

The Optical Biopsy: A Novel Technique for Rapid Intraoperative Diagnosis of Primary Pulmonary Adenocarcinomas

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Background: With increasing use of chest computed tomography scans, indeterminate pulmonary nodules are frequently detected as an incidental finding and present a diagnostic challenge. Tissue biopsy followed by histological review and immunohistochemistry is the gold standard to obtain a diagnosis and the most common malignant finding is a primary lung adenocarcinoma. Our objective was to determine whether an intraoperative optical biopsy (molecular imaging) may provide an alternative approach for determining if a pulmonary nodule is a primary lung adenocarcinoma.

Methods: Before surgery, 30 patients with an indeterminate pulmonary nodule were intravenously administered a folate receptor-targeted fluorescent contrast agent specific for primary lung adenocarcinomas. During surgery, the nodule was removed and the presence of fluorescence (optical biopsy) was assessed in the operating room to determine if the nodule was a primary pulmonary adenocarcinoma. Standard-of-care frozen section and immunohistochemical staining on permanent sections were then performed as the gold standard to validate the results of the optical biopsy.

Results: Optical biopsies identified 19 of 19 (100%) primary pulmonary adenocarcinomas. There were no false positive or false negative diagnoses. An optical biopsy required 2.4 minutes compared to 26.5 minutes for frozen section ($P < 0.001$) and it proved more accurate than frozen section in diagnosing lung adenocarcinomas.

Conclusions: An optical biopsy has excellent positive predictive value for intraoperative diagnosis of primary lung adenocarcinomas. With refinement, this technology may prove to be an important supplement to standard pathology for examining close surgical margins, identifying lymph node involvement, and determining whether suspicious nodules are malignant.

Keywords: intraoperative diagnosis, optical biopsy, surgical oncology

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Over the last decade, surveillance guidelines for high-risk patients and increasing use of computed tomography scans have resulted in a rise in incidentally discovered nodules and masses.^{1,2} A particular example of this problem is solitary pulmonary nodules. When a nodule is discovered, the gold standard for diagnosis is a biopsy for hematoxylin and eosin staining and reviewed by a pathologist. This is frequently supplemented by immunohistochemistry for specific tissue typing in the case of malignancies. Although this approach is sensitive and specific, it does not always provide useful information to surgeons in certain situations during an operation, such as resection with close surgical margins, identification of malignant lymph nodes, and determination of the malignancy of small or difficult to remove nodules.³

Our group has been interested in developing a diagnostic technology that could provide specific histologic information about tissues within the confines of the operating room.^{4–9} Such a technology would provide an important supplement to standard hematoxylin and eosin and immunohistochemistry. To achieve this goal, we postulated that molecular imaging may be a reasonable intraoperative diagnostic tool. Molecular imaging is a technique by which a targeted, fluorescent contrast agent is used to identify a specific receptor on a particular cell. Thus, when the targeted agent accumulates on the cell of interest, it will fluoresce.

As a proof-of-principle of this technology, we developed a molecular-targeted agent to lung adenocarcinoma because it is the most common pulmonary neoplasm in the United States. In preliminary studies, we first studied the specificity of a targeted fluorescent molecular imaging contrast agent, folate–fluorescein isothiocyanate (folate–FITC) to folate receptor- α (FR α) on human lung adenocarcinoma cell lines. Molecular imaging could identify as few as 10^4 cancer cells in ideal conditions. Follow-up studies in murine studies demonstrated an 8–10 fold increased fluorescent signal in orthotopic lung tumors compared to normal lung parenchyma, and molecular imaging could locate nodules as small as 0.5 mm. Finally, we performed in vitro analyses of more than 100 human tumors confirming that FR α is highly expressed (10^3 – 10^4 receptors/cell) on lung adenocarcinomas and folate–FITC has 80% to 90% specificity for pulmonary adenocarcinomas.¹⁰ Based on these data, we postulated FR α could be an appealing molecular target for optical imaging to identify lung adenocarcinomas during surgery in humans.

In this work, we propose a novel method that we refer to as an “optical biopsy,” which is an application of nonradioactive molecular imaging. Our objective was to determine the efficacy of the optical biopsy for determining whether a solitary pulmonary nodule is a primary lung adenocarcinoma.

MATERIALS AND METHODS

Study Design

We performed a prospective pilot study on 30 consecutive patients who presented with an indeterminate peripheral pulmonary

nodule. All patients had a preoperative computed tomography scan that identified a peripheral lung nodule less than 3 cm in diameter. Three patients had undergone nondiagnostic biopsies (2 transthoracic needle aspirations, 1 endobronchial ultrasound-guided biopsy) before surgery. The remaining patients did not have preoperative invasive diagnostic procedures due to patient preference, physician choice, and/or technical limitations. Clinical and demographic information including age, sex, indication for surgery, nodule size, maximum standardized uptake value (SUV) on 18 F-fluorodeoxyglucose positron emission tomography scan, and clinical stage were measured. The protocol was approved by the University of Pennsylvania and Philadelphia Veterans Affairs Institutional Review Boards and all patients provided informed consent.

