MKT_2021_lung_01 / 21-008276

nCLE Guided Randomized Controlled Trial for Lung Cancer Diagnosis

NCT05556525

Document Date: 12/07/2021

nCLE Guided Randomized Controlled Trial for Lung Cancer Diagnosis

Needle based confocal laser endomicroscopy guided For Lung Cancer Diagnosis: A Randomized Controlled Trial

Protocol Number: MKT_2021_lung_01

December 2021

Investigator-Initiated Multi-center Study:

Dr. Fernandez-Bussy, Mayo Clinic, Jacksonville, Florida

Dr. Reisenauer, Mayo Clinic, Rochester, Minnesota

Centers:

Mayo Clinic, Jacksonville, Florida

Mayo Clinic, Rochester, Minnesota

Financial Sponsor of the Study: The study will be funded by Mauna Kea Technologies, Paris, France

Abbreviations:

AE	Adverse Event
AR	Adverse ReactionCA Competent authority
CLE	Confocal Laser Endomicroscopy
CT	Computed tomography
EBUS	Endobronchial Ultrasound
EUS-B	Esophagus ultrasound using the EBUS scope
r-EBUS	Radial endobronchial ultrasound
ENB	Electromagnetic Navigational Bronchoscopy
EUS	Endoscopic Ultrasound
EUS-FNA	Endoscopic Ultrasound - Fine-Needle Aspiration
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IGRT	Image-Guided Radiation Therapy
IMRT	Intensity-Modulated Radiation Therapy
nCLE	Needle-based Confocal Laser Endomicroscopy
NLST	National Lung Screening Trial
NPV	Negative Predictive Value
OB	Optical Biopsy
pCLE	Probe-based Confocal Laser Endomicroscopy
PET	Positron Emission Tomography
PPN	Peripheral Pulmonary Nodule
ROSE	Rapid On-Site Evaluation
StO2	Microvascular oxygen saturation
SAE	Serious Adverse Event
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example, a pharmaceutical company, academic hospital, scientific
TBB	Transbronchial Biopsy
TTNA	Transthoracic Needle Aspiration
VATS	Video-Assisted Thoracoscopic Surgery
VB	Virtual Bronchoscopy
VMAT	Volumetric Modulated Arc Therapy

1. Summary 5
2. Objectives 9
Background 9
Primary objective: 9
Secondary objectives: 9
3. Study Design 10
4. Study Population 11
Population (base) 11
Exclusion criteria 11
Intra-operative Exclusion/Stopping Criteria: 11
Sample size calculation 11
5. Treatment of Subjects 12
Investigational product/treatment 12
Summary of known and potential risks and benefits 14
Study procedures 15
Preparation before the procedure 15
Steps during the procedure 16
Retrieval of the probe 17
After the Cellvizio procedure 18
Steps of standard - and study (in bold) procedures: 18
Pathological examination 19
Cytological aspiration (standard procedure) 19
Histological evaluation 20
Final Diagnosis or Definitive Diagnosis 20
Adverse Events 20
Definitions 20
Severity Rating 20
6. Data Collection 22
Subject demographics 22
Subject medical history and baseline status 22
Lesion characteristics 23
Procedural assessments 23
Follow-up assessment 23
nCLE image quality: 24
Diagnostic and Therapeutic Management 24
7. Statistical Analysis 25
Population demographics comparison in both arms: 25

Lesion characteristics comparison in both arms: 26
Navigation Accuracy 26
Navigation success 26
Navigation time 26
First- and cumulative-pass diagnostic yields and performance calculations 26
Per patient diagnostic yield and performance calculations 27
Patient diagnostic and management analysis: 27
Feasibility statistical analysis: 27
Safety: 28
Reproducibility: 28
8. End Points 28
First-pass diagnostic yield: 29
Per patient diagnostic yield and performance: 29
Patient management: 29
Safety: 29
9. Study Benefit/Risk Ratio 30
10. References: 30

1. Summary

Rationale: Lung cancer is the leading cause of malignancy-related mortality worldwide. While these start as small nodules in lung parenchyma, early and accurate diagnosis allows timely surgical resection of malignant nodules while avoiding unnecessary surgery in patients with benign nodules. The National Lung Screening Trial (NLST) showed that screening high-risk patients with low-dose chest computed tomography (CT) reduced mortality from lung cancer by 20% [1]. However, 96% of the positive screens were subsequently found not to be malignant, suggesting the need for an accurate diagnostic tool with high specificity [1].

Transthoracic needle aspiration (TTNA) is currently the preferred mode for sampling peripheral pulmonary nodule (PPN) because it has a high diagnostic yield ranging from 80% to 90% [2]. However, it has a 12% to 45% risk of pneumothorax, with 2% to 15% requiring chest tube placement [3]. Flexible bronchoscopy with brushing, washing, and transbronchial biopsy (TBB) is safe but provides a much lower diagnostic yield (14 to 63%) depending on the size and location of PPN [4,5]. A recent meta-analysis including 1033 PPNs revealed that the diagnostic yield of TBB can reach up to 65%, if guidance systems such as electromagnetic navigational bronchoscopy (ENB), virtual bronchoscopy (VB), or radial-probe ultrasound (r-EBUS) are used. However, the negative predictive value (NPV) was reported to be low (0.52) [6,7].

Robotic technology has recently been incorporated into navigational bronchoscopy to improve flexibility, reach, and stability [8,9]. While manual and robot-assisted navigational bronchoscopy technologies have been proven effective in directing the bronchoscopist to achieve proximity to peripheral nodules, further localization with radial ultrasound and/or fluoroscopy is often required to make fine adjustments to align the biopsy needle with the target nodule. Without independent confirmation that a needle is in the lesion, a bronchoscopic lung biopsy that does not show tumor cells is often considered non-diagnostic rather than negative. Needle biopsies may therefore require multiple needle passes, which can cause bleeding. While rarely life-threatening, bleeding can contaminate slides and make a rapid on-site evaluation (ROSE) of biopsy samples challenging. A technology offering independent confirmation that the tip of the needle is within the nodule may reduce the incidence of non-diagnostic biopsies.

Needle-based Confocal laser endomicroscopy (nCLE) is a novel high-resolution imaging technique that uses an excitation laser light to create 'real-time' microscopic images of tissues. nCLE can be integrated into needles allowing real-time cancer detection during endoscopy. In a recent study by Wijmans et al. [10] (ClinicalTrials.gov Identifier: NCT02689050) nCLE of lung tumors and mediastinal nodes was feasible, using an endoscopic ultrasound-guided approach from the esophagus. nCLE malignancy criteria were identified. This study demonstrated that the nCLE technique provides real-time information regarding the malignancy status of lung tumors and mediastinal nodes. Improved needle-based bronchoscopic diagnostics might result in improved diagnostic cancer rate, reduction of surgical diagnostic procedures, reduced costs, and shorter time-from lung nodule detection to final diagnosis allowing treatment at an earlier cancer stage. Ideally, in the future, a target lesion will be identified as being malignant in a real-time fashion and, in the same session, bronchoscopically treated.

The first steps towards real-time optical biopsies were made with EUS-guided nCLE in gastroenterology. Recently, promising data were published on EUS-guided nCLE in lung lesions and mediastinal lymph nodes. Wijmans et al. [10] identified three nCLE characteristics for the detection of malignancy in different subtypes of lung tumors and metastatic lymph nodes (dark enlarged pleomorphic cells; dark cell clusters consisting of overlapping cell structures ('dark clumps'); and the continuous movement of a part of the cells in one direction ('directional streaming')). In this study, nCLE accuracy for detecting malignancy was **90% in tumors** and **89% in metastatic lymph nodes**. Both **inter-observer agreement (k=0.68)** and **intra-observer agreement (k=0.70)** were **substantial.** To our best knowledge, data comparing PPN diagnostic yield of Robotic-nCLE-guided TBNA for lung cancer diagnosis with conventional Robotic-guided TBNA does not exist.

In the present study, we aim to compare the diagnostic yield of Robotic-nCLE-guided TBNA for lung cancer diagnosis to conventional Robotic-guided TBNA using the final diagnosis (histology or follow-up) as the standard reference. Furthermore, we will create an image atlas of the characteristics of nCLE-images in PPNs. Also, we aim to describe tumor types based on in-vivo characteristics. Improved characterization of distal lung lesions might lead to improved and quicker cancer diagnosis and patient care.

Background: nCLE imaging of PPNs under bronchoscopic guidance is feasible and safe.

Primary objective:
To compare the first-pass diagnostic yield of the sequential needle passes (ROSE) between the Robotic-nCLE-guided TBNA arm and the Robotic- guided TBNA arm in PPNs.

Primary endpoint:
Increase of the first-pass diagnostic yield by 20%, from 70% to 90%, between Robotic-nCLE-guided TBNA arm and the Robotic- guided TBNA arm in PPNs.

Secondary objectives:

1. To compare the per-patient diagnostic yield (cumulative pass diagnostic yield: cumulative number of passes until five cumulated passes) of Robotic-nCLE-guided TBNA arm to that of Robotic-guided TBNA arm in PPNs.
2. To compare the proportion of patients with lung cancer treatment in the Robotic-nCLE-guided TBNA arm to that of the Robotic-guided TBNA arm in PPNs.
3. To compare the proportion of patients with follow-up (video-assisted thoracoscopic surgery (VATS) or TTNA or TBNA procedures) in the Robotic-nCLE-guided TBNA arm to that of the Robotic-guided TBNA arm in PPNs.
4. To compare the number of passes needed to obtain a final diagnosis of Robotic-nCLE-guided TBNA to that of Robotic-guided TBNA in PPNs.
5. To assess the diagnostic performance (sensitivity, specificity, PPV, NPV, accuracy) of the sequential cumulative nCLE passes and the sequential cumulative ROSE passes using the final diagnosis as a reference.
6. Feasibility: obtain adequate CLE video footage in >80% of the PPN punctures.
7. To assess the safety of nCLE imaging, as defined by:
    a. The number and frequency of all AE/SAE from the start of the procedure until end of 12-month follow-up;
    b. The number and frequency of nCLE procedure-related AE/SAE from the start of the procedure until end of 12-month follow-up.
8. To assess the reproducibility of nCLE criteria with regard to the reference standard. Three nCLE characteristics for the detection of malignancy were identified during Wijmans et al. study [10]:
    a. Dark enlarged pleomorphic cells;
    b. Dark cell clusters consist of overlapping cell structures ('dark clumps') and
    c. Continuous movement of the cells in one direction ('directional streaming').
9. To create an nCLE image atlas for malignant characteristics in PPNs.

