CyPath® Lung in Practice: Cases 1-4

Dr Gordon Downie, MD, PhD
Director, Titus Regional Hospital Lung Nodule Clinic and Interventional Pulmonology

BACKGROUND

The clinical burden of competent pulmonary nodule identification and definitive diagnosis has exploded over the past 20 years. The National Lung Screening Trial, which began in 2002 and was published in 2011, proved that low dose CT scanning of the chest (LDCT) reduced mortality from lung cancer.[1] This research has helped drive the development of dynamic technologies, including imaging, biopsy devices, chemical assays, and diagnostic algorithms, which have made the identification of pulmonary nodules dramatically more accurate.[2] Additionally, Pulmonary Nodule Clinics have proliferated across hospital systems, increasing the number of at-risk individuals who undergo lung cancer screening by imaging. At the same time, however, the clinical costs for surveillance of at-risk patients and safe and accurate biopsies of cancer nodules in high-risk patients have become a financial burden to our healthcare system.[3] Primary care providers, pulmonologists, pulmonary nodule clinical programs, and patients subsequently are left with the high-stress clinical dilemma of determining the appropriate care path for presentations of mostly benign nodules with devastating malignancy potential if misdiagnosed. Universal invasive biopsies on all nodules are resource and cost prohibitive, and waiting for definitive radiographic growth to determine neoplastic potential often is clinically unacceptable. Therefore, non-invasive adjuvant diagnostic strategies that give the diagnostician a pathway with high sensitivity and specificity allowing for a confident decision tree process is extremely desirable. I present four cases employing non-invasive sputum flow cytometry analysis as a critical component of our practice's pulmonary nodule algorithm that allows for significant clinical confidence in our approach for me as the clinician as well as for my patients' diagnosis anxiety.

Case 1: "James" (negative test, saved bx)

Case 2: "Carol" (positive test 6 mm nodule, breast ca)

Case 3: "Barbara" (high risk, close surv)

Case 4: "Joan" (positive test, cancer)

DISCUSSION

The clinical burden of assessing discovered pulmonary nodules, whether a serendipitous finding or part of a high-risk lung cancer program, most often falls on the shoulders of pulmonary specialists. Certainly, medical and radiation oncologists, thoracic surgeons, and primary care specialists evaluate a fair number of these patients, but the vast majority of patients are assessed by pulmonology. Navigating the pitfalls of missing an early diagnosis of lung cancer or dealing with a serious complication from a biopsy of a benign nodule demand that clinical assessments give the clinician and the patient the most in-depth and accurate risk profile after the discovery of a pulmonary nodule.

In our practice, the number of pulmonary nodules that need to be evaluated has exploded as medical society recommendations for lung cancer surveillance have become hardwired into standard screening by primary care and hospitals.[4] LDCT scans of the chest in higher-risk patients (smoking history with or without COPD between 50-80 years of age) with previously unrecognized lung nodules (<30 mm in size) is the presenting abnormality in the majority of patients who come to our practice or pulmonary nodule clinic.[4] Nodules greater than 30 mm are biopsied, with a PET scan usually preceding the biopsy. This combination of imaging allows for staging biopsies as well as diagnostic biopsies in these patients and are relatively straightforward recommendations. It is the <30 mm noncalcified pulmonary nodules that cause stress, confusion, and conflicting opinions on whether to directly biopsy or follow indirectly.

Overlapping avidity in PET scans caused by high metabolic inflammatory processes and lower metabolic neoplasms often fail to significantly stratify nodules, and the recorded specificity of a PET scan hovers in the low 70% range, making this an inadequate test to solely determine malignancy.[5] Additional adjuvants are needed to give both clinicians and their patients the confidence to assume the risk of biopsies or to ease the anxiety of watchful waiting for nodules to grow. On a monthly basis, medical literature reports on indirect adjuvants being trialed to help improve the decision tree for sub 30 mm noncalcified nodules. Over the last two years, two such adjuvants have been added to our standard lung nodule algorithm: CyPath® Lung sputum flow cytometry analysis and serum marker analysis.

