on the cellular level, allowing for cytometry in 3 dimensions.<sup>32</sup> In addition, it has been demonstrated that automated cancer cell recognition based on 3-dimensional measurements is superior to 2-dimensional (slide-based) cell analysis.<sup>33</sup>

A common concern with sputum cytology is that lung tumors at an early stage may not shed sufficient numbers of cancer cells into sputum. In contrast, we observed that cancer stage had only a small influence on the presence of both premalignant cells and cancer cells. This is not surprising for premalignant cells: Dysplasia usually develops over a long time and is present in many foci. The finding that the cancer-cell-based incidence also was relatively stable across cancer stages suggests that even early cancers may shed significant numbers of cells into the sputum.

Another important question was whether the incidence of diagnostic cells in sputum depended on the histologic type of the tumor. Our analysis indicated that all cancer types shed diagnostic cells into sputum, and the incidence of dysplastic cells in sputum is relatively independent of cancer type, in contrast to a recent study by Byers et al<sup>20</sup> in which dysplastic cells in sputum were correlated more directly with squamous cell carcinomas than with adenocarcinomas. Nevertheless, the notion that squamous dysplastic lesions are indicative of the underlying lung cancer risk for not only squamous cell carcinomas but also for adenocarcinomas and other smoking-related cancer types<sup>34</sup> has become increasingly accepted in recent years. Our results support this view. Furthermore, we observed that not only dysplastic cells but also cancer cells were present with comparable frequency in sputa from patients with squamous cell carcinomas, adenocarcinomas, and even small cell carcinomas. Similar results have

been reported by Böcking et al.<sup>21</sup> Large cell carcinomas and carcinomas of mixed type had a somewhat lower incidence of diagnostic cells in sputum, although the case numbers for these types were very small; thus, the results might not be representative.

Tumor size and location did appear to play a role in the incidence of both premalignant cells and cancer cells in sputum. Patients who had larger and centrally located tumors more frequently had sputum that was positive for premalignant cells or cancer cells; however, the differences were relatively small for both tumor size and tumor location.

An important limitation of the current study was that it did not include a noncancer, high-risk control group. The follow-up of 120 days after enrollment was too short to assuredly exclude a cancer diagnosis in each case that was not clearly confirmed as cancer, and it was not possible to obtain retrospective data. Thus, only the confirmed cancer cases were selected for our analysis of sensitivity, and there was no negative control. Although we were not in a position to assess the question of specificity, the compilation of literature cited above confirms that sputum cytology achieves a high specificity: near 100%. Another limitation was that, in our study, the sputum specimens were not optimally collected and prepared. For example, each patient was asked to expectorate sputum spontaneously once before undergoing sputum induction. For spontaneous sputa, the best results usually are obtained by collecting the first morning cough over 3 days.<sup>21,35</sup> In addition, the subsequent induced sputa may have been of lower cellularity, because the lungs were “emptied out” by the preceding spontaneous cough. Hence, the sensitivities for abnormal cells in sputum obtained in our analysis may be underestimated.

In conclusion, the findings of the current study suggest the important diagnostic potential of sputum cytology for lung cancer detection and risk assessment across all stages, histologic types, tumor sizes, and locations. Because sputum cytology is a noninvasive method with almost 100% specificity, regular monitoring of the cellular profile of sputum from high-risk patients may be a useful tool for triaging further diagnostic workup, such as CT and bronchoscopy.

However, the high combined sensitivity but low average sensitivity for the 3 cytologists who participated in this study indicates that routine sputum cytology, as

currently practiced, would not generate sufficient sensitivities in large-scale screening settings. The diagnostic potential of sputum cytology may be exploited better through the standardization and automation of sputum preparation and analysis.

### Conflict of Interest Disclosures

Dr. Neumann, Mr. Meyer, Ms. Patten, and Dr. Nelson are stockholders and employees of VisionGate, Inc.

Dr. Johnson is a stockholder and employee of Inspire Pharmaceuticals, Inc.

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