Approximately 4 hours before surgery, patients were injected with a tumor-targeted fluorescent contrast agent via a peripheral antecubital vein. The operative sequence was as follows: first, the surgeon either performed a video-assisted thoracoscopic surgery or thoracotomy to locate the nodule. The lesion was then removed by a partial wedge excision of the lung. The nodule was bisected without affecting the margins and underwent molecular imaging immediately on the surgical field. If the nodule was fluorescent, it was presumed to be a primary lung adenocarcinoma. If the nodule was not fluorescent, then no prediction could be made about the nature of the nodule. All specimens were photodocumented with a white light and 490 nm filtered light.

Then, the specimen was carried to the Pathology Department. Frozen section evaluation was performed on all specimens and the results were phoned to the operating room. Due to the experimental nature of this study, the decision to perform a lobectomy was based on the frozen section results, not the optical biopsy results. All specimens were sent for permanent histopathology and immunohistochemical studies for final diagnosis as to the nature and cell type of the lesion.

Targeted Contrast Agent for Optical Biopsy of Lung Adenocarcinomas

Folate-FITC is a conjugate between folate and fluorescein isothiocyanate. This conjugate forms a negatively charged fluorescent molecule that binds weakly and nonspecifically to serum proteins, but with exquisite single-digit nanomolar affinity to folate receptor-positive tumors. FITC is a synthetic organic compound that is excited in the 465–490 nm wavelength range and emits light in the 520 to 530 nm range. Previous research has demonstrated that pulmonary adenocarcinoma cells significantly overexpress FR α on the basal (luminal) side of the cell allowing for selective uptake of systemically administered folate-FITC.^{11,12} A 0.1 mg/kg dose of this agent (On Target Laboratories, Inc., West Lafayette, IN) was dissolved in 10 mL of normal saline, and then given to the patients 4 hours before surgery. Patients were monitored for 30 minutes after injection for an adverse reaction.

Intraoperative Optical Biopsy System

Intraoperative molecular imaging was performed using the FlowCam imaging system developed in our laboratory.⁸ Positive and negative controls were used in all cases. At the time of optical biopsy, the surgeon graded the image as (+) fluorescent or (–) fluorescent (Fig. 1, column 4).

Frozen Section Pathologic Analysis

Each sample was embedded in OCT frozen tissue matrix (Sakura Finetek; Torrance, CA) and frozen at –25°C using a Leica-CM3050S cryostat. Samples were cut into 5 μ m-thick sections, treated with 95% alcohol, and stained with hematoxylin and

eosin according to standard clinical protocols. Samples were analyzed by a pathologist at the University of Pennsylvania or Philadelphia Veterans Affairs Medical Center.

Immunohistochemistry

For final pathological analysis, specimens were formalin-fixed and paraffin-embedded according to established protocols. Surgical specimens were stained for TTF-1, Napsin-A, CK5/6, and p63 for accurate subclassification of non-small cell lung carcinoma and for supporting primary lung origin. For permanent section, a specialized lung pathologist reviewed each sample. The samples were stained for FR α with mAb343 (1.8 mg/mL) by protocols previously described.¹² Staining was graded from 0 (no positive staining, no expression) to 3+ (>80% of cells staining, strong expression), and averaged for 3 high-powered fields.

Statistical Analysis

This trial was designed as a pilot study whose primary aim was to identify potential adverse events of the administration of the targeted contrast agent. Estimating the diagnostic properties of the method for identifying adenocarcinomas was a secondary objective.

Posthoc image analysis was performed to quantify the amount of fluorescence using region-of-interest software within ImageJ (<http://rsb.info.nih.gov/ij/>; public domain free software available through National Institutes of Health). Researchers were blinded to the nodule histopathology. A background reading was taken from adjacent normal lung tissue to calculate a tumor-to-background ratio (TBR). We assessed the significance of differences in mean values of TBR of (–) fluorescent versus (+) fluorescent tumors by the Mann-Whitney test. We tested for differences in paired samples using the Wilcoxon signed-rank test. We assessed correlation of continuous outcomes by the Pearson correlation coefficient. These analyses were performed in SAS Version 9.3 (SAS Institute, Inc., Cary, NC). We computed exact confidence bounds for sensitivity, specificity, and positive predictive value in PASS Version 12 (NCSS, LLC; Kaysville, UT).

RESULTS

Study Population

The median patient age in the study was 63 years (IQR: 61–73), with 14 males and 16 females. On preoperative imaging, the average nodule size and maximum SUV on PET were 1.8 cm (IQR: 1.2–2.3 cm) and 4.9 (IQR: 1.8–6.1), respectively. All 30 patients underwent injection of the folate-targeted contrast agent before surgery without any adverse events. The final extent of resection was wedge excision in 11 patients, lobectomy in 18 patients, and bilobectomy in 1 patient.