The central science that allows for a high sensitivity and high specificity sputum flow cytometry analysis for cancers within the lung are the traits of porphyrin biochemistry, specifically Meso Tetra (4-Carboxyphenyl) Porphyrin (TCPP).[6] Work conducted at the Los Alamos National Laboratory in the 1980s used the observation from the 1940s that porphyrins have a unique affinity for malignant cancer cells and additional findings from the 1960s when porphyrins were used as a fluorescent probe to test for lung cancers in uranium miners with different lung cancers. These studies confirmed that TCPP will localize in lung cancer cells found in subjects' sputum samples.[7,8]

Patriquin et al presented a proof-of-concept for a non-invasive quantitative assay to detect lung cancer in patient's sputum.[6] Using TCPP-incubated sputum cells, high-intensity fluorescent red cells correctly classified subjects into cancer or high-risk populations. Lemieux et al presented results of a clinical trial using flow cytometry and machine learning to analyze TCPP-incubated sputum cells to improve diagnostic sensitivity and specificity in a sputum-based test (CyPath® Lung).[9] Sensitivity and specificity were reported as 82% and 88% respectively, and the negative and positive predicted values were reported as 96% and 61%. Further work by Bederka et al, Grayson et al, and Elzi et al from 2020 to 2022 emphasized not only the TCPP intracellular fluorescence but also the role of the immune environment in which the cancer cells were suspended. These findings further improved the ability of flow cytometry to stratify and identify at-risk patients effectively.[10–12] Collectively, these results suggested that the CyPath® Lung test could be used to improve early lung cancer diagnosis in pulmonary nodules discovered by lung cancer screening CTs.

Morris et al studied the economic impact of the CyPath® Lung sputum analysis test in patients with newly discovered lung nodules found via low dose CT scanning for lung cancer screening.[3] The analysis projected significant cost savings for both patients covered by Medicare and privately insured patients. The economic benefit makes CyPath® Lung even more attractive as an adjuvant diagnostic test.

Over the past year, the CyPath® Lung sputum test has become an active component in our clinical assessment of newly discovered non-calcified pulmonary nodules. The four cases presented here are real-time, real-life examples of how CyPath® Lung testing augmented and reinforced confidence in our clinical recommendations to patients. Obviously, there are many scenarios where LDCT followed by PET scan gives a clear-cut diagnostic pathway with no need for further supportive tests. However, several clinical presentations seem particularly suited to the CyPath® Lung test's ability to augment decision making.

CASE STUDIES

Case 1: CyPath® Lung Helped Avoid Unnecessary Biopsy for James

CASE STUDY JAMES: Negative Test Result Prevented High-Risk Biopsy

PATIENT INFORMATION AND INITIAL WORKUP

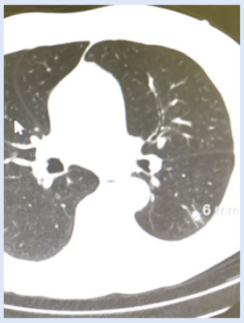
- **Age:** 85 years old
- **Sex:** Male
- **Smoking status:** Former smoker (>20 pack-years)
- **Medical history:** Asbestos exposure; COPD/OSA
- **Family history:** Unremarkable
- **Malignancy risk:** 99x risk vs nonsmoker without asbestos exposure
- Patient acutely aware of his high-risk status, requested LDCT for surveillance

Actual patient case, but name has been changed to ensure privacy.

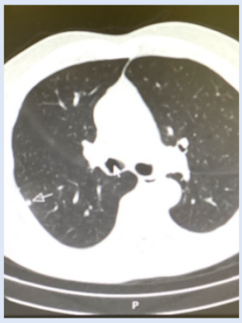
bx=biopsy; COPD=chronic obstructive pulmonary disease; CT=computed tomography; LDCT=low-dose computed tomography; LLL=left lower lobe; OSA=obstructive sleep apnea; PET=positron emission tomography; RLL=right lower lobe.

