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Medical Policy Manual Laboratory, Policy No. 73

Molecular Testing in the Management of Pulmonary Nodules

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Proteomic and gene expression tests have been proposed as methods for assessing the risk of malignancy in patients with pulmonary nodules found incidentally on radiological exam.

MEDICAL POLICY CRITERIA

Proteomic screening and/or gene expression profiling for the evaluation of pulmonary nodules is considered **investigational**, including but not limited to:

A. BDX-XL2
B. Nodify CDT™
C. Nodify XL2™
D. EarlyCDT®-Lung
E. Percepta® Genomic Sequencing Classifier
F. CyPath® Lung
G. REVEAL Lung Nodule Characterization

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Targeted Genetic Testing for Selection of Therapy for Non-Small Cell Lung Cancer (NSCLC), Genetic Testing, Policy No. 56
3. Analysis of Proteomic and Metabolomic Patterns for Cancer Detection, Risk, Prognosis, or Treatment Selection, Laboratory, Policy No. 41
4. Investigational Gene Expression, Biomarker, and Multianalyte Testing, Laboratory, Policy No. 77

BACKGROUND

PULMONARY NODULES

Pulmonary nodules are a common clinical problem that may be found incidentally on a chest x-ray or computed tomography (CT) scan or during lung cancer screening studies of smokers. The primary question after the detection of a pulmonary nodule is the probability of malignancy, with subsequent management of the nodule based on various factors such as the radiographic characteristics of the nodules (e.g., size, shape, density) and patient factors (e.g., age, smoking history, previous cancer history, family history, environmental/occupational exposures). The key challenge in the diagnostic workup for pulmonary nodules is appropriately ruling in patients for invasive diagnostic procedures and ruling out patients who should forgo invasive diagnostic procedures. However, due to the low positive predictive value of pulmonary nodules detected radiographically, many unnecessary invasive diagnostic procedures and/or surgeries are performed to confirm or eliminate the diagnosis of lung cancer.

PLASMA-BASED PROTEOMIC SCREENING FOR PULMONARY NODULES

Proteomics is the study of the structure and function of proteins. The concentration, structure, and other characteristics of proteins in various bodily tissues, fluids, and other materials has been proposed as a method to identify and manage various diseases, including cancer.

Plasma-based proteomic screening has been investigated to risk-stratify pulmonary nodules as likely benign to increase the number of patients who undergo serial CT scans of their nodules (active surveillance), instead of invasive procedures such as CT-guided biopsy or surgery. Additionally, proteomic testing may also determine a likely malignancy in clinically low-risk or intermediate-risk pulmonary nodules, thereby permitting earlier detection in a subset of patients.

Nodify XL2™ is a plasma-based proteomic screening test that measures the relative abundance of proteins from multiple disease pathways associated with lung cancer using an analytic technique called multiple reaction monitoring mass spectroscopy. The role of the test is to aid physicians in differentiating likely benign from likely malignant nodules. If the test yields a likely benign result, patients may choose active surveillance via serial CT scans to monitor the pulmonary nodule. If the test yields a likely malignant result, invasive diagnostic procedures would be indicated. The test is therefore only used in the management of pulmonary nodules to rule in or out invasive diagnostic procedures and does not diagnose lung cancer. Earlier generations of the test include Xpresys Lung®, Xpresys Lung 2®, and BDX-XL2. This current test combines measurements of two proteins with five clinical characteristics to assess the risk of malignancy.

Nodify CDT™ is a proteomic test that measures autoantibodies to tumor-associated antigens to assess risk of having lung cancer. It is offered alone or in conjunction with the Nodify XL2™ as the Nodify Lung™.

EarlyCDT-Lung is a serum-based test that measures seven autoantibodies associated with small cell and non-small cell lung cancer (NSCLC). Unlike the Xpresys tests, the role of this test is to aid physicians in “ruling in” a diagnosis of malignancy.

The REVEAL Lung Nodule Characterization (MagArray) is a plasma-protein biomarker test designed to characterize indeterminate pulmonary nodules (4 to 30 millimeters) in current smokers aged 25 years and older. The test is based on a multianalyte assay with a proprietary algorithmic analysis using immunoassay, microarray, and magnetic nanoparticle detection techniques to obtain laboratory data for calculation of the risk score for lung cancer. The REVEAL Lung Nodule Characterization is presented on a scale from 0 to 100 with a single cut point at 50. The score is based on the measurement of three clinical factors (age, sex, and nodule diameter) and three proteins (epidermal growth factor receptor, prosurfactant protein B, and tissue inhibitor of metalloproteinases associated with the presence of lung cancer. It may aid a clinician in the decision to perform a biopsy or to consider routine monitoring. It is not intended as a screening or stand-alone diagnostic assay.