Study design: Randomized, controlled, interventional, comparative prospective multi-center study. A maximum of 118 patients with (suspected) PPN will be enrolled (59 in each arm, Robotic-guided TBNA versus the Robotic-nCLE-guided TBNA). All of the enrolled patients will have an indication for tissue sampling in a PPN based on standard (PET-) CT imaging.

Study population:
<u>To be eligible to participate in this study, a study subject must meet all of the following criteria:</u>

- ≥21 years of age
- Suspected PPN
- Nodule size in CT between 8 mm and 30 mm (largest dimension)

- Ability to understand and willingness to sign a written informed consent and HIPAA consent document

Subjects meeting at least one of the following criteria will be excluded from the study

- Inability or non-willingness to provide informed consent
- Failure to comply with the study protocol
- Patients with known allergy for fluorescein or risk factors for an allergic reaction
- Use of beta-blocker within 24 hours before the start of the bronchoscopic procedure
- Possibly pregnant, pregnant or breastfeeding women
- Patients with hemodynamic instability
- Patients with refractory hypoxemia
- Patients with a therapeutic anticoagulant that cannot be held for an appropriate interval before the procedure
- Patients who are unable to tolerate general anesthesia according to the anesthesiologist
- Patient undergoing chemotherapy

Intervention: In patients scheduled for a standard bronchoscopic procedure (Robotic-guided TBNA) with cytological aspirations of the PPN, we will perform nCLE imaging during the bronchoscopy just before cytology.

Nature and extent of the burden and risks associated with participation, benefit, and group relatedness

A participating patient might not benefit from participating in this study. However, future patients might benefit from improved lung cancer diagnostics based on these study findings. nCLE combined with EUS has proven to be safe in the field of gastroenterology, lung tumors, and analysis of intrathoracic lymph nodes [10–12]. This minimally invasive imaging technique provides real-time information at a microscopic level about tissues before aspiration. There is little burden related to study participation: before the beginning of the bronchoscopic procedure, fluorescein will be administered intravenously through the existing venous entrance necessary for the sedation. Fluorescein is a standard used dye in hospitals. Adverse reactions are rare (1.1%) and mild in character [13]. According to the literature, in patients without a known allergy to fluorescein, beta-blocker use, or pregnancy, the use of fluorescein is safe. In 2010, Wallace et al. [14] published a retrospective study of all confocal laser endomicroscopy procedures performed between January 2003 and November 2008, or 2,272 procedures (performed under protocol only), and no serious adverse events related to fluorescein injection were identified.

nCLE acquisitions will be performed during Robotic bronchoscopic workup followed by conventional cytological aspirations (routine workup), without aspirations or biopsies for research purposes. The estimated prolonged endoscopy time due to study participation is a maximum of 10 minutes. The patient will not be aware of this as the procedure will be performed under midazolam (fentanyl) or Propofol sedation (following standard practice). Based on previous studies, adverse events are not expected; FNA combined with nCLE is reported to be safe, easy to perform, and a little time-consuming, without adverse events [10,11,15–17]. In conclusion, we believe that the burden and risks associated with study participation are low.

PROTOCOL SIGNATURE PAGE

Needle based confocal laser endomicroscopy for lung cancer diagnosis: A Randomized Controlled Trial

Protocol No.

REVISION 01

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision and my hospital ethics committee/institutional review board (EC/IRB). I will discuss this material with them and ensure they are fully informed regarding the Cellvizio CLE system and the conduct of the study according to this protocol, applicable laws, and applicable confidentiality and patient privacy requirements, US regulatory requirements, including hospital EC/IRB requirements.

Clinical Site Name: _____

Site Investigator: (*Name & Title)*

Signed: _____ Date: _____

2. Objectives

Background

The diagnostic yield is one of the main factors in evaluating the clinical effectiveness of an interventional medical technique, such as diagnostic bronchoscopy, intended to biopsy suspicious lesions.
The purpose of the diagnostic yield value is to determine the probability that the selected medical technology will provide the information needed to establish a definitive diagnosis [18].
As most lung cancers are diagnosed at an advanced stage with meager survival rates, it is essential to achieve a definitive diagnosis at an early stage to increase the chances of successful treatment. Since the goal is to reach a definitive decision on the nature and type of lesion that will dictate the next steps in a patient's medical treatment, the debate arises when a non-malignant result from the biopsy is received. The most accurate and conservative approach for non-malignant results would be to accommodate a 12-month follow-up to confirm or dismiss the malignancy [19]. However, a recent publication by the Fleischner Society has indicated that a shorter follow-up period might be more appropriate for selected subjects, depending on risk factors, nodule type, nodule morphology, and accuracy of measurements, suggesting that a follow-up period of 12 months would be sufficient [20,21].
The biopsy results can be classified into three categories: positive results, including malignant or benign lesions confirmed by a follow-up period, or negative results, including [18] intermediate histological samples, such as chronic inflammation, organizing pneumonia, or atypical cells without sufficient features to ascertain malignancy; or [22] indeterminate samples, such as normal lung tissue or insufficient sample size [6]. The tissue samples that produce intermediate and indeterminate samples should be further evaluated according to recommended guidelines for the management of pulmonary nodules.
In this study, the diagnostic yield of the bronchoscopic procedure (Robotic-guided TBNA or Robotic-nCLE-guided TBNA) will be defined as the proportion of patients in whom the bronchoscopic procedure yield a definitive diagnosis out of the total number of patients that have received the diagnostic procedure. This value is affected by the lesions' sizes, locations, and the existence of a bronchus sign or an airway leading directly into the lesion [23].

Primary objective:

To compare the first-pass diagnostic yield of the sequential needle passes (ROSE) between the Robotic-nCLE-guided TBNA arm and the Robotic- guided arm in PPNs.

Secondary objectives:

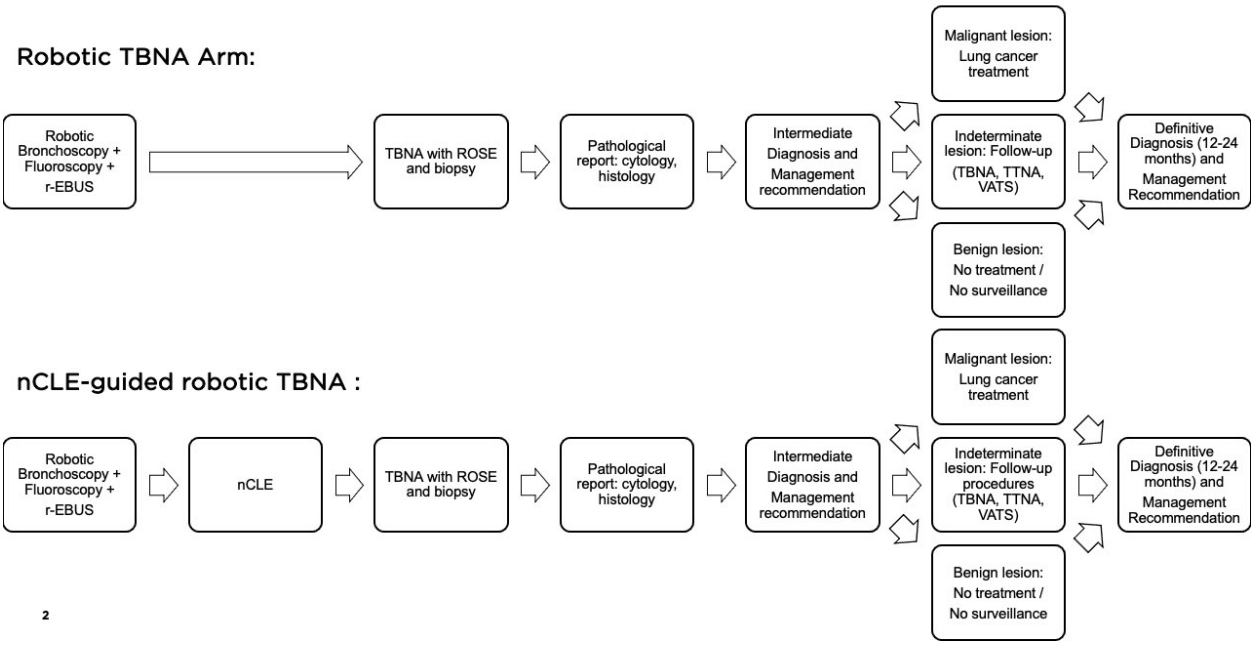
1. To compare the per-patient diagnostic yield (cumulative pass diagnostic yield: cumulative number of passes until five cumulated passes) of Robotic-nCLE-guided TBNA to that of Robotic-guided TBNA in PPNs.
2. To compare the proportion of patients with lung cancer treatment in the Robotic-nCLE-guided TBNA arm to that of the Robotic-guided TBNA arm in PPNs.
3. To compare the proportion of patients with follow-up (video-assisted thoracoscopic surgery (VATS) or TTNA or TBNA procedures in the Robotic-nCLE-guided TBNA arm to that of the Robotic-guided TBNA arm in PPNs.
4. To compare the number of passes needed to obtain a final diagnosis of Robotic-nCLE-guided TBNA to that of Robotic-guided TBNA in PPNs.
5. To assess the diagnostic performance (sensitivity, specificity, PPV, NPV, accuracy) of the sequential cumulative nCLE passes and the sequential cumulative ROSE passes using the final diagnosis as a reference.
6. To assess the feasibility by obtaining adequate CLE video footage in >80% of the PPN punctures.
7. To assess the safety of nCLE imaging, as defined by:

   a. The number and frequency of all AE/SAE from the start of the procedure until end of 12-month follow-up
   b. The number and frequency of nCLE procedure-related AE/SAE from the start of the procedure until end of 12-month follow-up
8. To assess the reproducibility of nCLE criteria to the reference standard. Three nCLE characteristics for the detection of malignancy were identified during Wijmans et al. study [10]:
   a. Dark enlarged pleomorphic cells;
   b. Dark cell clusters consist of overlapping cell structures ('dark clumps') and
   c. Continuous movement of the cells in one direction ('directional streaming').
9. To create an nCLE image atlas for malignant characteristics in PPNs.