IMAGING RESULTS

Initial LDCT: 4/24 scan revealed several new subcentimeter (≤8 mm) noncalcified nodules. 6-mm LLL noncalcified nodule concerning for malignancy was in a difficult-to-reach location.

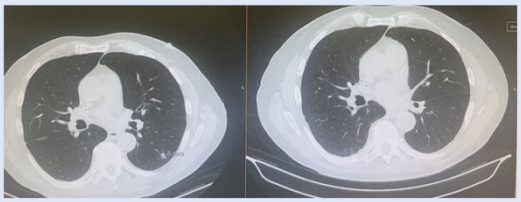


6 mm nodule LLL



4 mm nodule RLL

Follow-up chest CT scans: Complete resolution of nodules on 7/24 scan. 1/25 scan (not shown) revealed no new nodules.



ADDITIONAL FINDINGS/ NEXT STEPS

- **Brock model risk:** 1.5%
- **PET scan:** Not recommended for nodules <8 mm because of low sensitivity
- **Biodesix Nodify:** Not recommended for nodules <8 mm
- **Follow-up testing options:** Serial CT scans, robotic bronchoscopic bx, percutaneous bx
- Patient favored robotic biopsy despite low Brock risk score

OUTCOME WITH CYPATH® LUNG

- **CyPath® Lung:** 5/24 test result: 0.46; unlikely lung cancer
- Patient comfortable with serial CT scans instead of robotic bx or high-risk percutaneous bx
- Follow-up CT scans on 7/24 and 1/25 showed no nodules
- **CyPath® Lung prevented unnecessary biopsy**

CyPath® Lung

Case 1 illustrates a common diagnostic dilemma within our practice and a common presentation to any pulmonary nodule clinic—high-risk individuals with a sub 8 mm nodule in a difficult to biopsy location within the thorax. A typical clinician-patient encounter will outline the imaging findings including size, shape, location of the nodule, whether there is any presence of calcium, clinically significant lymphadenopathy, the malignancy probability based on an imaging probability model, and risks involved in any attempt to biopsy the nodule. This patient, despite being over 80 years old, was in excellent health with full activities of life. He understood the significance of both asbestos exposure and his smoking history in terms of his risk for developing lung cancer. While previous CT scans had been unremarkable, the most recent scan revealed new nodules. The modeling risk was relatively low, but the patient's mindset was to be proactive rather than passive. He understood the risks of a robotic bronchoscopic or percutaneous biopsy versus close follow-up serial CT scanning. PET scan and serum lung cancer markers were not indicated given the sub 8 mm nodule size, and the nodules' locations raised the risk of performing a biopsy. Without the "unlikely lung cancer" finding on the CyPath® Lung test, he would have opted for a biopsy; however, given the CyPath® Lung result,

the patient felt comfortable opting for close CT scan surveillance. Unnecessary invasive biopsy with the obvious risks for a patient over 80 years of age was avoided. A follow-up CT scan three months later showed that the nodules had resolved.

Case 2: CyPath® Lung Played Key Role in Next Step Care for Carol

CASE STUDY CAROL: Positive Test Result Helped Direct Next Step Care

PATIENT INFORMATION AND INITIAL WORKUP

- **Age:** 80 years old
- **Sex:** Female
- **Smoking status:** Tobacco abuse; quit in 2010
- **Medical history:** COPD; CAD with MI and cardiac stents
- **Breast cancer:** Diagnosed in 2019
- Patient chose to stop chronic breast cancer hormonal therapy
- Yearly LDCT screening for high-risk lung cancer

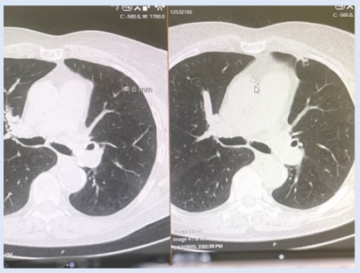
Actual patient case, but name has been changed to ensure privacy.