GENE EXPRESSION PROFILING FOR AN INDETERMINATE BRONCHOSCOPY RESULT

Gene expression profiling is the measurement of the activity of genes within cells. Messenger RNA serves at the bridge between DNA and functional proteins. An important role of gene expression profiling in molecular diagnostics is to detect cancer-associated gene expression of clinical samples to assess for the risk for malignancy.

The Percepta® Genomic Sequencing Classifier (GSC), previously Percepta Bronchial Genomic Classifier (BGC) is a 23-gene, gene expression profiling test that analyzes genomic changes in the airways of current or former smokers to assess a patient’s risk of having lung cancer, without the direct testing of a pulmonary nodule. The test is indicated for current and former smokers following an indeterminate bronchoscopy result to determine the subsequent management of pulmonary nodules (e.g., active surveillance or invasive diagnostic procedures), and does not diagnose lung cancer.

SPUTUM-BASED CANCER CELL SCREENING AFTER SUSPICOUS CT RESULT

Sputum flows against the lungs and can directly contact a potential tumor that sheds cancerous or cancer-related cells. Cancer-associated genes and proteins can be measured in sputum to detect cancerous cells in the lung micro-environment.

CyPath Lung® analyzes sputum using automated flow cytometry to detect cell types indicative of lung cancer. The test measures tetra(4-carboxyphenyl)porphyrin (TCPP), a compound that preferentially binds to cancer cells, and markers of immune cells associated with the lung tumor environment. The test is indicated for patients with a suspicious CT result to aid in risk stratification and management (e.g., more invasive testing or continued active surveillance).

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Xpresys Lung® and Xpresys Lung 2® (Indi, acquired

in 2018 by Biodesix), and Percepta® Genomic Sequencing Classifier (GSC) (Veracyte) are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any diagnostic test focuses on three main principles: (1) analytic validity, which refers to the technical accuracy of the test; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility, which refers to how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review is focused on clinical validity and utility, particularly evidence from well-designed studies related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention; and
- Improve health outcomes as a result of those decisions

PLASMA-BASED PROTEOMIC SCREENING OF PULMONARY NODULES

Nodify XL2, BDX-XL2, Xpresys Lung® and Xpresys Lung 2®

Pritchett (2023) published a propensity score-matched cohort study of the clinical utility of the Nodify XL2 test among 197 patients with undiagnosed pulmonary nodules detected by CT in the ORACLE study.[2] This study reported on clinical management changes but not on outcomes after invasive procedures were performed. Patients with low to moderate (≤50%) pretest probability of malignancy were compared to a retrospective chart review of control patients who received typical care. Of the 197 patients tested in the Nodify XL2 classifier group, 162 (82%) were benign and 35 (18%) were malignant. Patients with a benign nodule in the classifier arm were 74% less likely to undergo an invasive procedure compared to patients in the control group (absolute difference, -14%; 95% CI, -19.5% to -7.9%; p<0.001). There was one invasive procedure per 20 patients in the benign nodule classifier group compared to one invasive procedure per five patients in the control group (odds ratio, 0.23; 95% CI, 0.09 to 0.53; p<0.001). In other words, for every seven benign nodules tested with the Nodify XL2 test, one unnecessary invasive procedure was avoided. The rate of patients in the classifier group with a malignant nodule was not statistically different than the control group. This study is limited by lack of a prospective control group.

Silvestri (2018) reported the validation of the Xpresys Lung version 2 (BDX-XL2)/Nodify XL2in a prospective multicenter observational study (Pulmonary Nodule Plasma Proteomic Classifier [PANOPTIC]) that enrolled 685 patients with lung nodules of 8 to 30 mm and a low pretest probability of malignancy < 50%.[3] After exclusions for missing clinical data or a pretest probability of > 50%, 178 patients remained in the intended use population. Of these, 66 were classified as likely benign, 65 of which had a benign nodule, while 1 of 29 malignant nodules (3%) was misclassified as likely benign. Of the 149 benign nodules in the study, 44% were correctly classified as likely benign. Of the 71 patients who had invasive procedures, 42 had benign nodules. Use of the integrated proteomic classifier would have reduced the number of patients undergoing an invasive procedure to 27, a 36% relative risk reduction, with one malignant nodule misclassified as benign. A high number of patients were excluded from the study due to incomplete clinical data or because they were subsequently determined to be outside of the intended use population. It is unclear if the intended use population was determined a priori.