3. Study Design

This is a randomized, controlled, interventional, comparative prospective multi-center study. In total, a maximum of 118 consecutive patients with a (suspected) PPN who are referred for a diagnostic bronchoscopic (Robotic-guided TBNA) procedure will be considered for enrollment. The estimated duration of the study is 24 months. Patients will be consecutively recruited and randomly assigned to undergo Robotic-guided TBNA (n=59) or Robotic-nCLE-guided TBNA (n=59).

Only patients in the nCLE arm will receive intravenously administered fluorescein before the start of the Robotic procedure. During the nCLE procedure, an 18G needle enters the targeted tissue, followed by an nCLE AQ-Flex™ 19N Confocal Miniprobe™ (Mauna Kea Technologies, France) through the biopsy needle, to obtain real-time imaging. nCLE imaging will be performed in the PPNs with a total estimated time for research measurements of 10 minutes. nCLE imaging will be performed for each sequential TBNA pass/sampling within the lung nodule lesion and each TBNA path cytopathological result will be correlated with the nCLE sequence acquired for that TBNA pass. TBNA sampling will end when sufficient diagnostic material will be found with ROSE (as assessed by the cytopathologist) or when the physician performing the procedure judges that the number of samplings/passes is adequate. ROSE will be performed by a cytopathologist.



4. Study Population

Population (base)

Patients with a PPN referred for diagnostic bronchoscopic/endosonographic workup tissue sampling based on standard (PET-) CT imaging

Inclusion criteria

To be eligible to participate in this study, a study subject must meet all of the following criteria:

- ≥21 years of age
- Suspected PPN
- Nodule size in CT between 8 mm and 30 mm (largest dimension)
- Ability to understand and willingness to sign a written informed consent and HIPAA consent document

Exclusion criteria

- Inability or non-willingness to provide informed consent
- Failure to comply with the study protocol
- Patients with known allergy for fluorescein or risk factors for an allergic reaction
- Use of beta-blocker within 24 hours before the start of the bronchoscopic procedure
- Possibly pregnant, pregnant or breastfeeding women
- Patients with hemodynamic instability
- Patients with refractory hypoxemia
- Patients with a therapeutic anticoagulant that cannot be held for an appropriate interval before the procedure
- Patients who are unable to tolerate general anesthesia according to the anesthesiologist
- Patient undergoing chemotherapy

Intra-operative Exclusion/Stopping Criteria:

- The lesions are unable to be localized/confirmed by bronchoscopy
- The procedure will be terminated if the patient develops a significant procedural complication as determined by the treating physician
- The procedure will be terminated if the patient develops hemodynamic instability as determined by the treating physician

Sample size calculation

A bronchoscopic TBNA (Robotic-guided TBNA or Robotic-nCLE-guided TBNA) will be regarded as successful when histological confirmation of the diagnosis is achieved and matches the final diagnosis. If additional TBNA passes or tests, e.g., further bronchoscopies, surgery, etc., are needed to establish the diagnosis, the TBNA pass will be regarded as non-diagnostic.

We will calculate TBNA diagnostic yield for the cumulative number of passes until five cumulated passes for both the Robotic-guided TBNA and Robotic-nCLE-guided TBNA arms. It is anticipated that sequential passes at a target site will result in stepwise yield increments to a plateau. Every needle pass at a location will be reported separately and entered into a database to provide yields after each sequential pass.

Proportional data will be compared using a Chi-squared test of contingency tables or Fisher's exact test on 2x2 contingency tables in the case of very small counts (<5).

First- and cumulative-pass diagnostic yields:

Diagnostic yields for the different cumulative passes are defined as follows:

1. First-pass diagnostic yield is calculated as the proportion of patients for which first-pass TBNA during the bronchoscopic procedure yielded a definitive diagnosis out of the total number of patients that have received a diagnostic procedure.
2. First-two-passes cumulative diagnostic yield is calculated as the proportion of patients for which first-two-passes cumulative TBNA pass during the bronchoscopic procedure yielded a definitive diagnosis out of the total number of patients that have received a diagnostic procedure.
3. First-three-passes cumulative diagnostic yield is calculated as the proportion of patients for which first-three-passes cumulative TBNA pass during the bronchoscopic procedure yielded a definitive diagnosis out of the total number of patients that have received a diagnostic procedure.
4. Etc…. until the fifth pass.

The number of patients has been calculated to demonstrate an increase of the TBNA first-pass diagnostic yield by 20%, from 70% to 90%, between the Robotic-guided TBNA and the Robotic-nCLE-guided TBNA, with a statistical power of 80% with a 2-sided significance level of 0.05. A group size of 59 patients per arm has been derived. By including 59 patients in each arm, we expect to image at least 29 malignant nodules (prevalence of 0.5) in each arm.
The study will enroll 118 consecutive patients randomly assigned 1:1 to Robotic-guided TBNA arm or Robotic-nCLE-guided TBNA arm.

5. Treatment of Subjects

Investigational product/treatment

In recent years, confocal laser endomicroscopy (CLE) has emerged as a novel technique that enables in vivo microscopic analysis during ongoing endoscopy [24]. CLE is based on tissue illumination with a low-power laser with subsequent detection of the fluorescence light reflected from the tissue. It is a contrast-based technique, the most widely used agent being the intravenously administered fluorescein. The probe of the endomicroscopy system consists of a flexible catheter probe representing a bundle of optical fibers linked to a micro-objective, a laser scanning unit, and the control and acquisition software (Cellvizio® I.V.E system, Mauna Kea Technologies, France). The principle of the technique is based on a laser beam of the defined wavelength being focused towards the targeted tissue, and the recaptured signal is displayed as 'optical biopsies' in a single horizontal plane.

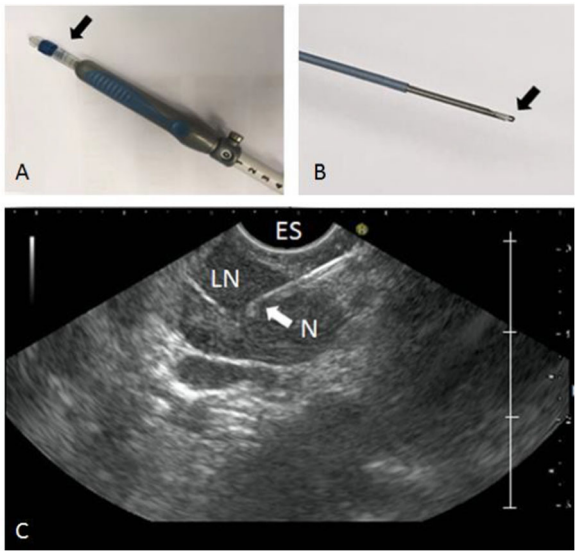


Figure 1: A- Locking of the CLE probe to the aspiration needle. B- CLE probe inside endosonography needle. C- Endosonography images of n-CLE measurements (white arrow) inside a lymph node (LN) (Wijmans/Annema et al. [10]*).*

The advantage of nCLE is that it can provide real-time information on a microscopic level of tissues within a solid organ. Therefore, immediate validation of a biopsy location and outcome is possible. Since a needle is used to deliver the optical imaging probes, cell seeding is probably minimal since no tissue is removed by force or taken out of position. For the nCLE procedure, the Confocal Miniprobe™ (AQ-Flex™ 19 N Confocal Miniprobe™, Mauna Kea Technologies, France, was cleared by the FDA for TBNA procedures (K183640)) has been recently designed. This probe is small enough to fit through an 18G aspiration needle, has a resolution of 3.5 µm, and has a penetration depth of 40-70 µm.

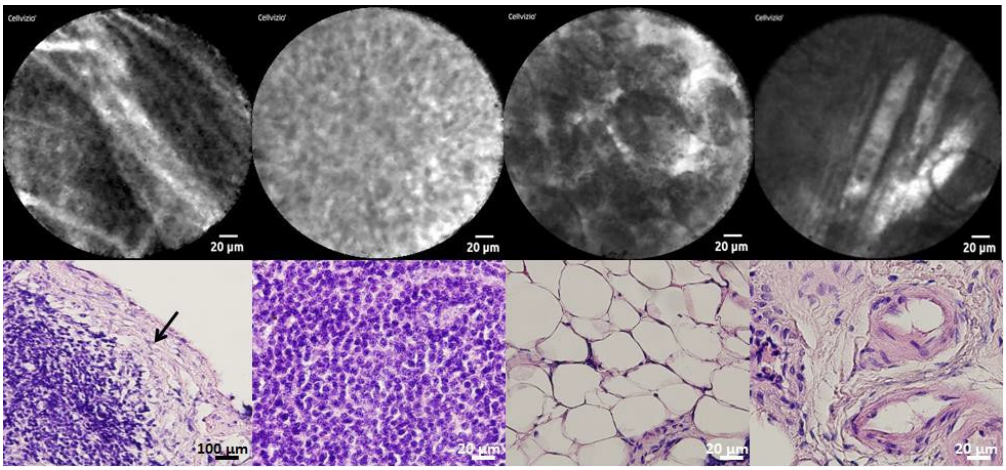


Figure 2: Image (Wijmans/Annema et al. [10]) of EUS guided nCLE in lung cancer patients. nCLE imaging on top with corresponding cytology (lymph node capsule, stroma, vet, vasculature – from left to right)

Summary of known and potential risks and benefits

Fluorescein is a standard used dye in hospitals, where it is used primarily as a contrast dye to visualize the ocular vascular network. In patients without a known allergy to fluorescein, beta-blocker use, or pregnancy, the use of fluorescein is safe. In an extensive prospective review of 11,898 patients undergoing an angiogram with intravenous administration of fluorescein, 132 adverse events were recorded (1.1%) with the most typical reactions being nausea and vomiting [13]. No severe adverse reactions or deaths occurred. Adverse reactions on intravenous administration are rare and primarily mild reactions (Table 1). Fluorescein is widely used in studies with CLE, where it has proved to be safe and easy to perform [10–12].