An Optical Biopsy Can Accurately Diagnose Primary Pulmonary Adenocarcinomas

After partial lung wedge excision, all tumors were bisected and graded as (+) fluorescent or (–) fluorescent by optical biopsy (Table 1). Nineteen of 30 nodules were (+) fluorescent thus were predicted to be lung adenocarcinomas (Fig. 1). On frozen section of these nodules, the pathologist read 13 as pulmonary adenocarcinomas, 4 as cancers of unknown origin, 1 as a squamous cell carcinoma, and 1 as containing no malignant cells. Based on the frozen section results, 18 of the 19 patients underwent a lobectomy. Ultimately, after permanent sectioning and immunohistochemistry, final pathological analysis determined that all 19 nodules were primary pulmonary adenocarcinomas: 16 invasive adenocarcinomas, 2 minimally invasive adenocarcinomas, and 1 primary mixed adenosquamous cell carcinoma. If the optical biopsy had been the guide for surgery, all 19 patients would have correctly

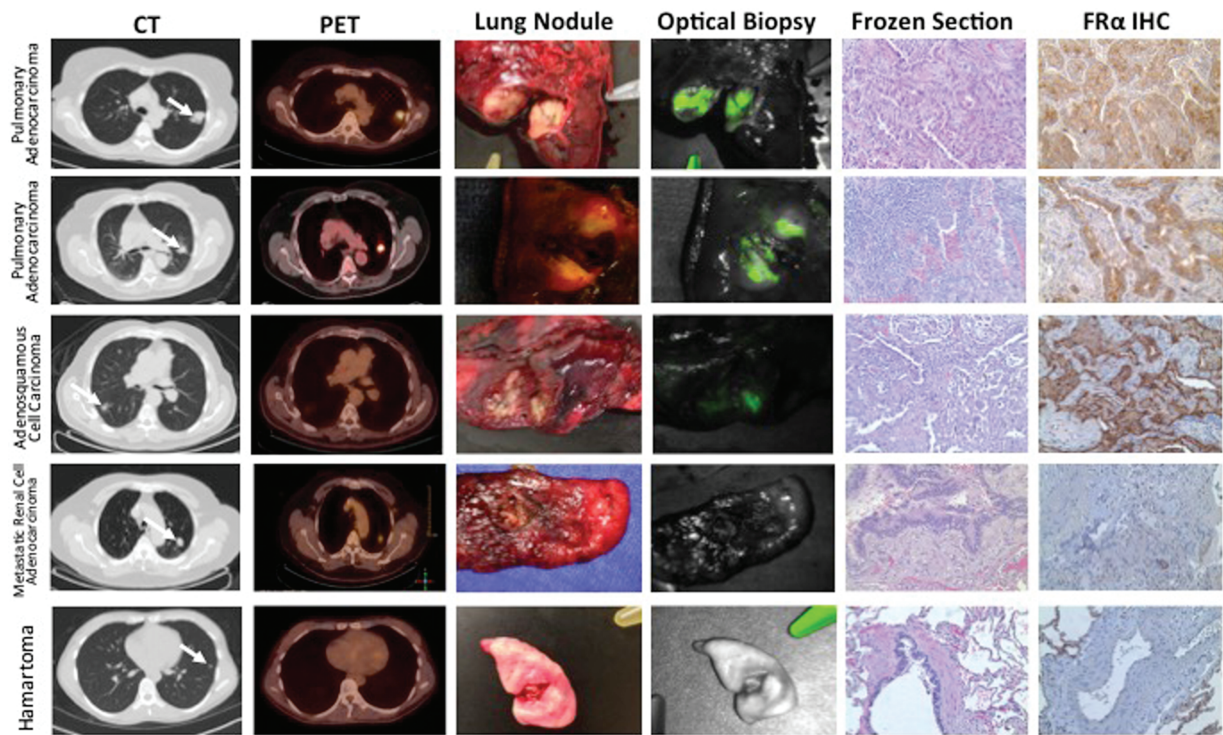


FIGURE 1. Five representative cases of indeterminate pulmonary nodules: 3 primary pulmonary adenocarcinomas, a metastatic renal cell adenocarcinoma, and a hamartoma. The first 2 columns represent preoperative imaging studies (computed tomography and ¹⁸fluoro-deoxyglucose positron emission tomography studies). The third and fourth columns demonstrate the optical biopsy that is performed immediately on the surgical field. The fifth column is the frozen section pathology. Finally, the sixth column demonstrates FRα immunohistochemistry.

undergone a lobectomy. However, because the frozen section was the basis for the standard-of-care, 18 patients underwent a lobectomy.