In an extended analysis and two-year follow-up of the PANOPTIC trial, Tanner (2021) found that all nodules designated as benign at year one remained benign by imaging at year two with no change in pathologic diagnoses or nodule size by CT. Additionally, the area under the curve of the integrated classifier was 0.76 (95% confidence interval [CI] 0.69 to 0.82), which outperformed the physician pretest probability for malignancy (0.69, 95% CI 0.62 to 0.76) and the Mayo (0.69, 95% CI 0.62 to 0.76), Veterans Administration (0.6, 95% CI 0.53 to 0.67), and Brock (0.71, 95% CI 0.63 to 0.77) models in the lower risk pretest probability (≤50%) cohort.

Kearney (2017) conducted an exploratory study that combined the 11-protein plasma classifier (Xpresys Lung) with clinical risk factors using 222 samples associated with a lung nodule of 8 to 20 mm in diameter from the reclassification study by Vachani (2015) described below.[4] The study determined that the ratio of LG3BP to a normalizer protein C163A was the diagnostic and normalizer protein pair with the highest area under the curve (60%). At sensitivity of 90% and specificity of 33%, the ratio of the proteomic marker was more accurate than clinical risk factors, and the combination of the clinical risk factors with the proteomic markers was more accurate than either alone. This study led to the development of the Xpresys Lung version 2, (now marketed as the Nodify XL2™) which includes LG3BP, C163A, and clinical risk factors.

Pecot (2012) validated a seven-peak matrix-assisted laser desorption ionization mass spectrometry proteomic signature in two prospective cohorts of patients with one or more pulmonary nodules on chest CT (total n=379 [cohort A: n=265, mean nodule size 31.2 mm; cohort B: n=114, mean nodule size 19.4 mm]).[5] The area under the curve for the matrix-assisted laser desorption ionization mass spectrometry score alone for cohort A was 0.64 (95% confidence interval [CI] 0.58 to 0.71) and for cohort B was 0.64 (95% CI 0.52 to 0.75). For cohort A, adding the proteomic signature to clinical and chest CT data did not significantly improve prognostic value. For cohort B, however, prognostic ability improved when the proteomic signature was added to clinical and chest CT data, as measured by the integration discrimination improvement index (integration discrimination improvement, 20%; p<0.001). Similarly, in a subgroup of 100 nodules from 5 to 200 mm in diameter, the proteomic signature added prognostic value (integration discrimination improvement, 15%; p<0.001).

Li (2013) reported on the development and validation of the 13-protein version, proposed to differentiate benign from malignant pulmonary lung nodules.[6] The test identifies classifier proteins likely modulated by a few transcription regulators (NF2L2, AHR, MYC, and FOS) associated with lung cancer and inflammation. The classifier was developed in a set of 143

serum samples from subjects with either benign or stage IA lung cancer, with a nodule size 4 to 30 mm. The test was locked and validated in a set of 52 benign and 52 tumor samples. Test characteristics are shown in Table 1. These results were independent of age, nodule size, or smoking history.

Vachani (2015) reported on the validation of an 11-protein plasma classifier designed to identify likely benign lung nodules in a sample of 141 plasma samples associated with indeterminate pulmonary nodules 8 to 30 mm in diameter.[7] This retrospective, blinded analysis evaluated existing samples. The 11 proteins in this assay were reported to be derived from the 13-protein sample in Li (2013), described above. The performance of the classifier in identifying benign nodules was tested at predefined reference values. For example, using a population, based non-small-cell lung cancer prevalence estimate of 23% for indeterminate pulmonary nodules 8 to 30 mm in diameter, the classifier identified likely benign lung nodules with a 90% negative predictive value (NPV) and a 26% positive predictive value, at 92% sensitivity and 20% specificity, with the lower bound of the classifier's performance at 70% sensitivity and at 48% specificity. Additional sample diagnostic characteristics, selected to keep the study's target NPV of 90%, are shown in Table 1. Classifier scores for the overall cohort were statistically independent of patient age, tobacco use, nodule size, and chronic obstructive pulmonary disease diagnosis. The classifier also demonstrated incremental diagnostic performance in combination with a four-parameter clinical model. However, this test is very different from the current marketed version.