Table 1. Different types of adverse reactions

Classification	Types of adverse reaction	No. of adverse reactions (%)
Mild	Nausea	87 (0.7)
	Vomiting	47 (0.4)
	Dizziness	34 (0.3)
Moderate	Faint	17 (0.1)
	Localized reactions	14 (0.1)
	Urticaria	28 (0.2)
Severe	Seizure	0 (0.0)
	Myocardial infarction	0 (0.0)
	Anaphylactic attack	0 (0.0)
Death		0 (0.0)

Table 1 Type and number of adverse reactions after intravenous administration of fluorescein in patients undergoing fluorescein angiography (n=11,898). (Kwan et al. 2006)

In 2010, Wallace et al. [14] published a retrospective study of all CLE procedures performed between January 2003 and November 2008, or 2,272 procedures (performed under protocol only), and no serious adverse events related to fluorescein injection were identified.
Most recently, in January 2018, Cellvizio® was recommended by the Korean National Health Agency (NECA, New Health Technology Assessment Committee) and obtained the Innovative Technology status. This recommendation was based on a systematic review of the literature on CLE and allowed the NECA to recognize endomicroscopy as "a safe and effective method that can help identify cancerous lesions and target biopsies for patients with suspected dysplasia in the esophagus, stomach, and bile duct stenosis." In this evaluation (nHTA Report / Submission No. HTA-2018-16 *Abstract (English)* : https://nhta.neca.re.kr/nhta/publication/u0601vfd.ecg?pub_seq=572&file_kind=E *Full text* : https://nhta.neca.re.kr/nhta/publication/u0601vfd.ecg?pub_seq=572&file_kind=F), the NECA evaluated the safety of CLE by examining 17 articles to identify complications and side effects associated with endomicroscopy with fluorescein injection. From this review, the NECA subcommittee evaluating the safety of endomicroscopy noted:

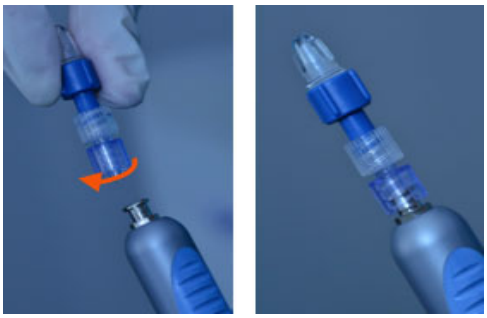
- that the complications or side effects identified in this literature review showed no difference between an endoscopy procedure without endomicroscopy and an endoscopy with endomicroscopy and fluorescein injection, and,
- most studies have not reported any serious side effects or complications.

The NECA subcommittee, therefore, concluded that the safety of the technology is of an acceptable level. So far, all the studies that investigated nCLE use showed that no adverse events occurred. Most studies investigated nCLE combined with EUS, and only one case report examined the Robotic guided nCLE and reported no adverse events.

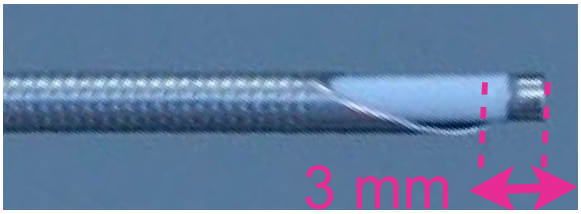
Study procedures

Preparation before the procedure

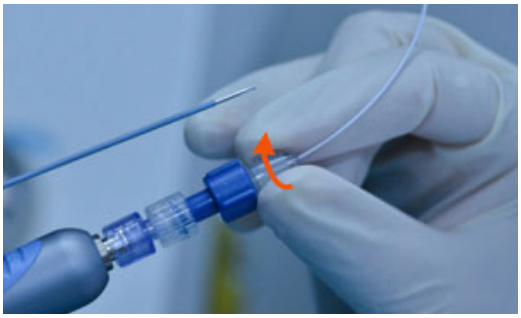
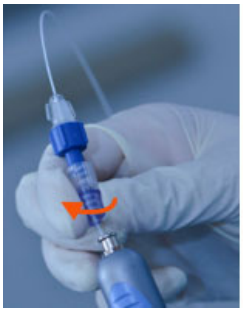
Before beginning the procedure, a locking device is attached to the proximal end of a standard TBNA puncture needle (Bronchus 18G needle). To do so, the metallic stylet originally in the needle must be removed.

1. Removal of the stylet from the needle	2. Locking of the locking device onto the proximal needle hub
	

The AQ-Flex™ 19 N Confocal Miniprobe™ is then inserted inside the needle through the locking device, taking the place of the stylet. Once the tip of the Confocal Miniprobe™ extends out from the needle's bevel, the Confocal Miniprobe™'s insertion distance in the needle is slightly adjusted to be in the correct position (e.g., the Confocal Miniprobe™'s tip is almost perfectly aligned with the needle's tip). More precisely, the bevel of the needle must be in contact only with the 3mm long distal metallic tip of the Confocal Miniprobe™ (called a ferrule). This ensures that the needle will not damage the probe. By doing so, the Confocal Miniprobe™ is allowed to protrude from the distal end of the needle by about 3mm (the needle bevel has approximately a 4mm length). Reference photograph included in Step 4 below.

3. Fully open the locking device and insert the AQ-Flex™ 19 N Confocal Miniprobe™ inside the needle through the locking device	4. Stop the Confocal Miniprobe™ insertion once it has reached the safe position: the Confocal Miniprobe ™ must protrude from the needle distal bevel tip by no more than 3 mm
 	

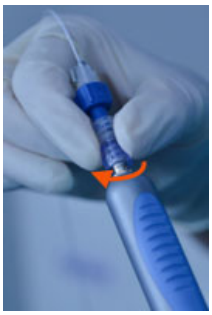
Once the AQ-Flex™ 19 N Confocal Miniprobe™ is correctly positioned inside the needle, the locking device is locked on the Confocal Miniprobe™ and should not be unlocked until the end of the procedure. The Confocal Miniprobe™ can now be fully withdrawn inside the needle for the insertion inside the endoscope and the ultrasound-guided puncture and TBNA needle positioning. Retracting the Confocal Miniprobe™ about 2cm inside the needle both protects it during TBNA needle positioning and avoids interfering with the TBNA needle positioning procedure. To do this, the purple screw of the locking device must be unlocked, and the Confocal Miniprobe™ slightly retracted inside the needle.

5. Twist the locking device firmly onto the Confocal Miniprobe™ to secure it in the safe position	6. Untwist the locking device (still tightened to the probe) from the needle until the probe tip reaches the base of the needle bevel Retract the needle inside the sheath
	 

Steps during the procedure

nCLE requires the administration of exogenous fluorescent contrast. Minutes before CLE positioning, the patient will be administered 2.5 mL of 10% fluorescein IV. A repeat dosage of 2.5 ml of 10% fluorescein can be provided after 15-30 minutes to a maximum dosage of 5 ml of 10% fluorescein in total. Patients will be monitored for allergic reactions, including anaphylaxis. Fluorescein Sodium can be used as a contrast agent with Cellvizio® I.V.E system with Confocal Miniprobes™ without change of formulation, mode of action, approved dose, or route of administration. Refer to the indications for using the fluorescein to perform the injection or to chapter 1: "Fluorescein Angiography: Basic Principles and Interpretation" of Ryan et al. book on Optical Imaging Technologies [25].

The target area will then be identified on the ultrasound and/or fluoroscopy monitor and accessed in the usual manner per standard of care. Once the nodule is localized, an 18G needle with the Confocal Miniprobe™ inside will be introduced through the bronchoscope's working channel and advanced into a lung nodule. After the puncture, the AQ-Flex™ 19 N Confocal Miniprobe™ will then be advanced through the needle into the nodule. Imaging will take place, which will be evaluated by the physician performing the procedure. The on-site image interpretation will be recorded. The AQ-Flex™ 19 N Confocal Miniprobe™ will be removed, and tissue will be aspirated at the imaging site from the tip of the needle that is still in place. ROSE will be performed by the cytology team per standard of care. If no lesional tissue is identified, then the robot may be repositioned for additional needle passes. For each needle pass, a separate CLE movie will be recorded. Multiple needle passes and nCLE imaging may occur, specifically if no tumor is identified on ROSE. In the case of re-localization of the needle, nCLE imaging may be repeated before further needle aspirations. Duration will be recorded after each pass. Chest ultrasound or fluoroscopy will be performed to evaluate for pneumothorax.

7. Puncture the identified target lesion with the needle per standard of care and usual procedure (the Confocal Miniprobe™ is pre-loaded but retracted)	8. Maintain the needle as straight as possible and parallel to the working channel of the scope. (to limit the retraction of the probe within the working channel) Twist the locking device and the probe back onto the needle
	

Microscopic, real-time image sequences are recorded, and cellular structures are visualized as desired. If at any time the ~3 mm Confocal Miniprobe™ protrusion interferes with the TBNA procedure, the Confocal Miniprobe™ can be pulled back into the needle by unlocking the purple screw to change directions, and then advanced again and locked. As long as the white screw remains locked, the Confocal Miniprobe™ cannot be advanced further than ~ 3mm beyond the distal needle tip and will not be damaged.