The remaining 11 nodules were (–) fluorescent on optical biopsy. Of these nodules, frozen section reported 5 benign nodules, 4 carcinoma of unknown origin, and 2 metastatic renal cell adenocarcinomas (Table 1). Based on frozen section results, 3 of the 11 patients underwent a

lobectomy. On permanent section analysis, the (–) fluorescent nodules were 3 noncaseating granulomas, 2 primary squamous cell carcinomas, 2 hamartomas, 2 metastatic renal cell adenocarcinomas, 1 metastatic leiomyosarcoma, and 1 mucoepidermoid carcinoma.

In summary, as a diagnostic test for a primary pulmonary adenocarcinoma, an optical biopsy had a positive predictive value of

TABLE 1. Results of Intraoperative Optical Biopsy Versus Frozen Section Pathology		
	(+) Fluorescent (n = 19)	(–) Fluorescent (n = 11)
	Intraoperative Diagnosis	
Optical biopsy	Primary pulmonary adenocarcinoma (19)	Not primary pulmonary adenocarcinoma (11)
Frozen Section	Primary pulmonary adenocarcinoma (13) Cancer of unknown origin (4) Squamous cell carcinoma (1) Benign (1) [†]	Benign (5) Cancer of unknown origin (4) Metastatic renal cell carcinoma (2)
	Final Diagnosis	
Permanent Section	Primary pulmonary adenocarcinoma (19)	Granuloma (3) Hamartoma (2) Squamous cell carcinoma (2) Metastatic renal cell adenocarcinoma (2) Metastatic leiomyosarcoma (1) Mucoepidermoid carcinoma (1)

[†]Sectioned 3 times.

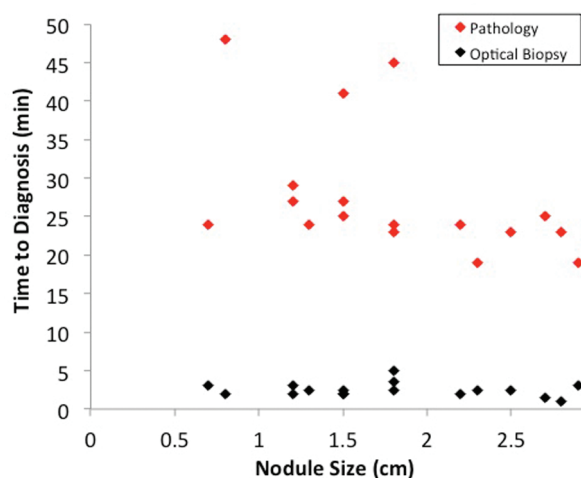


FIGURE 2. Time to intraoperative diagnosis by optical biopsy versus frozen section pathology relative to nodule size.

100% (95% lower confidence bound: 85.4%) for undergoing pulmonary lobectomy. There were no false positive or false negative diagnoses.

Optical Biopsy Is Rapid

To assess the efficiency of optical biopsies, we measured the time to diagnosis for each nodule. The mean time to a diagnosis from when the nodule was removed from the patient to when the surgeon made a diagnosis was 2.4 minutes (range: 1–5 min). The mean time from when the specimen left the surgical field to when the frozen section results were reported was 26.5 minutes (range: 19–48 min) (Fig. 2). Six cases required additional time: 3 cases required resectioning for additional tissue to improve diagnostic yield, and in 3 cases, the pathologist consulted a specialized lung pathologist to review the case before rendering a decision (time to diagnosis 41, 45, and 48 min). Time to frozen section diagnosis improved with larger nodules, but this trend was not statistically significant ($P = 0.10$).

Posthoc Image Analysis of Optical Biopsies

In our posthoc analysis, we selected several tumor characteristics to determine if these features correlated with the degree of tumor fluorescence. Of the 19 primary pulmonary adenocarcinomas, there were 9 acinar predominant tumors, 3 lepidic predominant, 3 solid predominant, 2 minimally invasive carcinomas, 1 micropapillary predominant, and 1 adenosquamous cell carcinoma (Table 1). Five tumors were graded as well differentiated, 7 were moderately differentiated, and 7 were poorly differentiated. The degree of tumor fluorescence was quantified based on the TBR. In the 19 (+) fluorescent nodules, the mean TBR was 5.6 (IQR: 4.8–6.3). In the 11 (–) fluorescent nodules, the average TBR was 1.1 (IQR: 0.9–1.2) ($P < 0.001$). There was no overlap in the TBR range of (+) fluorescent versus (–) fluorescent tumors (Fig. 3). The TBR did not correlate with tumor subtype or differentiation. Also, TBR did not correlate with tumor size or SUV on PET.

DISCUSSION

Patients with a solitary pulmonary nodule frequently undergo thoracic surgery for diagnosis. The standard-of-care operative approach is partial lung wedge excision followed by frozen section pathology. If the nodule is a non-small cell lung cancer (eg, pulmonary adenocarcinoma and pulmonary squamous cell carcinoma) on frozen section analysis, the patient undergoes a completion pulmonary lobectomy.