CAD=coronary artery disease; COPD=chronic obstructive pulmonary disease; CT=computed tomography; DDx=differential diagnosis; LDCT=low-dose computed tomography; LUL=left upper lobe; PET=positron emission tomography; RUL=right upper lobe.

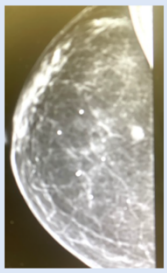
IMAGING RESULTS

Initial LDCT: 9/23 scan was negative. 10/24 scan revealed new noncalcified sub 8 mm nodules in LUL and RUL, as well as others.

12/24 mammogram: Revealed new suspicious areas in the right breast.

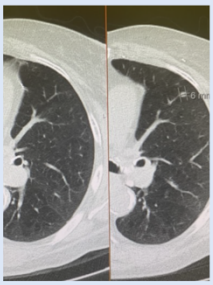


9/23 no nodule 10/24 6 mm nodule



12/24 mammogram

Follow-up LDCT: 2/25 scan shows resolution of LUL nodule vs 10/24 scan.



2/25 scan 10/24 scan

ADDITIONAL FINDINGS/ NEXT STEPS

- **Brock model risk:** 4.3%
- **PET scan:** Not recommended for nodules <8 mm because of low sensitivity
- **Serum markers:** Contraindicated given breast cancer within 5-year period
- **Follow-up testing options:** Bronchoscopy high-risk and poor-yield without robotic augmentation

OUTCOME WITH CYPATH® LUNG

- **CyPath® Lung:** 11/24 test result: 0.72, likely lung cancer
- 12/24 mammogram to recheck patient's status revealed new suspicious areas in the breast
- Breast biopsy was positive for recurrent breast cancer; hormonal therapy was restarted
- 2/25 LDCT showed resolution of LUL nodule
- Patient is at high risk for lung cancer and will continue close CT surveillance
- DDx remains metastatic breast cancer to the lung now suppressed vs inflammatory process resolved
- **CyPath® Lung played a key role in next step care**

CyPath Lung

Case 2 details the other end of the sub 8 mm nodule spectrum, noncalcified small nodules in a high-risk patient with relatively low-risk imaging probability by modeling. Her CyPath® Lung test result was "likely cancer." It is still relevant to mention that PET scanning for sub 8 mm nodules has an unacceptable sensitivity, and serum markers were contraindicated on the basis of size and previous cancer history within the last five years. Because of her previous breast cancer history, the patient was referred to surgical oncology where an abnormal mammogram led to a positive breast biopsy for recurrent breast cancer. The patient who had self-directed stopping her treatment was restarted on chronic breast cancer hormonal-based therapy. A follow-up CT scan of the chest demonstrated resolution of the pulmonary nodules. The differential diagnosis included breast cancer metastasis to the lung, which resolved after resuming therapy, versus an inflammatory process that resolved spontaneously. Lung cancer was ruled out due to the resolution of the nodules without any lung cancer-specific treatment. Without the complicating factor of a possible breast cancer diagnosis, the patient would likely have undergone an invasive biopsy to confirm the diagnosis. The "likely cancer" result

from the CyPath® Lung test played a critical role in guiding the next steps in patient management.

Cases 1 and 2 illustrate the powerful tool that CyPath® Lung brings to the evaluation of sub 8 mm noncalcified nodules. It has helped direct patient conversations and has given our practice greater confidence when recommending the next diagnostic steps, whether that next step is invasive or watchful waiting. Cases 3 and 4 illustrate our use of CyPath® Lung in clinical presentations that unfortunately are not infrequent in our practice.