Table 1. Summary of Diagnostic Performance Studies for Xpresys Lung® Tests to Predict Malignancy

Study	Prevalence, %	Cutoff Value	Sensitivity, %	Specificity, %	NPV, %	PPV, %
Li (2013)[6]	15	0.60	71	44	90	18
	20	0.46	83	29	87	23
	25	0.42	90	27	89	29
Vachani (2015)[7]	23.1	0.35	93.2	18.5	90.1	26
	23.1	0.34	93.7	18.5	90.1	25.6
	23.1	0.33	94.7	17.6	90.3	25.5
Silvestri (2018)		NR	97 (82 to 100)	44 (36 to 52)	98 (92 to 100)	

NPV: negative predictive value; NR: not reported; PPV: positive predictive value.

Vachani (2015) reported on a multicenter prospective-retrospective study of patients with indeterminate pulmonary nodules.[8] A plasma protein classifier was used on 475 patients with nodules 8 to 30 mm in diameter who had an invasive procedure to confirm the diagnosis. Using the classifier, 32.0% (95% CI 19.5% to 46.7%) of surgeries and 31.8% (95% CI 20.9% to 44.4%) of invasive procedures (biopsy and/or surgery) on benign nodules could have been avoided, while 24.0% (95% CI 19.2% to 29.4%) of patients with malignancy would have been triaged to CT surveillance. By comparison, 24.5% (95% CI 16.2% to 34.4%) of patients with malignancy were routed to CT surveillance using clinical parameters alone.

No evidence directly demonstrating improved outcomes in patients managed with proteomic testing was identified. Indirect evidence suggests that 36% of invasive procedures (biopsy and/or surgery) on benign nodules could have been avoided, if the test is used in patients with a low to moderate pretest probability of malignancy. Three percent of malignant lesions may be missed, although these patients would be followed by CT to verify lack of progression. Compared with the standard care plan, some patients without cancer will have avoided an unnecessary invasive procedure, which is weighed against the increase in missed cancers in

patients who had lung cancer but tested as negative on the proteomic classifier with high NPV test.

EarlyCDT®-Lung

Duarte (2022) conducted a systematic review to evaluate the diagnostic accuracy and clinical effectiveness of EarlyCDT®-Lung.[9] Data from 695 patients with pulmonary nodules across 47 publications were included in the analysis. All patient cohorts were small or at high risk of bias. EarlyCDT®-Lung had poor diagnostic accuracy, with a summary sensitivity of 20.2% (95% CI 10.5% to 35.5%) and specificity of 92.2% (95% CI 86.2% to 95.8%). This sensitivity was substantially lower than the manufacturer's estimate of 41.3%. Limited evidence among patients with pulmonary nodules prevented meta-analyses. The authors concluded that the evidence on EarlyCDT®-Lung was insufficient to draw conclusions about diagnostic accuracy or clinical value and that prospective cohort studies, in which EarlyCDT®-Lung is used among patients with identified pulmonary nodules, are required for assessment of the clinical value of this test.

Borg (2021) published a prospective cohort study of 246 high-risk patients that were referred from their general practitioner for suspicion of lung cancer.[10] The patients received the EarlyCDT®-Lung test as well as a complete diagnostic work-up. In this group, 30% had a diagnosis of lung cancer, 5% had lung metastases from a non-lung primary cancer, and 65% had no cancer detected. The overall sensitivity of the EarlyCDT®-Lung test in this cohort was 33% for lung cancer and 31 % for primary lung cancer and lung metastases combined. A subgroup analysis indicated that the test performed best in older, late-stage patients with history of smoking. The authors concluded that the test did not have sufficient sensitivity for use in a low-dose CT lung cancer detection program.

Sullivan (2020) reported results of the Early Diagnosis of Lung Cancer Scotland (ECLS) study, which randomized 12,208 patients at risk of developing lung cancer to receive either the EarlyCDT®-Lung test plus subsequent CT scanning or standard of care.[11] Patients in the EarlyCDT®-Lung group who had a positive test result had low-dose CT scans every six months for up to two years. At the two-year follow-up, 127 lung cancers had been detected: 56 in the EarlyCDT®-Lung group and 71 in the control group. Of the patients with cancer diagnosed, those in the control group were more likely to be diagnosed at stage III/IV (73.2% vs. 58.9% in the test group). No significant differences were seen for lung cancer and all-cause mortality. The authors noted that the study was not intended to evaluate the incremental contribution of the EarlyCDT®-Lung test alone.