Retrieval of the probe

When the physician has completed imaging tissue with the Confocal Miniprobe™, it can be removed from the needle by unlocking the purple screw and extracting the probe. The locking device is a single-use device and is discarded after the procedure.

9. After imaging, extract the Confocal Miniprobe™ and locking device assembly from the needle	10. Remove the locking device from the Confocal Miniprobe™ and discard it

Further biopsies may then be performed with a 20 g needle, forceps, or brush per standard of care at the discretion of the bronchoscopist. After the biopsies, a bronchial-alveolar lavage may be performed per standard of care at the discretion of the bronchoscopist. Endobronchial ultrasound (EBUS) with lymph node biopsies will also be performed when indicated as part of the standard of care.
If a second lesion requires a biopsy, the bronchoscope will be withdrawn, and the chest will be imaged by fluoroscopy or chest ultrasound to rule out pneumothorax per standard of care. The bronchoscope can then be re-inserted, followed by navigation to the second lesion. However, only one lesion will be imaged with nCLE per patient.

After the Cellvizio® procedure

After removal of the Confocal Miniprobe™, the tissue can be routinely aspirated per the standard TBNA procedure and analyzed for chemistry, cytology, and/or tumor markers. nCLE imaging will not interfere with tissue / fluid specimen collection during the remainder of the conventional TBNA procedure.

Steps of standard - and study (in bold) procedures:

1. Patients will be sedated following standard procedure.
2. **(research-purposes) After starting the procedure, we will administer 2.5 ml of fluorescein 10% intravenously to visualize the CLE images only in the patients in the Robotic-nCLE-guided TBNA arm).**
3. Lidocaine 10ml will be orally administered to provide local throat and tracheal anesthesia
4. The bronchoscope will be introduced into the trachea.
5. After target localization (lung tumor), a guiding needle (FlexNeedle 18G, Broncus Medical) will be introduced in the working channel of the scope and on the placement of the endoscope within the bronchus in proximity to the target, the needle is introduced through the bronchial wall and into the lung tumor using fluoroscopy and r-EBUS guidance.
6. **(research-purposes) nCLE acquisitions will be obtained (only in patients in the ROBOTIC-nCLE-guided TBNA arm) by fitting the optical fiber into the TBNA needle and introducing it in the target lesions under fluoroscopy control.**
7. After the nCLE data is acquired and the probe is retrieved, cytological samples will be conducted following a standard protocol (ROSE).
8. Steps 6 and 7 will be performed for each pass.
9. The bronchoscope will be retrieved.

On completion of each procedure, the patients will be extubated and transferred to the recovery area. All patients will be monitored until discharge per standard institutional clinical protocol. This includes a chest x-ray post-procedure to determine whether the patient has suffered a pneumothorax. In the case of a pneumothorax, the complication will be treated with the institutional standard of care. The risks associated with the procedure are no different from a routine bronchoscopy and biopsy. There is a small increased risk

that a patient may have an allergic reaction to fluorescein. Again, this complication will be treated with the institutional standard of care.

nCLE analysis

Each nCLE-video will be analyzed in a blinded fashion to evaluate nCLE lung nodule and lymph node characteristics. The results of the nCLE video reviews will be compared to the corresponding cytological or histopathological results.
Three blinded raters will review nCLE videos for image quality, lung lesion detection and CLE characteristics.

Training session for CLE raters

nCLE raters with various backgrounds will be trained in the nCLE lung cancer criteria using powerpoint slides, hand-drawn schematics and a preselected set of training videos that had not been used previously. Training slides with hand-drawn nCLE lung cancer criteria schematics will also be provided.

Intra-procedure Interpretation of nCLE

During the nCLE procedure the bronchoscopist will judge quality of the image, lung lesion detection, and presence of malignancy. The bronchoscopist will be trained in nCLE image evaluation.

Validation session

The trained raters will evaluate nCLE videos of all 59 patients as a randomized data-set and be blinded to the patient history and cytologic/pathologic diagnosis. Videos used for the training session will be excluded. The results of the nCLE video reviews will be compared to the corresponding cytological or histopathological results. The first session will be performed to assess the nCLE characteristics, image quality and confidence and will be used to calculate the inter-observer agreement (IOA) between the raters for detecting malignancies based on nCLE videos. The second session will be performed by the same group of raters, after a 2-week wash-out period, using the same set nCLE videos in a different order to assess the intra-observer reliability (IOR).

Pathological examination

During bronchoscopy, the sites suspected of malignancy will be evaluated with nCLE. In the case of positive tumor cytology (nodes/tumor) or histology (tumor), this will be the reference standard for the nCLE images. In the case of tumor negative cytology or histology, additional surgical/pathological staging or clinical/radiological follow-up will be available.

Cytological aspiration (standard procedure)

During the bronchoscopic procedure, the cytological aspirations will be evaluated on-site by a cytopathologist (ROSE). The appearance of cancer cells will be reported and shown on screen to the endoscopist. Definitive diagnosis of the sample follows after re-evaluation by a staff member of the pathological department. No additional samples will be taken for the research protocol, nor will the research protocol interfere with the pathological diagnostic protocol.

Histological evaluation

Suppose a patient suspected of lung cancer has a negative result of the bronchoscopic procedure. In that case, a surgical procedure (mediastinoscopy or lobectomy/pneumectomy with lymph node dissection) could follow to obtain tissue. This surgical procedure will be performed regardless of the result of nCLE imaging. If a surgical procedure is indicated, we will collect the histological images to compare with nCLE imaging. This study will not interfere with the standard cytology or the standard histology process. Digital copies of the cytological and histological slides of the measured nodules will be obtained and compared with the frames of the nCLE sequences.

Final Diagnosis or Definitive Diagnosis

For this 12-month analysis, pathology results of Robotic-aided biopsy samples that will be diagnostic of a non-malignant condition or indeterminate will be referred to as negative for malignancy or negative for brevity. Follow-up will be conducted to determine the final diagnosis (malignant or non-malignant). All cases will be followed according to the practitioner's judgment and standard of care testing (e.g., surgical tissue biopsy, repeat Robotic, CT-guided transthoracic needle biopsy or aspiration (TTNA), serial CT imaging, and lung health visits). Cases with subsequent diagnostic tests confirming a non-malignant diagnosis or without lesion progression on radiographic follow-up will be considered true-negative at 12 months, consistent with prior Robotic studies. If the follow-up diagnostics reveal malignancy or lesion growth on repeat imaging with appropriate follow-up diagnostic testing, this will be considered a false-negative. The following will also be regarded as false-negative: death due to lung cancer within 12 months; treatment without a confirmed diagnosis; and new diagnosis of cancer in the lung from any site (including non-index lesions or lymph nodes diagnosed as malignant by linear bronchoscopy during or after the index procedure).

Twelve-month diagnostic yield will be calculated per subject as the rate of true-positives (for malignancy) plus true negatives (for malignancy) of all subjects with attempted lung lesion biopsies.

Adverse Events

Definitions

Adverse Events (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, treatment, or procedure regardless of whether it is considered related to the medical treatment or procedure *(NCI CTEP Guidelines March 28, 2011)*.
Serious Adverse Event (SAE) is an AE that is fatal or life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours), persistent or significant incapacity, or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly. Important medical events that may not result in death but may require hospitalization may be considered an SAE, based upon appropriate medical judgment. A 'life-threatening' adverse event places the patient at immediate risk of death in the judgment of the investigator or sponsor.

Severity Rating

The investigator will evaluate the severity of each adverse event. NCI Common Terminology Criteria for Adverse Events (CTCAE v.5.0: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5.0.xlsx) or study

specific toxicity tables provided in the protocol define severity. If not included in CTCAE v.5.0, severity is expressed in numerical grade using the following definitions:

- Grade 1: Mild-asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate-minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
- Grade 3: Severe-severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE

Attribution / Relationship to nCLE or Standard robotic procedure

- Definite – related
- Probable – likely related
- Possible – maybe related
- Unlikely – doubtfully related
- Unrelated – clearly not related

Possible Adverse Events

This is a minimal risk clinical study that poses no additional risk than the standard of care biopsy.

- Hypoxia
- Respiratory failure
- Bleeding at the puncture site
- Pneumothorax
- Infection including pneumonia, bronchitis, or mediastinitis
- Sore throat
- Fever

There is a small risk of allergic reaction to fluorescein:

- Anaphylaxis/hypersensitivity reactions

AEs and SAEs recording and reporting

Upon identification of an AE or SAE, the site investigator will utilize the above definitions to properly classify attribution and expectedness of the event. All SAEs must be recorded for each event.

All AEs and SAEs will be recorded in the “AE case report forms” (CRF) and in progress reports with details about the grade and attribution of each episode, action taken with respect to the study drug, and the patient’s outcome will be recorded in the CRF. All events will be recorded on case report forms for the duration of the study until they resolve.

The investigator/ site is responsible to report all SAEs that occur on or after the first day of study treatment to the sponsor within 24 hours of becoming aware of the event. It may be necessary to submit a final SAE report if the initial report did not have the complete information. All SAEs will be recorded on the FDA MedWatch form 3500a. The attribution and expectedness must be recorded on the MedWatch form. If this information is not available at the time of initial reporting, a final report must be documented with attribution and expectedness. It may be necessary to submit follow up reports to the Sponsor should the event require further investigation. All subsequent SAEs must be recorded for up to 5 after the last treatment. EachinvestigatorisresponsibletoreportallAEs/SAEstotheirlocalIRBfollowing guidelines set by that IRB.

Follow-up of adverse events

After the initial AE/SAE report, the Investigator is required to follow-up on each subject who experienced the AE at subsequent visits or contacts.