Case 3: CyPath® Lung Provided Increased Clinical Confidence to Barbara

CASE STUDY BARBARA: Test Gives Added Confidence to High-Risk Patient

PATIENT INFORMATION AND INITIAL WORKUP

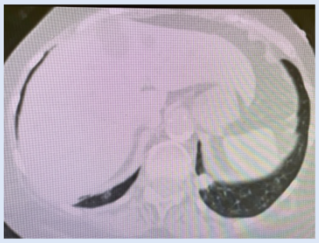
- **Age:** 84 years old
- **Sex:** Female
- **Smoking status:** 45 pack-year smoker; quit 2004
- **Medical history:** OSA, COPD
- Significant COVID-19 pneumonia, 8/22
- Report of 8/22 hospital CT not communicated with 7-month delay in care
- Patient has aged out of lung cancer screening guidelines with LDCT

Actual patient case, but name has been changed to ensure privacy.

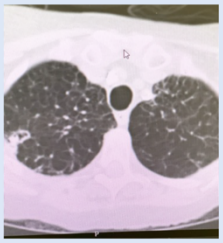
COPD=chronic obstructive pulmonary disease; COVID-19=coronavirus disease 2019; CT=computed tomography; c/w=consistent with; LDCT=low-dose computed tomography; LLL=left lower lobe; OSA=obstructive sleep apnea; PET=positron emission tomography; RUL=right upper lobe; q=every.

IMAGING RESULTS

Initial CT: 8/22 scan revealed 14-mm LLL nodule and 10-mm cavitary RUL nodule; bilateral bronchiectasis also seen.

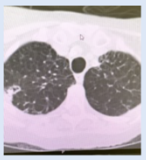


14 mm nodule LLL

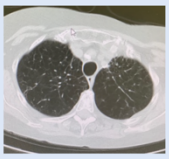


10 mm nodule RUL

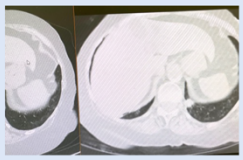
Follow-up: Complete resolution of nodules on 2/23 PET scan. Subsequent close follow-up CT scans have remained stable without evidence of nodules.



2/23 PET



8/23 CT



5/24, 8/24, and 12/24 CTs (not all shown)

ADDITIONAL FINDINGS/ NEXT STEPS

- **Brock model risk:** 55.2%
- **Serum markers:** Ordered 1 week prior to PET c/w high probability for lung cancer
- **2/23 PET:** Revealed resolution of LLL and RUL processes and less prominent bronchiectasis
- Initial nodules appear related to COVID-19 with resolution

OUTCOME WITH CYPATH® LUNG

- **CyPath® Lung:** 7/24 test result: 0.86, likely lung cancer
- Given positive CyPath® Lung and serum marker, close follow-up is recommended
- Patient uncomfortable with yearly LDCT, so have gone to q 3-month serial CT scans without evidence of new nodules
- Will change to 6-month CT scans at 1 year
- **CyPath® Lung:** Patient appreciates the increased clinical confidence the test provides

CyPath Lung

Case 3 is a high-risk individual who aged out of recommended LDCT surveillance but as part of a COVID-19 infection evaluation had a CT scan of the chest with serendipitous discovery of pulmonary nodules. Probability modeling gave a ≥50% probability of lung cancer. As outlined above, our algorithm included ordering a PET scan, serum markers, and CyPath® Lung testing. Both serum markers and CyPath® Lung testing came back as high probability for cancer; however, when the PET was reviewed both nodules had resolved. The patient refused a surveillance bronchoscopy to inspect her bilateral airways but agreed to CT chest scans every three months for one year. The clinician-patient discussion to pursue close follow-up is not infrequent. Having CyPath® Lung testing has repeatedly helped to help direct these conversations.