In a prospective registry trial, Massion (2017) assessed the value of the EarlyCDT®-Lung test in patients with an identified lung nodule.[12] A cohort of 1,987 individuals were evaluated, and 451 had at least one nodule. Of those, 296 met inclusion criteria and received imaging, pathology, and testing with EarlyCDT®-Lung. Patients with a positive EarlyCDT®-Lung result had a twofold greater relative risk of developing lung cancer as compared with those with a negative test result. When EarlyCDT®-Lung was added to risk models, diagnostic performance with high specificity (>92%) and positive predictive value (>70%) were improved.

Jett (2014) published the results from the first 1,699 patients for whom US physicians ordered EarlyCDT®-Lung test.[13] Six-month outcome analysis was based on 1,613 patients. Six-month follow-up for the positives/negatives was 99%/93%. Sixty-one patients (4%) were identified with lung cancer, only 25 of whom tested positive by EarlyCDT®-Lung (sensitivity of 41%). A positive EarlyCDT®-Lung test on the current panel was associated with a 5.4-fold increase in

lung cancer incidence versus a negative test result. Comparing performance of the seven-autoantibody panel (7AAB) and the six-autoantibody panel (6AAB), the 7AAB showed highly statistically significant ($p<0.0001$) improved specificity over the 6AAB panel (91% versus 83%, respectively). The sensitivities of the 6AAB and 7AAB panels were not statistically different (46% versus 37%), respectively. The positive predictive value (PPV) offered by the 7AAB panel was nearly twice that of the previous 6AAB panel (16% versus 9%, respectively). Eight out of fourteen NSCLCs (57%) detected as positive were early stage cancer (I or II). the investigators concluded that EarlyCDT®-Lung may be a complementary tool to CT for detection of early lung cancer.

Chapman (2012) published the results of a case-control study involving 235 subjects with newly diagnosed lung cancer and 235 healthy controls used to evaluate both six- and seven-antigen versions of the EarlyCDT®-Lung test.[14] In addition, two prospective consecutive series of 776 and 836 individuals at an increased risk of developing lung cancer were also evaluated with both versions of the EarlyCDT®-Lung test. The six-antigen panel gave a sensitivity of 39% and a specificity of 89%, while the seven-antigen panel resulted in a sensitivity of 41% and a specificity of 91%. Once adjusted for occult cancers in the population, this resulted in a specificity of 93%.

Lam (2011) published a case-control study describing the sensitivity of the EarlyCDT®-Lung test, which evaluated samples for tumor associated autoantibodies found in individuals with lung cancer, including 574 subjects from four separate cohorts.[15] Group one (n=122) included subjects with only small cell lung cancer (SCLC); group two (n=249) was composed of 97% of subjects with non-small cell lung cancer (NSCLC); group three (n=122) included only subjects with NSCLC; and group four (n=81), was made up of 62% of subjects with NSCLC. For group one the results indicated a sensitivity of 57% for SCLC (specificity data not calculated). The sensitivity and specificity for group two was 34% and 87% for NSCLC. For group three sensitivity and specificity was 31% and 84% for NSCLC. Finally, in group four sensitivity and specificity was 35% and 89% for NSCLC and 43% and 89% for SCLC. No significant difference in positivity was reported for the EarlyCDT®-Lung test with regard to different lung cancer stages.

Initial clinical validation of the EarlyCDT®-Lung test was reported by Boyle (2011).[16] This study used the same three populations as the Murray study.[17] The optimal assay cut-off point was calibrated to target a 90% specificity, which provided the optimal overall accuracy based on Monte Carlo simulations. For the three separate populations, sensitivity was 36%, 39% and 37%. The specificity was 91%, 89%, 90%, approximating the 90% specificity of test calibration. Using a population prevalence of 2.0%, the PPV ranged from 7.0%-7.2% and the NPV was 98.6%. The area under the curve by ROC analysis was 0.63. There were no significant differences in accuracy of the test by lung cancer stage.

REVEAL Lung Nodule Characterization (MagArray)

Trivedi (2018) published a clinical validity study of the REVEAL Lung Nodule Characterization test using retrospective human plasma samples and associated clinical data from current smokers aged 25 to 85 years with indeterminate lung nodules measuring 4 to 30 millimeters in diameter.[18] Plasma samples from patients with metastatic disease or previously diagnosed lung cancer were excluded. The REVEAL test was used in conjunction with the Veteran's Affairs (VA) Clinical Factors Model, with the objective to add discriminatory information when the VA model classified samples as inconclusive or intermediate risk. 97 samples were

included in the validation study. Of the 97 samples, 68 were grouped as having intermediate risk by the VA model. The REVEAL model correctly identified 44 (65%) of these intermediate-risk samples as low (n=16) or high (n=28) risk. The REVEAL assay NPV was 94% and its sensitivity was 94%, suggesting potential application as a rule-out test to increase the confidence of providers to avoid aggressive interventions for patients for whom the VA model result is inconclusive or intermediate risk. This study is limited by retrospective design and small sample size.