All patients will be evaluated for adverse events (AEs) and serious adverse events (SAEs) at the procedure day, 7 days, and 30 days post-procedure. On the day of the procedure the principal investigator will evaluate possible signs and symptoms such as shortness of breath, chest pain, dizziness, low blood oxygen saturation (≥ 90%), hemoptysis (cough bright red blood) and fever (> 37.3 C). At Day 7(post procedure), a study team will contact the patient by the phone and ask about any symptoms of shortness of breath, chest pain, cough with yellow or greenish sputum, hemoptysis (cough bright red blood) and fever. At Day 30, the study team will review patient's medical records and collect data of medications, doctor visits (primary care and specialist visits), procedures and other medical records information. In addition, patients will be instructed to call in with concerns and/or present to the research staff or Principal Investigator. . The study staff will be recording and reporting in the electronic medical record all the adverse events with an attribution of possibly, probably, or definitely related to the study intervention.

Depending on the event, follow up may require additional tests or medical procedures as indicated and/or referral to the general physician or a medical specialist.

Stopping rules based on safety considerations

Investigators will be responsible for data and safety monitoring for this trial. Investigators will discuss with the statistician and/or the study Sponsor if higher than expected rate of adverse events of unacceptable grades are documented. If the rate is more than 10 % of the patient with bleeding, more than 5% with pneumothorax or respiratory failure and 1 patient with anaphylaxis, the principal investigator will consider stopping the study. Statistician and/or Sponsor, after discussion with the Investigator, may recommend to close the study to IRB if the rate of study related adverse events is between the parameters mentioned previously.

6. Data Collection

Subject demographics

- Age
- Sex
- Ethnicity
- Race

Subject medical history and baseline status

- Prior invasive lung procedures and surgeries
- Lung function and diffusing capacity
- Antithrombotic medication current and prior status, including duration of any discontinuation
- Subject risk factors
- Pre-procedure probability of malignancy

Lesion characteristics

- Size
- Location
- Presence of bronchus sign on computerized tomography (CT)
- Lung zone (peripheral, middle, and proximal thirds)
- Visibility on fluoroscopy (if applicable)
- Positron emission tomography (PET)-positive (yes/no)
- Associated lymphadenopathy
- Distance to closest fissure
- Distance from lesion to pleura
- Pre-procedure probability of malignancy (investigator assessment)

Procedural assessments

- Indication for procedure
- Anesthesia type
- Catheter type
- Procedure duration
- Imaging used (fluoroscopy, PET, radial endobronchial ultrasound [EBUS])
- Ability to successfully navigate to the lesion
- Use of associated tools and type (e.g., access tools, biopsy forceps, cytology brush, aspiration needle)
- Number of lesions biopsied
- Distance between the tip of the locatable guide/nCLE Confocal Miniprobe for each lesion
- Cases in which the operator can successfully navigate to the lung target with Robotic-guidance and Robotic-nCLE guidance, based on investigator self-assessment
- Number of lymph nodes biopsied (if applicable)
- Placement of fiducial markers (if applicable), the type used, indication, and status at follow-up imaging
- Surgical resection, including use of dye marker, the type used, and adequacy for surgical resection
- Diagnosis by both cytologic rapid on-site evaluation (ROSE) and pathology
- Cancer type (primary or metastatic), if applicable
- Cancer stage, if applicable
- Adequacy of sample for molecular testing and mutation type (if applicable)
- Number and type of repeat bronchoscopy procedures or other biopsies
- Other health services (e.g., imaging, transfusion, surgery, emergency room admission, prescriptions) received during admission for the index procedure
- Hospital admission duration
- Adverse events, action taken, relationship to device, and outcome

Follow-up assessment

- All health services (e.g., imaging, transfusion, surgery, emergency room admission, prescriptions) received since the last visit
- Since index procedure, all healthcare services related to lung health (e.g., primary care and specialist visits, hospital, emergency room, oncology, radiology, and pain management).
- All therapeutic and diagnostic procedures and diagnoses related to lung health since the last visit
- Adverse events, action taken, relationship to device, and outcome

- Twelve months follow-up data: repeat diagnostic procedures or new diagnoses on any lung nodule evaluated during the initial bronchoscopic index procedure, as well as healthcare utilization since the bronchoscopic index procedure (including healthcare visits, repeat bronchoscopic procedures, transthoracic biopsy, bronchoscopy, chemotherapy, brachytherapy, radiation therapy, surgical resection, and lymph node dissection).

nCLE image quality:

nCLE image quality for each nodule will be rated by the interventional pulmonologist as:

- **Very poor**: impossibility to observe any structure or criteria
- **Poor**: The observed criteria and structures are hardly recognisable and interpretable
- **Moderate**: Structures and criteria are recognisable and interpretable with close attention, but confidence in the interpretation remains low (may require replaying the sequence frame by frame)
- **Good**: Image quality is sufficient to enable an easy structures and criteria observation and interpretation (may require replaying the sequence frame by frame)
- **Very good**: criteria and structures are recognisable and interpretable very easily

Diagnostic and Therapeutic Management

Lung cancer treatment options are the following:

- **Minimally invasive surgery.** Thoracic surgeons can use minimally invasive techniques when possible, including video-assisted thoracoscopic surgery (VATS) and robotic surgery. Surgeons remove the smallest amount of lung tissue necessary through wedge resection, sleeve lobectomy, and bilobectomy.
- **Highly targeted radiation therapy.** Radiation oncologists have access to the latest technology to carefully shape and precisely deliver radiation to the target. Targeted treatment is available through intensity-modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT), image-guided radiation therapy (IGRT), and proton therapy. These techniques reduce the risk of side effects and improve outcomes.
- **Carefully selected systemic therapy.** Mayo Clinic oncologists have access to various systemic treatments, including chemotherapy, targeted therapy, and immunotherapy. Systemic treatments are selected using many factors, including the genetic makeup of your cancer cells.

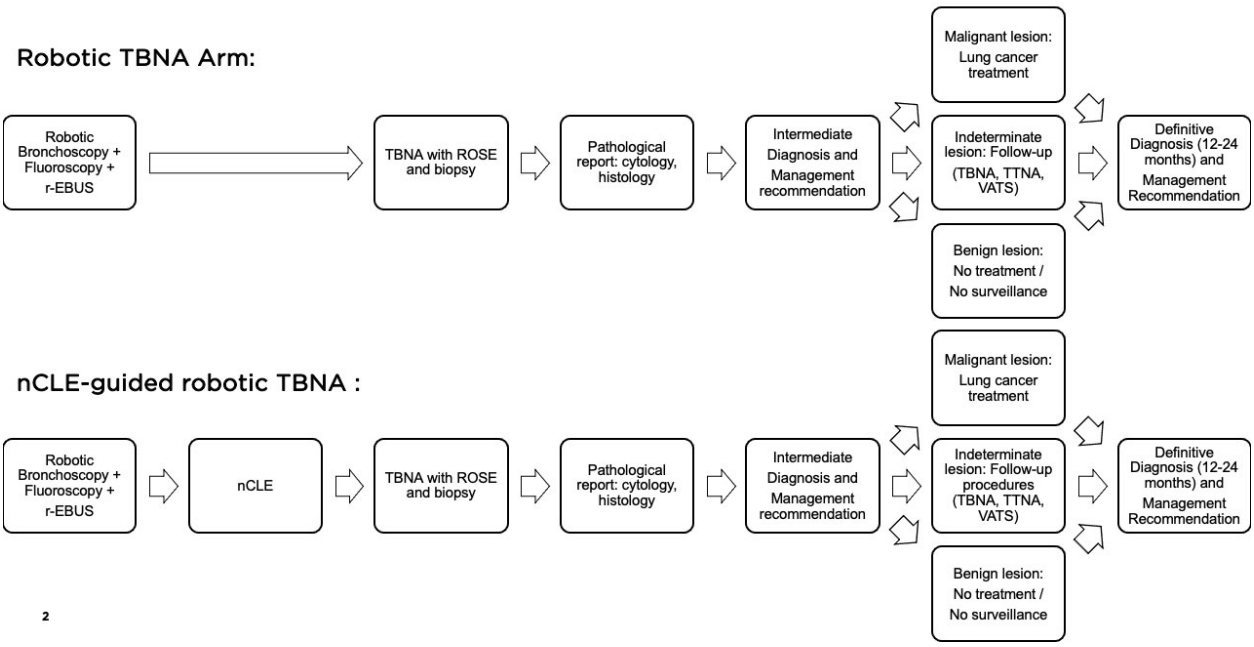


Figure 3: Randomized controlled study device with two arms: Robotic-guided TBNA and Robotic-nCLE-guided TBNA with the different steps

The tumor board will determine intermediate diagnosis and management recommendations for each patient at the intermediate and final diagnosis phases (see Figure 3). Three therapeutic management options will be considered: "neither treatment nor follow-up", "follow-up", or "treatment" (see above). If the diagnosis is certain, "neither surgery nor surveillance" will be recommended for benign nodules; treatment will be recommended for malignant lesions; either treatment or follow-up will be recommended for indeterminate nodules.

An intermediate diagnosis and an intermediate management recommendation will be provided after the tumor board receives TBNA histological results and recorded in the Case Report Form for each patient of both arms.
A final diagnosis and a final management recommendation will be provided just after the final diagnosis results are received by the tumor board and recorded in the Case Report Form for each patient of both arms.

These intermediate diagnosis and management recommendations will also be compared to the definitive diagnosis and final patient management decisions.

7. Statistical Analysis

Population demographics comparison in both arms:

Statistical analysis will be performed to compare the patient population in terms of demographics to assess whether there are any differences between the populations of the two arms in terms of age, sex, race, type of lung cancers, and cancer prevalence.

Lesion characteristics comparison in both arms:

Statistical analysis will be performed to compare the lesion characteristics to assess whether there is any difference in lesion characteristics between the two arms, in terms of size, location of the nodule, Presence of bronchus sign on computerized tomography, lung zone, positive PET, associated lymphadenopathy, distance closest to fissure, distance from lesion to pleura, and pre-procedure probability of malignancy.