Case 4: CyPath® Lung Helped With Diagnosis of Second Primary Cancer for Joan

CASE STUDY JOAN: Positive Test Result Led to Diagnosis of 2nd Primary Cancer

PATIENT INFORMATION AND INITIAL WORKUP

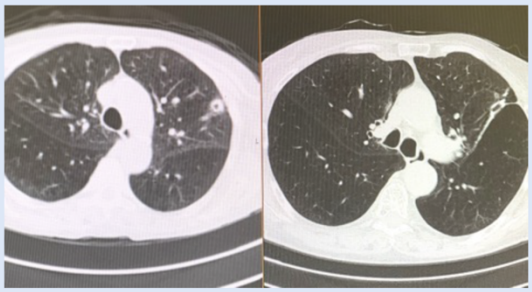
- **Age:** 72 years old
- **Sex:** Female
- **Smoking status:** 50 pack-year smoker; quit 2021; using vape pen
- **Medical history:** COPD/OSA with chronic respiratory failure on O_2
- **Cancer history:** 10/23 LUL wedge resection for clinical Stage IA NSCCA of the lung
- Patient with Stage IA NSCCA of the lung requires close follow-up

Actual patient case, but name has been changed to ensure privacy.

COPD=chronic obstructive pulmonary disease; CT=computed tomography; ES-SCLC=extensive-stage small cell lung cancer; LUL=left upper lobe; NSCCA=non-small cell carcinoma; OSA=obstructive sleep apnea; PET=positron emission tomography.

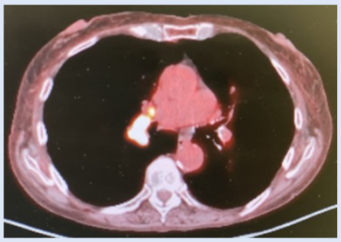
IMAGING RESULTS

Initial CT: 4/24 scan revealed subtle changes to LUL post-op resection site.



Pre- and post-op wedge resection, post-op increased nodularity from 10/23 to 4/24

Follow-up PET: 7/24 scan showed post-op scar without avidity, but right hilum and mediastinum grossly positive. Unusual presentation for metastatic NSCCA from LUL lesion.



ADDITIONAL FINDINGS/ NEXT STEPS

- **Serum markers:** Contraindicated given cancer within 5-year period
- Subtle post-op changes common and present a diagnostic dilemma

OUTCOME WITH CYPATH® LUNG

- **CyPath® Lung:** 5/24 test result: 0.83, likely lung cancer
- Because this represents a real-time possible recurrence, follow-up PET ordered 7/24
- PET showed right lung, right mediastinal and liver uptake
- Liver biopsy confirmed new small cell lung cancer as second primary
- Patient is currently being treated for ES-SCLC
- **CyPath® Lung positive result led to PET scan and diagnosis of a second primary cancer**

CyPath Lung

Case 4 outlines a complex clinical presentation when nodules or other suspicious imaging findings appear in a patient with an earlier biopsy-proven lung cancer who has undergone surgery and/or other definitive oncologic interventions. Real-time evaluations in this setting are hindered by post-treatment tissue changes that affect imaging and the contraindication of serum-related testing within five years of a cancer diagnosis. One of the great advantages of the CyPath® Lung platform is that testing is done on real-time viable cells in the setting of the lung microenvironment. Therefore, a "likely cancer" finding suggests the probability that recurrent cancer or a second cancer is present. The tightrope conversation with cancer patients exploring the possibility of recurrence or a new cancer is raw, emotional, and potentially devastating to the patient. In this setting, confidence in your clinical approach is imperative to quickly move beyond the imagined possibilities ahead into new treatment and outcomes. The real-time information CyPath® Lung testing has given our practice has been invaluable during these interactions.