GENE EXPRESSION PROFILING OF INDETERMINATE BROCHOSCOPY RESULTS

Mazzone (2022) conducted a prospective, multicenter, blinded, clinical validation study of 412 current or former smokers undergoing bronchoscopy for suspected lung cancer from the Airway Epithelium Gene Expression In the DiagnosiS of Lung Cancer (AEGIS-1/AEGIS-2) trial cohorts and the Percepta Registry. [19] The sensitivity, specificity, and predictive values were calculated using predefined thresholds, and the ability of the Percepta classifier to decrease unnecessary invasive procedures was estimated. 29% of intermediate-risk lung lesions were down-classified to low-risk with a 91.0% NPV, and 12.2% of intermediate-risk lesions were up-classified to high-risk with a 65.4% PPV. 54.5% of low-risk lesions were down-classified to very low risk with greater than 99% NPV, and 27.3% of high-risk lesions were up-classified to very high risk with a 91.5% PPV. Investigators noted that Percepta GSC performance was similar between the AEGIS-1/AEGIS-2 cohorts and the Percepta Registry with an overall area under the curve of 0.73 (95% CI, 68.3 to 78.4), demonstrating the robustness of the classifier performance across different patient cohorts. Investigators also estimated the potential utility of Percepta GSC in decreasing invasive procedure utilization, had the classifier result been available to manage these lesions. It was determined that, if the classifier results were used in nodule management, 50% of patients with benign lesions and 29% of patients with malignant lesions undergoing additional invasive procedures could have avoided these procedures. Limitations of this study are that only patients with a history of smoking were included, and follow-up was only required at 12 months to determine benign status. Therefore a few indolent lung cancers could have been present.

Whitney (2015) reported on the development and initial validation of an RNA-based gene expression classifier from airway epithelial cells designed to be predictive of cancer in current and former smokers undergoing bronchoscopy for suspected lung cancer.[20] Samples were from patients in the prospective, observational, cohort AEGIS-1 and AEGIS-2 trials. Cohort details are described in Silvestri (2015), below. A total of 299 samples from AEGIS-1 (223 cancer-positive and 76 cancer-free subjects) were used to derive the classifier. Data from 123 patients in a prior study with a nondiagnostic bronchoscopy were used as an independent test set. In the final model, the classifier included 17 genes, patient age, and gene expression correlates and was reported as a dichotomous score (≥0.65 as cancer-positive, <0.65 as cancer-negative). The performance characteristics of the classifier in the training and test set are shown in Table 2.

Silvestri (2015) reported on the diagnostic performance of the gene expression classifier developed in Whitney (2015), in a sample of 639 patients enrolled in two multicenter prospective studies (AEGIS-1, n=298 patients; AEGIS-2, n=341 patients).[21] The study enrolled patients who were undergoing clinically indicated bronchoscopy for a diagnosis of possible lung cancer and had a history of smoking. Before the bronchoscopy, the treating physician assessed each patient’s probability of having cancer with a five-level scale (<10%, 10-39%, 40-60%, 61-85%, >85%). Patients were followed until a diagnosis was established

(either at the time of bronchoscopy or subsequently by another biopsy means) or until 12 months after bronchoscopy.

A total of 855 patients in AEGIS-1 and 502 patients in AEGIS-2 met enrollment criteria. After exclusions due to sample quality issues, loss to follow-up, lack of final diagnosis, or nonprimary lung cancer, 341 subjects were available in the validation set for AEGIS-2. For AEGIS-1, patients were randomized to the development (described above) or validation (n=298) sets. Of the 639 patients in the validation study who underwent bronchoscopy, 272 (43%; 95% CI 39 to 46%) had a nondiagnostic examination. The prevalence of lung cancer was 74% and 78% in AEGIS-1 and AEGIS-2, respectively. The overall test characteristics in AEGIS-1 and AEGIS-2 are summarized in Table 2. The classifier improved prediction of cancer compared with bronchoscopy alone, but comparisons with a clinical predictor were not reported. For the subset of 272 patients with a nondiagnostic bronchoscopy, the classifier performance was presented by the pretest physician-predicted risk of cancer. For most subpopulations, there was a very high NPV. However, there were 13 false negatives, 10 of which were considered at high (>60%) risk of cancer pre-bronchoscopy.