Navigation Accuracy

Distance between the tip of the locatable guide/nCLE Confocal Miniprobe™ and the targeted lung lesion will be measured for each nodule. The median and standard deviation of the distance will be calculated for both arms. Using a statistical test (Student T-test), they will be compared between arms (Robotic-guided TBNA and Robotic-nCLE-guided TBNA).

Navigation success

Based on investigator self-assessment, the proportion of cases in which the operator can successfully navigate to the lung target with Robotic guidance and Robotic-nCLE guidance will also be recorded and compared for both arms. Using a statistical test (Student T-test), the navigation success will be compared between arms (Robotic-guided TBNA and Robotic-nCLE-guided TBNA).

Navigation time

Total time that the locatable guide/ nCLE Confocal Miniprobe™ is used in the subject during the Robotic-guided TBNA or Robotic-nCLE-guided TBNA procedure. The navigation time medians and standard deviations will be calculated for both arms. Using a statistical test (Student T-test), they will be compared between arms (Robotic-guided TBNA and Robotic-nCLE-guided TBNA).

First- and cumulative-pass diagnostic yields and performance calculations

First-pass diagnostic yield was defined above (see section Sample size calculation for the definition). Two first-pass diagnostic yields will be calculated one for the Robotic-nCLE-guided TBNA arm and a second one for the Robotic-guided TBNA arm. 95% confidence intervals will be calculated, and the p-value to compare diagnostic yield values between the two arms. Sequential cumulative pass diagnostic yields will also be calculated for each arm.

The diagnostic performance of the sequential cumulative ROSE passes and the sequential cumulative nCLE passes for the Robotic-nCLE-guided TBNA arm and of ROSE only for the Robotic guided TBNA will also be assessed at the end of a follow-up period according to their sensitivities, specificities, positive predictive value (PPV), and negative predictive value (NPV). The sensitivity test will measure the ability of sequential cumulative ROSE passes or sequential cumulative nCLE passes to characterize malignant nodule (true positive rate) correctly. In contrast, the specificity test will measure the ability for sequential cumulative ROSE passes or sequential cumulative nCLE passes to correctly characterize benign nodule (true negative rate) using the final/definitive diagnosis as the reference.

Where a = true positive, b = false positive, c = false negative, and d = true negative:

- Sensitivity: Probability that sequential cumulative ROSE passes will be positive when malignancy is present (true positive rate): = a/(a + c)

- Specificity: Probability that a sequential cumulative ROSE passes will be negative when malignancy is not present (true negative rate): = d/(b + d)
- PPV: Probability that malignancy is present when sequential cumulative ROSE passes is positive: = a/(a + b)
- NPV: Probability that malignancy is not present when sequential cumulative ROSE passes is negative: = d/(c + d)

The same calculations will be performed for the different sequential cumulative nCLE passes.
Diagnostic performances for both techniques will be compared using the DeLong and DeLong statistical method [26] with the final diagnosis as the reference diagnosis.

Per patient diagnostic yield (cumulative pass diagnostic yield) and performance calculations

Per patient diagnostic yield of each bronchoscopic procedure (Robotic-guided TBNA and Robotic-nCLE-guided TBNA) is defined as the proportion of patients whose procedure will yield a definitive diagnosis out of the total number of patients that have received that procedure. Two diagnostic yields will be calculated: one for the Robotic-guided TBNA arm and one for the Robotic-nCLE-guided TBNA arm. 95% confidence intervals will be calculated, and the p-value to compare diagnostic yield values between arms.

The diagnostic accuracies of a bronchoscopic procedure (Robotic-guided TBNA and Robotic-nCLE-guided TBNA) will be measured at the end of a follow-up period according to their sensitivities and specificities. The sensitivity test will measure the ability of a technique to correctly identify those patients with the disease (true positive rate). In contrast, the specificity test will measure the ability of the technique to correctly identify those patients without the disease (true negative rate). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) will be calculated as the accuracy of the Robotic-guided TBNA and that of Robotic-nCLE-guided TBNA diagnosis (based on final pathology results) compared to the definitive diagnosis based on all available procedures and follow-up.

Where a = true positive, b = false positive, c = false negative, and d = true negative:

- Sensitivity: Probability that a Robotic-guided biopsy will be positive when malignancy is present (true positive rate): = a/(a + c)
- Specificity: Probability that a Robotic-guided biopsy will be negative when malignancy is not present (true negative rate): = d/(b + d)
- PPV: Probability that malignancy is present when a Robotic-guided biopsy is positive: = a/(a + b)
- NPV: Probability that malignancy is not present when a Robotic-guided biopsy is negative: = d/(c + d)
- Accuracy: Probability that a Robotic-guided biopsy gives correct prediction among the total number of cases examined= (a + d)/(a + b + c + d)

Patient diagnostic and management analysis:

Intermediate and final diagnosis and management recommendation proportion differences for the two patient populations (Robotic-guided TBNA arm and Robotic-nCLE-guided TBNA arm) will be compared using a chi-square test. Intermediate and final diagnosis and management recommendation proportions will also be compared for the two patient populations. Differences between Robotic-guided TBNA and Robotic-nCLE-guided TBNA arms regarding the specific diagnostic and management categories will be compared using McNemar's test both at the intermediate diagnosis and the final diagnosis stage.

Feasibility statistical analysis:

The feasibility of needle-based lung cancer diagnosis will be assessed by investigating the following items:

- Assessing nCLE-image quality
- Assessing the feasibility of the integrated needle/Cellvizio® I.V.E Confocal Miniprobe™.

The imaging feasibility yield for the Robotic-nCLE-guided TBNA procedure is defined as the proportion of patients in whom procedure will yield at least good nCLE image quality out of the total number of patients that will receive that procedure.
The mechanical feasibility yield for the Robotic-nCLE-guided TBNA procedure is defined as the proportion of patients for whom Robotic-nCLE-guided TBNA procedure will be possible mechanically (meaning that the nCLE probe will be guided to the nodule successfully) out of the total number of patients that will receive that procedure.

Safety:

The safety will be assessed by carefully monitoring adverse events related to both procedures. Serious adverse event rates will be calculated by dividing the number of events for each procedure divided by the total number of procedures for each arm.

Reproducibility:

To assess the reproducibility of bronchoscopy guided nCLE:

- Estimating the reproducibility of nCLE criteria will be evaluated by calculating the inter-observer agreement of nCLE criteria between 3 readers (Fleiss Kappa).

In Wijmans et al. [10], three nCLE characteristics for the detection of malignancy were identified in different subtypes of lung tumors and metastatic lymph nodes:

1) dark enlarged pleomorphic cells;
2) dark cell clusters consisting of overlapping cell structures ('dark clumps'); and
3) the continuous movement of a part of the cells in one direction ('directional streaming')

These criteria will be evaluated qualitatively.

New nCLE characteristics in malignant and benign lung lesions could also be identified during this study. An imaging atlas of nCLE images will also be built for the different tumor types on in-vivo characteristics. The department of pathology will be strongly involved in this project.

nCLE-video images will be reviewed and diagnosed retrospectively, independently, and blindly by investigators, expert readers, and pathologists.
The diagnostic yield will be tested to give the likelihood that nCLE will provide the information needed to establish a diagnosis.
Correlation analysis will also be performed between:

- nCLE imaging results and digital images of cytological or histopathological results
- nCLE imaging results in different tumor types.

8. End Points

Primary endpoint: The definitive diagnosis will serve as the reference standard for the calculation of study endpoints. The study pathologists will not be aware of nCLE results. The primary endpoint of this study will be the first-pass diagnostic yield, as defined in the section “Sample size calculation”, i.e. and is calculated as the proportion of patients for which first-pass TBNA during the bronchoscopic procedure yielded a definitive diagnosis out of the total number of patients that have received a diagnostic procedure

Secondary endpoint: The secondary outcome of this study will be the per-patient diagnostic yield, calculated as the proportion of patients in whom the bronchoscopic procedure yielded a definitive diagnosis out of the total number of patients that have received a diagnostic procedure.

Third endpoint: The third endpoint for patient management will be assessing the clinical impact of nCLE by determining the proportion of patients with a correct change in definitive diagnosis and the associated change in management plan with nCLE.

First-pass diagnostic yield:

The study is designed to enroll 118 patients who will be randomly assigned 1:1 to Robotic-guided TBNA alone or Robotic-nCLE-guided TBNA for a statistical power of 80% with a 2-sided significance level of 0.05 to detect an increase of the primary study endpoint (per pass TBNA diagnostic yield) of at least 20%, from 70% to 90%, between the Robotic-guided TBNA and the Robotic-nCLE-guided TBNA.

Per patient diagnostic yield (cumulative pass diagnostic yield) and performance:

Per patient diagnostic yield of each bronchoscopic procedure (Robotic-guided TBNA and Robotic-nCLE-guided TBNA) will be calculated, and the accuracy of each technique using the final/definitive diagnosis as the reference. The diagnostic yield and accuracy for each method will be compared.

Patient management:

Three therapeutic management options will be considered right after the Robotic-guided TBNA or Robotic-nCLE-guided TBNA: 'neither treatment nor follow-up', 'follow-up', or 'treatment' (see section 6). If the diagnosis is certain, 'neither surgery nor surveillance' will be recommended for benign nodules; treatment will be recommended for malignant lesions; either treatment or follow-up will be recommended for indeterminate nodules.
Overall diagnostic and therapeutic management proportion differences in the two patient populations (Robotic-guided TBNA arm and Robotic-nCLE-guided TBNA arm) will be compared using a chi-square test. Differences between Robotic-guided TBNA and Robotic-nCLE-guided TBNA arms regarding the specific diagnostic and therapeutic management categories will be compared using McNemar's test.