CONCLUSION

Over the last four years, clinical validation and insurance approval for the CyPath® Lung test have been completed. The test became available for clinical use after the clinical validation was completed in September 2021. In

November 2021, it was certified for sale under the Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP) Accreditation Program based on an evaluation of Precision Pathology Services, the clinical laboratory where the test was developed. In June 2023, the American Medical Association (AMA) publicly released a newly approved Current Procedural Terminology (CPT) Propriety Laboratory Analysis (PLA) code specifically for use with CyPath® Lung. CPT code 0406U became effective October 1, 2023. Medicare/CMS finalized payment for CPT 0406U, effective January 1, 2024, and CyPath® Lung is listed on the CMS clinical laboratory fee schedule.

No diagnostic test offers 100% specificity or sensitivity, and understanding the shortcomings and limitations of any new test is a learned journey. Presented here are clinical scenarios that are common in my lung nodule practice, where we have found that adding CyPath® Lung to our algorithm has accelerated diagnosis, helped guide difficult clinical discussions, and prevented unnecessary invasive procedures. I am certain that as more clinical data become available, additional situations where this test provides significant benefit will be identified.

REFERENCES

1. National Lung Screening Trial Research Team. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med.* 2011;365(5):395-409. doi:10.1056/NEJMoa1102873
2. Weinberg SE, McDermont S. Diagnostic evaluation of incidental pulmonary nodule. *UpToDate.* Updated March 2025. Accessed April 11, 2025. https://www.uptodate.com/contents/diagnostic-evaluation-of-the-incidental-pulmonary-nodule
3. Morris JM, Habib SA, Do Valle ML, Schneider JE. Economic evaluation of a novel lung cancer diagnostic in a population of patients with a positive low-dose computed tomography result. *J Health Econ Outcomes Res*. 2024;11(2):74-79. doi:10.36469/001c.121512
4. Moran A, Daly ME. Surveillance imaging for non-small cell lung cancer: mounting evidence that less is more. *Transl Lung Cancer Res*. 2019;8(Suppl 4):S343-S346. doi: 10.21037/tlcr.2019.05.07
5. Alduraibi AK. FDG avid solitary pulmonary nodule mimicking lung cancer. *Radiol Case Rep*. 2022;17(4):1047-1049. doi:10.1016/j.radcr.2022.01.038
6. Patriquin L, Merrick DT, Hill D, et al. Early detection of lung cancer with Meso-Tetra (4-Carboxyphenyl) Porphyrin-labeled sputum. *J Thorac Oncol*. 2015;10(9):1311-1318. doi:10.1097/JTO.0000000000000627
7. Cole DA, Moody III D, Ellinwood EL, Gerard KM. Patent US-5162231-A. Method of using 5,10,15,20-tetrakis(carboxyphenyl)porphine for detecting cancers of the lung. Granted November 10, 1992. Accessed April 24, 2025. https://pubchem.ncbi.nlm.nih.gov/patent/US-5162231-A
8. Figge FH, Weiland GS, Manganiello LO. Cancer detection and therapy; affinity of neoplastic, embryonic, and traumatized tissues for porphyrins and metalloporphyrins. *Proc Soc Exp* Biol Med. 1948;68(3):640. doi:10.3181/00379727-68-16580
9. Lemieux ME, Reveles XT, Rebeles J, et al. Detection of early-stage lung cancer in sputum using automated flow cytometry and machine learning. *Respir Res*. 2023;24(1):23. doi:10.1186/s12931-023-02327-3
10. Bederka LH, Sanchez JR, Rebeles J, et al. Sputum analysis by flow cytometry; an effective platform to analyze the lung environment. *PLoS ONE*. 2022;17(8):e0272069. doi:10.1371/journal.pone.0272069
11. Grayson M, Lai SC, Bederka LH, et al. Quality-controlled sputum analysis by flow cytometry. *J Vis Exp*. 2021;(174). doi:10.3791/62785
12. Elzi DJ, Bauta WE, Sanchez JR, et al. Identification of a novel mechanism for meso-tetra (4-carboxyphenyl) porphyrin (TCPP) uptake in cancer cells. *FASEB J*. 2021;35(3):e21427. doi:10.1096/fj.202000197R