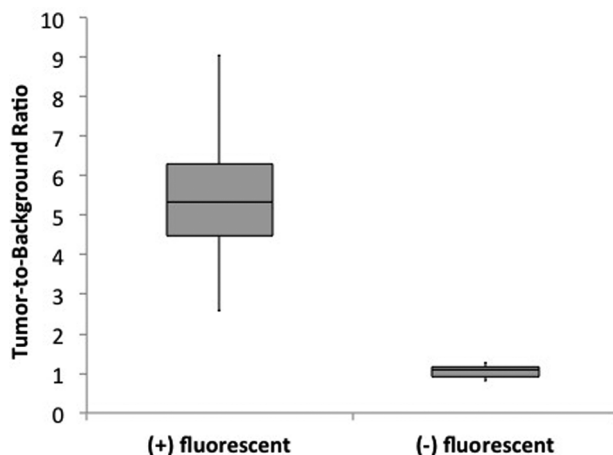


FIGURE 3. Posthoc analysis of the TBR of the nodule to the surrounding lung correlated strongly with the intraoperative fluorescence during the optical biopsy.

Here, we evaluated a novel intraoperative imaging technique that we refer to as an “optical biopsy” as a supplement to pathology. In this work, we used an optical biopsy to determine whether a pulmonary nodule is a lung adenocarcinoma, though this concept is generalizable to any malignancy with a known surface receptor.

We enrolled 30 patients with an indeterminate pulmonary nodule that necessitated a wedge excision for diagnosis. Intraoperative optical biopsies required less than 3 minutes for diagnosis and accurately identified all 19 primary pulmonary adenocarcinomas, which should have undergone a lobectomy without further delay. Frozen section diagnosis required 25 minutes and identified 13 of the 19 primary pulmonary adenocarcinomas. This study suggests intraoperative diagnosis using targeted tracers can substantially improve surgical oncology by reducing anesthetic time, operative costs, and incorrect operations.

By frozen section, one of the 19 nodules was misdiagnosed as a squamous cell carcinoma, and another nodule was incorrectly read as a benign lesion. Both of these nodules fluoresced at the time of surgery, and they were labeled as pulmonary adenocarcinomas by optical biopsy. These 2 incorrect frozen section diagnoses illustrate the limitations of pathology due to human error and sampling error, respectively. Ultimately, frozen section analysis incorrectly led the surgeon to terminate the second case without performing a lobectomy or a mediastinal lymph node dissection. Although immunohistochemistry is the gold standard for obtaining a definitive diagnosis, this approach can take several days and does not provide information to the surgeon during the critical portions of the operation.

In this small study, we could not define the lower size limit to the optical biopsy. In laboratory settings, we could identify a 0.3 mm nodule that is composed only of cancer cells. However, the smallest nodule that we removed in our human study was 1.2 cm, which was fluorescent. Due to practical issues, we have no method to control tumor size in patients. However, we do not predict that size will be a limitation due to advances in camera systems and magnification ability. More important will be the density of tumor cells in comparison to the background stromal cells and immune cells, which are not fluorescent. Thus, tumors that have a low proportion of FR α ⁺ cancer cells compared to the surrounding tissues will be difficult to discriminate due to noise.

Although this pilot study in intraoperative molecular imaging was a proof-of-principle, these data have several more far-reaching implications to pulmonary surgery beyond an optical biopsy. First,

this technology may have a major impact on minimally invasive surgery. Unlike traditional open surgery, minimally invasive surgery by video-assistance or robotics limits palpation of the lung and is the main disadvantage of these approaches. Thus, with miniaturization of imaging devices, intraoperative imaging may be useful for inspecting and locating nodules in the patient.

Second, because the fluorescent contrast agent is administered systemically, this method will be useful in bringing attention to synchronous and metachronous lesions that may have not been suspected by the surgeon. This may be particularly important to detecting metastatic cancer cells in lymph nodes that may seem grossly disease-free. Third, there has been an increase in sublobar pulmonary resections for smaller or minimally invasive adenocarcinomas. Because these lesions are small and difficult to find, the risk of a positive margin increases substantially. Intraoperative optical biopsies will allow for rapid assessment of parenchymal staple lines in these clinical situations. Ultimately, intraoperative molecular imaging may decrease local recurrence rates and improve survival for patients who undergo pulmonary resection.

Our novel technique—for which we coin the term “optical biopsy”—is based on molecular imaging using fluorescein as the fluorescent contrast agent coupled to folate as the ligand. The FR α receptor marker is highly tumor specific. Preclinical studies in our laboratory have shown that all lung adenocarcinomas express some level of FR α , and that 85% express extremely high levels of FR α .¹² This suggests that even low levels of FR α expression are sufficient for tumor diagnosis. Furthermore, most metastatic adenocarcinomas other than ovarian cancer do not express FR α .