Table 2. Summary of Clinical Validity Studies for GEC to Predict Malignancy in Bronchial Samples

Study	Population	AUC (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Whitney (2015)[20]	Training set, entire population (n=299)	0.78 (0.73 to 0.82)	93	57		
	Training set, subset with nondiagnostic bronchoscopy (n=134)	0.78 (0.71 to 0.85)				
	Test set with nondiagnostic bronchoscopy (n=123)	0.81 (0.73 to 0.88)	92 (78 to 98)	53 (42 to 63)	47 (36 to 58)	94 (83 to 99)
Silvestri (2015)[21]	AEGIS-1 (n=298)	0.78 (0.73 to 0.83)	88 (83 to 95)	47 (37 to 58)		
	AEGIS-2 (n=341)	0.74 (0.68 to 0.80)	89 (84 to 92)	47 (36 to 59)		
	Subset of all patients with nondiagnostic bronchoscopy, by pretest cancer probability risk					
	Risk <10% (n=61)				7 (1 to 24)	100 (89 to 100)
	Risk 10%-60% (n=84)				40 (27 to 55)	91 (75 to 98)
	Risk >60% (n=108)				84 (75 to 81)	38 (15 to 65)
	Risk unknown (n=19)				47 (21 to 73)	100 (40 to 100)

AUC: area under the curve; CI: confidence interval; GEC: gene expression classifier.

Vachani (2016) reported on rates of invasive procedures from AEGIS-1 and -2.[22] Of 222 patients, 188 (85%) had an inconclusive bronchoscopy and follow-up procedure data available for analysis. Seventy-seven (41%) patients underwent an additional 99 invasive procedures, which included surgical lung biopsy in 40 (52%) patients. Benign and malignant diseases were ultimately diagnosed in 62 (81%) and 15 (19%) patients, respectively. Among those undergoing surgical biopsy, 20 (50%) were performed in patients with benign disease. If the classifier had been used to guide decision making, procedures could have been avoided in 21 (50%) of 42 patients who had additional invasive testing. Further, among 35 patients with an inconclusive index bronchoscopy who were diagnosed with lung cancer, the sensitivity of the classifier was 89%, with four (11%) patients having a false-negative classifier result. Invasive procedures after an inconclusive bronchoscopy occur frequently, and most are performed in patients ultimately diagnosed with benign disease.

No evidence directly demonstrating improved outcomes in patients managed with the Percepta® GSC was identified. Decision impact studies have reported on clinical management changes, but not on outcomes after decisions for invasive procedures were made.[23, 24] The results of these studies indicate that a negative (low-risk) result might reduce invasive procedure recommendations in patients diagnosed with benign disease.

SPUTUM-BASED CANCER CELL SCREENING AFTER SUSPICOUS CT RESULT

Lemieux (2023) published a multi-center prospective cohort study of 182 individuals enrolled in either a non-cancer or cancer group.[25] The non-cancer group included 148 participants who were either smokers with a smoking history of at least 20 pack-years, or non-smokers with a smoking history of at least 20 pack-years who quit smoking within the past 15 years. Most individuals in the non-cancer group received a CT or other imaging result that was not suspicious for cancer. If participants in the non-cancer group required a follow-up imaging analysis or biopsy, they were followed until their health status was confirmed. If participants in the non-cancer group were diagnosed with lung cancer, they were switched to the cancer group. The cancer group included 34 individuals with high suspicion for lung cancer based on medical history and CT or other imaging results. Diagnosis was confirmed by biopsy after sputum samples were provided for the study, and participants with no cancer were moved to the non-cancer group. Participant-collected sputum samples, obtained over three consecutive days, were evaluated with the CyPath® Lung test. Overall, CyPath® Lung classified samples as cancer or non-cancer with a sensitivity of 82% and specificity of 88%. NPV and PPV values were 96% and 61%, respectively. For cases when pulmonary nodules were greater than 20 mm, the test was 92% sensitive and 87% specific.