Safety:

One of the secondary goals of the study is to evaluate the safety of Robotic-nCLE-guided TBNA in comparison to Robotic-guided TBNA. According to the validated Common Terminology Criteria for Adverse Events scale (CTCAE: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5.0.xlsx). The primary serious adverse event of concern is treatment-induced pneumothorax. Based on data from a recent sizeable multicentric study [27], Robotic-related Common Terminology Criteria for Adverse Events grade 2 or higher pneumothoraces (requiring admission or chest tube placement) occurred in 2.9%. The Robotic-related Common Terminology Criteria for Adverse Events grade 2 or higher bronchopulmonary hemorrhage and grade 4 or higher respiratory failure rates were 1.5% and 0.7%, respectively. Based on these results, we assume that the pneumothoraces rate among similar patients undergoing Robotic-nCLE-guided TBNA procedures will be equivalent to that study.

9. Study Benefit/Risk Ratio

The benefit of study participation is that the results of this study may be significant for future patients. nCLE is a promising safe, minimally-invasive imaging technique that, in conjunction with conventional endoscopic workup procedures, can provide real-time, high-resolution microscopic imaging of tissue characteristics within different organs in reach of bronchoscopy/endosonography. There is little burden related to study participation. After needle placement within the lymph node or lung tumor (standard procedure), we will hold the needle into place to obtain a CLE video.
Fluorescein, a commonly used stain in hospitals, will be administered (only in patients in the nCLE arm). In patients not at risk for allergic reactions to fluorescein (allergic reaction to fluorescein in the medical history), this is safe, and the risk of an adverse reaction is low (1.1 %). The most typical adverse reactions were mild, such as nausea (0.7%) [13]. Based on the fluorescein manufacturer's manual, patients with a known allergy to fluorescein and patients who used a beta-blocker within 24 hours before the intervention, and patients who are pregnant or lactating will be excluded. Based on the literature, we will not exclude patients with a previous reaction to another type of drug than fluorescein, as the literature shows that the incidence rate of an adverse reaction due to intravenous administration of fluorescein in 'special patient groups (such as: above 80 years old, drug allergy history, system disease) was similar to that in the healthy population.
The estimated time for a bronchoscopic procedure is 25-35 minutes. The estimated prolonged endoscopy time for nCLE imaging is 10 minutes.
The patient will not notice anything due to the use of midazolam (fentanyl) or Propofol sedation (following standard procedure). Adverse events are not expected. A recent study by Karstensen et al. [28], combined EUS-FNA with nCLE in abdominal lymph nodes and pancreas, and no adverse events occurred during the 30-day follow-up. Also, no adverse events were reported in the study by Wijmans et al. [10] in the same target population. As stated by the hospitals, the standard diagnostic, therapeutic (lobectomy) process, and pathological evaluation, this study will not affect the internal protocol. In conclusion, we believe that the burden and risk associated with participation are negligible.

10. Administrative

This study will be conducted in accordance with local, state and Federal regulations and according to accepted good clinical practice guidelines.

11. Informed Consent

The IRB approved informed consent documents must be signed by the patient before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient. If applicable, they will be provided in a certified translation of the local language.
Original signed consent forms must be filed in each patient’s study file with a copy in the study file

12. References:

[1] The NLST Research Team. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. N Engl J Med 2011; 365: 395–409. doi:10.1056/NEJMoa1102873
[2] Hiraki T, Mimura H, Gobara H, et al. Incidence of and Risk Factors for Pneumothorax and Chest Tube Placement After CT Fluoroscopy–Guided Percutaneous Lung Biopsy: Retrospective Analysis of the Procedures Conducted Over a 9-Year Period. Am J Roentgenol 2010; 194: 809–814. doi:10.2214/AJR.09.3224
[3] Gupta S, Wallace MJ, Cardella JF, et al. Quality improvement guidelines for percutaneous needle biopsy. J Vasc Interv Radiol JVIR 2010; 21: 969–975. doi:10.1016/j.jvir.2010.01.011
[4] Baaklini WA, Reinoso MA, Gorin AB, et al. Diagnostic yield of fiberoptic bronchoscopy in evaluating solitary pulmonary nodules. Chest 2000; 117: 1049–1054

[5] Rivera MP, Mehta AC. Initial Diagnosis of Lung Cancer. Chest 2007; 132: 131S-148S. doi:10.1378/chest.07-1357
[6] Gex G, Pralong JA, Combescure C, et al. Diagnostic Yield and Safety of Electromagnetic Navigation Bronchoscopy for Lung Nodules: A Systematic Review and Meta-Analysis. Respiration 2014; 87: 165–176. doi:10.1159/000355710
[7] Wang Memoli JS, Nietert PJ, Silvestri GA. Meta-analysis of Guided Bronchoscopy for the Evaluation of the Pulmonary Nodule. Chest 2012; 142: 385–393. doi:10.1378/chest.11-1764
[8] Rojas-Solano JR, Ugalde-Gamboa L, Machuzak M. Robotic Bronchoscopy for Diagnosis of Suspected Lung Cancer: A Feasibility Study. J Bronchol Interv Pulmonol 2018; 25: 168–175. doi:10.1097/LBR.0000000000000499
[9] Chen AC, Gillespie CT. Robotic Endoscopic Airway Challenge: REACH Assessment. Ann Thorac Surg 2018; 106: 293–297. doi:10.1016/j.athoracsur.2018.01.051
[10] Wijmans L, Yared J, de Bruin DM, et al. Needle-based confocal laser endomicroscopy (nCLE) for real-time diagnosing and staging of lung cancer. Eur Respir J 2019: 1801520. doi:10.1183/13993003.01520-2018
[11] Giovannini M, Sample Organization. Feasibility of Intratumoral Confocal Microscopy under Endoscopic Ultrasound Guidance. Endosopic Ultrasound 2012; 1: 80. doi:10.7178/eus.02.005
[12] Konda V, Meining A, Jamil L, et al. A pilot study of in vivo identification of pancreatic cystic neoplasms with needle-based confocal laser endomicroscopy under endosonographic guidance. Endoscopy 2013; 45: 1006–1013. doi:10.1055/s-0033-1344714
[13] Kwan AS, Barry C, McAllister IL, et al. Fluorescein angiography and adverse drug reactions revisited: the Lions Eye experience. Clin Experiment Ophthalmol 2006; 34: 33–38. doi:10.1111/j.1442-9071.2006.01136.x
[14] Wallace MB, Meining A, Canto MI, et al. The safety of intravenous fluorescein for confocal laser endomicroscopy in the gastrointestinal tract. Aliment Pharmacol Ther 2010; 31: 548–552. doi:10.1111/j.1365-2036.2009.04207.x
[15] Becker V, Wallace MB, Fockens P, et al. Needle-based confocal endomicroscopy for in vivo histology of intra-abdominal organs: first results in a porcine model (with videos). Gastrointest Endosc 2010; 71: 1260–1266. doi:10.1016/j.gie.2010.01.010
[16] Benias P, D'Souza L, Papafragkakis H, et al. Needle-based confocal endomicroscopy for evaluation of malignant lymph nodes – a feasibility study. Endoscopy 2016; 48: 923–928. doi:10.1055/s-0042-109775
[17] Su Z, Zhong C, Li S, et al. Needle-based confocal laser endomicroscopy in the diagnosis of peripheral pulmonary nodule: a preliminary report. J Thorac Dis 2017; 9: 2608–2612. doi:10.21037/jtd.2017.06.10
[18] Blandin Knight S, Crosbie PA, Balata H, et al. Progress and prospects of early detection in lung cancer. Open Biol 2017; 7: 170070. doi:10.1098/rsob.170070
[19] Folch EE, Bowling MR, Gildea TR, et al. Design of a prospective, multicenter, global, cohort study of electromagnetic navigation bronchoscopy. BMC Pulm Med 2016; 16. doi:10.1186/s12890-016-0228-y
[20] MacMahon H, Naidich DP, Goo JM, et al. Guidelines for Management of Incidental Pulmonary Nodules Detected on CT Images: From the Fleischner Society 2017. Radiology 2017; 284: 228–243. doi:10.1148/radiol.2017161659
[21] Bueno J, Landeras L, Chung JH. Updated Fleischner Society Guidelines for Managing Incidental Pulmonary Nodules: Common Questions and Challenging Scenarios. RadioGraphics 2018; 38: 1337–1350. doi:10.1148/rg.2018180017
[22] Bowling MR, Kohan MW, Walker P, et al. The Effect of General Anesthesia Versus Intravenous Sedation on Diagnostic Yield and Success in Electromagnetic Navigation Bronchoscopy: J Bronchol Interv Pulmonol 2015; 22: 5–13. doi:10.1097/LBR.0000000000000120

[23] Ost DE, Ernst A, Lei X, et al. Diagnostic Yield and Complications of Bronchoscopy for Peripheral Lung Lesions. Results of the AQuIRE Registry. Am J Respir Crit Care Med 2016; 193: 68–77. doi:10.1164/rccm.201507-1332OC
[24] Bhutani MS, Koduru P, Joshi V, et al. EUS-Guided Needle-Based Confocal Laser Endomicroscopy: A Novel Technique With Emerging Applications. Gastroenterol Hepatol 2015; 11: 235–240
[25] Retina. 5th ed. London: Saunders/Elsevier; 2013
[26] DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988; 44: 837–845
[27] Folch EE, Pritchett MA, Nead MA, et al. Electromagnetic Navigation Bronchoscopy for Peripheral Pulmonary Lesions: One-Year Results of the Prospective, Multicenter NAVIGATE Study. J Thorac Oncol 2019; 14: 445–458. doi:10.1016/j.jtho.2018.11.013
[28] Karstensen J, Cârţână T, Constantinescu C, et al. Endoscopic ultrasound guided needle-based confocal laser endomicroscopy in solid pancreatic masses – a prospective validation study. Endosc Int Open 2018; 06: E78–E85. doi:10.1055/s-0043-121987