The FR α -targeted fluorophore accurately detected all 19 primary pulmonary adenocarcinomas, including 2 minimally invasive adenocarcinomas and a mixed adenosquamous cell carcinoma. This technique differentiated 2 metastatic adenocarcinomas [(–) fluorescent] from 19 primary lung adenocarcinomas [(+) fluorescent]. We acknowledge there may be a sampling bias, and future expanded studies will evaluate this possibility. Though there was a small number ($n = 5$) of benign lesions in the study, we had no false positive diagnoses by optical biopsy. Thus, if these data are confirmed in larger studies, the surgeon can feel confident that fluorescent nodules will require completion lobectomy.

Another notable discovery is the reduction in the average diagnostic time from 26.5 minutes for frozen section to 2.3 minutes for optical biopsy ($P < 0.001$). This reduction in time to diagnosis is critically important to real-time surgical decision making in the operating room particularly in situations where a surgeon needs to assess a close margin or suspicious lymph node without removing the concerning lesion and sending it to pathology. This approach may be especially beneficial to patients with marginal lung function and the elderly for whom long operative times are a major risk factor for perioperative morbidity.¹³ Furthermore, reduced operative times and less reliance on frozen section pathology will facilitate significant cost savings.

We acknowledge several limitations. First, although our test has a high positive predictive value, it does not detect primary lung malignancies that are not adenocarcinomas (eg, primary pulmonary squamous cell carcinoma). Thus, we emphasize that actionable changes can only be taken when a nodule is fluorescent. A negative scan does not rule out malignancy and standard frozen section pathological analysis should be performed. Second, there are costs and risks of performing an optical biopsy. Patients must come to the hospital 4 hours before surgery to receive the contrast agent, and there is a small risk of an allergic reaction to the infusion. There are also costs associated with purchasing imaging devices with suitable sensitivity. Nevertheless, these costs are marginal relative to the

dangers of tissue damage, bleeding, excessive tissue removal, incorrect diagnoses, and prolonged anesthesia associated with conventional frozen section. Finally, we acknowledge that the number of patients with nodules studied was relatively small, and only 5 of the patients had nonmalignant inflammatory nodules and 2 patients had adenocarcinomas of nonpulmonary origin. A larger study will be necessary to confirm these results.

In the future, more targeted contrast agents can be developed for other cancer subtypes to expand the diagnostic utility of optical biopsies beyond primary pulmonary adenocarcinomas. By fusing a fluorescent dye to a receptor ligand, a contrast agent could theoretically be developed for any tumor type. This would be an important supplement to standard pathology in evaluating close surgical margins, lymph node involvement, or the malignancy of the primary nodule. Of note, in this study, we used as imaging device (FlowCam) that was developed in our laboratory. However, many companies do have commercially available imaging devices but are costly for research purposes.

The potential of this work is far reaching. If an optical biopsy could be performed in real time inside the patient and the surgeon visualized a fluorescent nodule, then the surgeon could immediately perform an anatomic lobectomy and mediastinal lymph node dissection. This would eliminate up to an hour of surgical time as the surgeon performs the wedge excision and waits for the frozen section analysis. In the future, we envision administering patients a cocktail of tracers that can identify multiple tumor types including other non-small cell lung cancer histological subtypes. In fact, groups are developing multiple “color-coded” contrast agents to distinguish various histologies (eg, squamous cell carcinoma could be labeled red, large cell carcinoma could be labeled yellow). Ultimately, we envision that an optical biopsy will be able to be performed in the patient without removing the specimen. As imaging devices improve, this vision is close to reality. This technology is broadly applicable and can be extended to all solid tumors by using tumor-specific fluorescent agents as they become available.

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DISCUSSANTS

D.H. Harpole, Jr. (Durham, NC):

I have no disclosures.

Rapid intraoperative assessment of suspicious nodules is a major obstacle to decreasing not only operating room costs but also, importantly, eliminating unnecessary anatomic resection for benign lesions. This novel molecular imaging technique seems to go a long way in optimizing selection of tumors for completion lobectomy.

The authors should be congratulated for taking a pertinent clinical problem to the laboratory to refine the process. This is a translational science project in action, and the presentation was clearly articulated by Sunil. My only major critique is that this feasibility project lacks the power to verify its utility as stated and that a multiinstitution cohort analysis needs to be initiated.

I have 2 questions.

One of the major difficulties we now encounter after the approval of CT screening for high-risk individuals is a noninvasive method to separate cancerous from noncancerous small detected lesions. Although ¹⁸fluorodeoxyglucose positron emission tomography is helpful, there are areas in North America with high endemic rates of granulomatous disease that create very high rates of false positivity and make the positron emission tomography (PET) additional information not useful. Could this technology be labeled with a radioisotope that would identify adenocarcinomas using a nuclear medicine imaging before the operating room?