Avoiding invasive procedures in situations where patients are at very low likelihood of having lung cancer is likely beneficial, given the known complications of invasive procedures (e.g., pneumothorax). However, reductions in unnecessary invasive procedures must be weighed against outcomes and harms associated with a missed diagnosis of lung cancer at earlier, more treatable stages.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF CHEST PHYSICIANS

The American College of Chest Physicians (2013) has published evidence-based clinical practice guidelines on the diagnosis and management of lung cancer, including pulmonary nodules.[26] These guidelines make an number of recommendations, including:

- In the individual with a solid, indeterminate nodule that measures > 8 mm in diameter, we suggest that clinicians estimate the pretest probability of malignancy either qualitatively by using their clinical judgment and/or quantitatively by using a validated model (Grade 2C).
- In the individual with a solid, indeterminate nodule that measures > 8 mm in diameter and low to moderate pretest probability of malignancy (5%–65%), we suggest that functional imaging, preferably with PET, should be performed to characterize the nodule (Grade 2C).
- In the individual with a solid, indeterminate nodule that measures > 8 mm in diameter and a high pretest probability of malignancy (> 65%), we suggest that functional imaging should not be performed to characterize the nodule (Grade 2C).

NATIONAL COMPREHENSIVE CANCER NETWORK

NCCN guidelines for non-small cell lung cancer include recommendations for pulmonary nodule risk assessment.[27] For patients presenting with an incidental finding of a nodule suspicious for lung cancer, the guidelines recommend:

- Multidisciplinary evaluation including thoracic surgeons, thoracic radiologists, and pulmonologists to determine the likelihood of a cancer diagnosis and the optimal diagnostic or follow-up strategy.
- Risk assessment including patient factors (age, smoking history, cancer history, etc) and radiologic factors.

For patients with an incidental finding of a solid nodule >8 mm on chest CT in a low-risk patient, the guidelines recommend considering CT at three months, PET/CT, or biopsy.

AMERICAN THORACIC SOCIETY

In 2017, the American Thoracic Society published a position statement on the evaluation of molecular biomarkers for the early detection of lung cancer.[28] The Society states that "a clinically useful molecular biomarker applied to the evaluation of lung nodules may lead to expedited therapy for early lung cancer and/or fewer aggressive interventions in patients with benign lung nodules." To be considered clinically useful, a molecular diagnosis "must lead to earlier diagnosis of malignant nodules without substantially increasing the number of procedures performed on patients with benign nodules" or "fewer procedures for patients with benign nodules without substantially delaying the diagnosis of cancer in patients with malignant nodules."

SUMMARY

It appears that plasma-based proteomic tests may be helpful for assessing risk of cancer in patients that have pulmonary nodules, but there is not enough research to show that these tests can improve health outcomes for these patients. In addition, clinical guidelines based on research do not recommend this testing. Therefore, proteomic screening, including but not limited to Nodifiy XL2™, Nodify CDT™, BDX-XL2, Xpresys Lung®, Xpresys Lung 2®, and REVEAL is considered investigational for the evaluation of pulmonary nodules.

There is not enough research to show that gene expression tests can improve health outcomes for patients with pulmonary nodules. In addition, clinical guidelines based on

research do not recommend this testing. Therefore, gene expression profiling, including but not limited to Percepta® Genomic Sequencing Classifier, is considered investigational for the evaluation of pulmonary nodules.

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CODES

Codes	Number	Description
CPT	0092U	Oncology (lung), three protein biomarkers, immunoassay using magnetic nanosensor technology, plasma, algorithm reported as risk score for likelihood of malignancy
	0080U	Oncology (lung), mass spectrometric analysis of galectin-3-binding protein and scavenger receptor cysteine-rich type 1 protein M130, with five clinical risk factors (age, smoking status, nodule diameter, nodule-spiculation status and nodule location), utilizing plasma, algorithm reported as a categorical probability of malignancy
	0360U	Oncology (lung), enzyme-linked immunosorbent assay (ELISA) of 7 autoantibodies (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4, and HuD), plasma, algorithm reported as a categorical result for risk of malignancy
	0395U	Oncology (lung), multi-omics (microbial DNA by shotgun next generation sequencing and carcinoembryonic antigen and osteopontin by immunoassay), plasma, algorithm reported as malignancy risk for lung nodules in early-stage disease
	0406U	Oncology (lung), flow cytometry, sputum, 5 markers (meso-tetra [4-carboxyphenyl] porphyrin [TCPP], CD206, CD66b, CD3, CD19), algorithm reported as likelihood of lung cancer
	83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
	84999	Unlisted chemistry procedure
HCPCS	None	

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