My second question is, although this adenocarcinoma technique is feasible, it still does not seem to solve a major problem that we encounter, which is metastatic colorectal versus primary adenocarcinoma in a solitary nodule. You showed some specificity with respect to renal cell carcinoma, but has your team refined the molecular imaging to a point that it would have the specificity to separate those metastatic adenocarcinomas from colorectal origin versus those that are primary lung cancer, because, as you know, this is a major stopping point for us in the operating room if the frozen section cannot differentiate those 2 lesions.

Response From S. Singhal:

Dr Harpole, thank you for your kind comment and questions. The ability to tag a radioactive isotope is feasible, and it is currently being performed for a host of ligands including folate. I think there are companies that are pursuing this approach. The goal of this particular study was to develop a specific molecular agent that would be relevant to the surgeon. PET scans require radioactivity and the sensitivity of this approach is closer to a centimeter. Intraoperative optical imaging is much more sensitive, almost down to 0.3 mm in laboratory conditions.

With regard to your second question regarding specificity, folate receptor-alpha (FR α) is 80% to 90% sensitive for lung adenocarcinomas. It is quite specific to lung and ovarian carcinomas, both of which express FR α on a high proportion of tumors. We have looked at several other cancers, including colorectal, thyroid, breast, and prostate. They do not uniformly express high levels of FR α . Nor do they bind our molecular contrast agent.

F. Michelassi (New York, NY):

I have no conflicts.

I am rising to congratulate you on this very nice presentation. This is a very interesting and intellectually stimulating application of modern technology.

I am not a thoracic surgeon. Yet, I am curious to know whether this technology could help us in better guiding us to biopsy areas of chronic inflammation in search of early signs of neoplastic transformation. Examples such as chronic ulcerative colitis or Barrett esophagus come to my mind: could this new methodology guide us in the search of areas of early malignant transformation?

Response From S. Singhal:

Thank you for your question Dr Michelassi. As long as your cell of interest has a unique receptor, you can construct a targeted fluorescent contrast agent. To the best of my knowledge, there are no limitations to what you can target with a properly constructed fluorescent contrast agent. Ideally, you want to select a ligand that will bind a surface receptor. I do not think antibody approaches are likely to be successful because they will be trapped in the reticuloendothelial system of the body. I should mention that our agent does bind pulmonary adenocarcinoma in situ. So the fact that it is a precursor to an invasive cancer is not an issue.

L. Kaiser (Philadelphia, PA):

My only disclosure is I have known the speaker since he was a medical student. He is a thoracic surgeon because I encouraged him in that direction.

Sunil, I am particularly interested in those lesions that are not PET positive that turn out to be carcinoma. The ground-glass opacities in particular, have you looked at any of those, what we used to call bronchoalveolar carcinomas, some of these minimally invasive carcinomas? Have those shown to be positive?

I guess the other thing is, if they are positive on PET, and it did not light up, would you be comfortable leaving it in there?

Response From S. Singhal:

Thank you for your question, and I do admit that my interest in thoracic surgery originated as a first-year medical student in Dr Kaiser's laboratory. In answer to your question, we have investigated FR α expression on adenocarcinoma in situ and minimally invasive adenocarcinomas. We do have 80% sensitivity for those tumors.

With regard to leaving lesions in the chest, I want to reemphasize that a major value of this technology is to draw the attention of the surgeon to a tissue that is glowing. A glowing tissue should raise the suspicion of the surgeon. It may identify synchronous and metachronous lesions. It may identify cancer cells in a lymph node.

Based on our small data set of 30 patients, if the lesion is glowing, we predict it will most likely be a lung adenocarcinoma and most probably needs to be removed. Ultimately, that is a clinical judgment. If the lesion is not glowing on optical biopsy, then you must use your standard-of-care approach. Larger studies need to be performed to validate these data.

V. Rusch (New York, NY):

This is a wonderful, novel, and interesting work that I think has real future application.

Were there any adverse events related to the infusion of this agent?

Second, do you have any experience with deeply located lung nodules that are hard to identify at video-assisted thoracoscopic surgery or thoracotomy? Does this agent fluoresce sufficiently to localize such nodules?

I would also emphasize that this technique does not supplant pathology, especially with respect to identifying adenocarcinoma histological subtype. The histological information may be important in helping to determine whether to perform a lobectomy rather than a sublobar resection.

Finally, I agree with Dr Harpole that this technique certainly merits assessment in a multicenter trial, particularly for the management of the 2 cm or less primary tumors, where we are considering sublobar resection. In that situation, molecular imaging may define the necessary margins of resection and the extent of lymph node dissection.

Response From S. Singhal:

Dr Rusch, thank you for your questions. We had no adverse events. The contrast agent is composed of folate, a naturally occurring vitamin. It is fused to a fluorophore, fluorescein, which occurs naturally in fireflies. We do ask our patients if they have an allergy to mosquitoes or bugs; however, in our study we encountered no issues.

With regard to our depth of penetration, that is the Achilles heel of the optical biopsy in the patient. If you put your endoscope into the chest, and the tumor is located 3 cm deep into the lung parenchyma, then this technology will not be able to tell you whether the tumor is glowing. The nodule needs to be removed or an incision needs to be made into the lung to get closer to the nodule. For a surgeon, this is usually not an issue. For example, if the surgeon is examining a staple line and sees the staple line is fluorescent, then the surgeon should suspect that there are tumor cells left behind.

With regard to subtyping, an optical biopsy is not meant to supplant nor replace pathology. This technology is meant to be a supplement to standard pathological techniques. The primary value of this work is to draw the surgeons' attention to an area that may be overlooked. Once that area is identified, it can be harvested and then the specific subtyping can be done by pathology. We have already done a tissue microarray of 220 patients with lung adenocarcinomas, but we have not done specific subtype analysis of FR expression on these.

With regard to small tumors, our smallest tumor was 1 cm in this study and the tumor fluoresced nicely. Although I do not know the lower bound for this technology, I believe that size is not the only important factor. Other important factors include the density of tumor cells in the nodule. For example, a large nodule that is composed of stromal cells, immune cells, or necrosis may be less likely to fluoresce than a small subcentimeter pulmonary nodule that is primarily composed of FR α positive cancer cells.

I did not fully address the impact of an optical biopsy on interrogating lymph nodes. There is no doubt that one of the most important implications of this method will be determining which lymph nodes contain metastatic cancer cells. Because most lymph nodes are less than 1 cm, depth of penetration will be less of an impediment. This may prove to be the most valuable application of this technology, and it is the focus on our current work.

L. Way (San Francisco, CA):

Have you had any experience with abdominal tumors, such as pancreatic or gastric adenocarcinoma, where extent of lymph node dissection is often an issue? Do you expect this technology will help stage the disease during the operation and guide the dissection?

Response From S. Singhal:

Thank you Dr Way. This particular agent targets FR α , which is chiefly expressed on lung and ovarian adenocarcinomas. I do not have any data on pancreatic or gastric cancers, though based on preliminary work in our laboratory, these 2 cancers do not express FR α .

R. Aft (St. Louis, MO):

I am just wondering what volume of tumor or what density of receptors is required on the cells in order for it to be detected.

Response From S. Singhal:

Thank you Dr Aft. The volume of tumor and density of receptors alone are not the only limiting issue, and the story is much more complex. Dr Phil Low, one of the pioneers of folate biology, and a coauthor on this abstract, has studied this topic in detail. On lung adenocarcinomas, there can be up to 1000 to 10,000 FR α per cell. However, the density of the receptors is not the only important factor. If a lung nodule is full of stroma, fibroblasts, and immune cells, then the nodule is going to have a weak fluorescent signal. On the other hand, a nodule that is composed of primary lung adenocarcinoma cells, even if the receptor density per cell is low or the size of the entire nodule is quite small, it is likely to fluoresce quite brightly. Another consideration is the blood supply coming to the nodule. A nodule that is well-vascularized is more likely to accumulate these contrast agents over time.

I would like to mention there is a major difference between laboratory conditions and the operating room. When we are doing in vivo intraoperative imaging in the chest, it can be technically challenging to image a small fluorescent nodule behind fat or other tissues because of the natural movements of the lung and the heart. The same nodule may be easier to image and detect in perfect laboratory conditions where there is no ambient light and no motion.

Broadly speaking, the most important goal of imaging is to improve the signal to background ratio. The signal depends on not just the number of receptors but also the imaging device and excitation energy source. As imaging devices improve, it will be easier to identify smaller and smaller quantities of fluorescent signal during surgery.

S. Demeester (Los Angeles, CA):

Cytology is frequently negative or falsely negative with a malignant pleural effusion. Can you use this to diagnose a malignant pleural effusion related to lung adenocarcinoma?

Response From S. Singhal:

Thank you for your question Dr DeMeester. Yes, you can use this technology for malignant pleural effusions if the cellular content is high enough.

C. Slingluff (Charlottesville, VA):

No disclosures relevant to this.

I just had a question about the specificity of FR α . It looks beautiful here. It is reported to be expressed in about half of colorectal cancers and a lot of ovarian cancers, but is it overexpressed at higher levels in lung adenocarcinoma or more on the cell surface?

Response From S. Singhal:

Thank you for bringing up this point, Dr Slingluff. The literature can be quite confusing on this topic. It is important to review the data carefully because much of the analysis of FR α expression on tumors is somewhat old. Since the earlier data, several things have happened. First, we now know that some of the older antibodies were polyclonal and were not truly staining FR α . Second,

we also now know that there are 4 folate receptors—alpha, beta, delta, and gamma. In some of the older studies, macrophages in the tumors were binding folate receptor-beta and the actual tumor cells did not express FR α .

With regards to your specific question, we have not seen our antiFR α antibodies stain colorectal cancers. Nor have they taken up our contrast agent.