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Design of a multicenter, randomized controlled trial on needle-based confocal laser endomicroscopy guided bronchoscopy for peripheral lung nodule diagnosis (CLEVER trial)

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Abstract

Introduction: Despite many technological advances, the diagnostic yield of bronchoscopic peripheral lung nodule analysis remains limited due to frequent mispositioning. Needle-based confocal laser endomicroscopy (nCLE) enables real-time microscopic feedback on needle positioning, potentially improving the sampling location and diagnostic yield. Previous studies have defined and validated nCLE criteria for malignancy, airway, and lung parenchyma. Larger studies demonstrating the effect of nCLE on diagnostic yield are lacking. We aim to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE.

Methods and analysis: This is a parallel-group randomized controlled trial. Recruitment is performed at pulmonology outpatient clinics in university and general hospitals in six different European countries and one hospital in the United States. Consecutive patients with a suspected peripheral lung nodule (10-30 mm) with a bronchus sign and an indication for diagnostic bronchoscopy will be screened, and 208 patients will be included. Web-based randomization (1:1) between the two procedures will be performed. The primary outcome is diagnostic yield. Secondary outcomes include diagnostic sensitivity for malignancy, needle repositionings, procedure and fluoroscopy duration, and complications. Pathologists will be blinded to procedure type; patients and endoscopists will not.

Discussion: Results of the CLEVER trial will inform on the added value of nCLE for the bronchoscopic diagnosis of peripheral lung nodules.

Ethics and dissemination: Approved by the Ethics Committee of the Amsterdam University Medical Center. Dissemination involves publication in a peer-reviewed journal.

Support: Financial and material support from Mauna Kea Technologies.

Trial registration: ClinicalTrials.gov NCT06079970.

Keywords: Respiratory tract neoplasms, bronchoscopy, confocal laser scanning microscopy, nCLE, rapid-on-site evaluation (ROSE), histology/cytology, lung cancer, peripheral lung nodule

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Article summary

Strengths and limitations

- This is the first multicenter randomized controlled trial on needle-based confocal laser endomicoscopy (nCLE) for bronchoscopic diagnosis of peripheral lung nodules; this study will provide interventional pulmonologist with important information about the added benefit of the nCLE technique.
- The outcomes will offer pulmonologist information to identify specific patients that may benefit from the addition of nCLE.
- The definition of diagnostic yield is under debate. In this study, the diagnostic yield will be reported based on two different definitions for better comparison with existing and future studies.
- Each participating center uses their own methods for conventional bronchoscopic diagnosis of peripheral lung nodules and will therefore not be completely uniform across all centers; this is a

limitation to the study design. Each center will keep conventional methods uniform in both the control and intervention group to ensure differences can be attributed to the nCLE technique.

Introduction

Lung cancer remains the leading causes of cancer-related deaths, with 2.09 million new diagnoses and 1.76 million deaths worldwide per year.(1, 2) The increased use of chest computed tomography (CT) and the future implementation of low-dose CT lung cancer screening programs result in an increased detection of lung nodules.(3, 4) Consequently, more early-stage lung cancer is detected, which is most often located in the periphery of the lung.(5, 6) Depending on lesion characteristics and associated risk factors, tissue sampling is needed to establish a definitive diagnosis and determine the appropriate treatment.

Bronchoscopic analysis of peripheral lung nodules remains challenging despite many technological innovations. The procedure comprises three essential pillars needed for a diagnostic success: [1] navigation to the lesion, [2] confirmation of tool location within the lesion (i.e., tool-in-lesion confirmation) and [3] successful tissue sampling. In the past years, fluoroscopy, radial probe endobronchial ultrasound (r-EBUS), electromagnetic navigation (EMN), virtual bronchoscopy (VB) or cone beam computed tomography (CBCT) combined with augmented fluoroscopy have improved navigation with or without tool-in-lesion confirmation.(7) Additionally, rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%, (8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern and pre-test probability of malignancy.(9, 10) The arrival of robotic bronchoscopy platforms combined with existing techniques have shown promising results with high navigation success rates. However, diagnostic yield remains behind due to substantial mispositioning rates, retaining a large gap between navigation success and diagnostic yield.(11-13) The persistently low diagnostic yield rates calls for complementary techniques providing real-time information for fine-tuning the needle position.

Confocal laser endomicroscopy (CLE) is a microscopic technique that visualizes individual cells in real-time with high resolution. It has proven useful in the field of gastroenterology, where it has been demonstrated that CLE could be used for rapid diagnosis, targeting of biopsies, and prediction of neoplasms.(14) CLE has been recently introduced in the respiratory tract, including for the peripheral lung nodule analysis.(15-17) Current CLE probes are thin enough to fit through 18G biopsy needles to provide microscopic feedback at the tip of the needle (needle-based CLE (nCLE)). Fluorescein dye is

used as a contrast agent and binds to the extracellular matrix, resulting in a highly fluorescent background in which individual cells can be seen. Previous studies have identified three nCLE image characteristics for malignancy in the lung (dark clumps, enlarged pleomorphic cells, and directional streaming),(17) and criteria for airway and lung parenchyma (elastic fiber bundles, bronchial epithelium, and alveoli).(16) The identification of malignancy and distinction from airway and lung parenchyma were accurate based on these criteria.(16, 17)

A recent study demonstrated a high needle mispositioning rate, as nCLE-imaging resulted in a repositioning of the biopsy needle in 9 out of 20 patients.(18) nCLE could therefore potentially bridge the gap between navigation success and diagnostic yield.

To date, larger studies investigating the effect of the addition of nCLE to bronchoscopic peripheral lung nodule analysis are lacking. The improved diagnostic yield could reduce the necessity further or more invasive diagnostic interventions such as CT-guided transthoracic biopsies or diagnostic surgery. In this multicenter randomized controlled trial, we aim to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules.

Methods and analysis

Study design

This study is an investigator-initiated, international, multicenter, parallel-group randomized controlled trial comparing two bronchoscopy procedures (with or without nCLE) for the diagnosis of suspected peripheral lung nodules. The study flowchart is shown in Figure 1.

Participating centers

The study is executed in university or general hospitals in six countries in Europe and one hospital in the United States.

Randomization

After the participant has given written informed consent, patient data is entered into a digital database (CASTOR Electronic Data Capture (EDC) electronic case report form (eCRF)). We will use a web-based block-randomization module in Castor to randomize participants into the control and interventional group (1:1). Randomization will be stratified by participating center to ensure that the

nCLE and non-nCLE group is of the same size in each center. As nodule size has significant impact on diagnostic yield,(8) we will stratify for nodule size (≤20 mm and >20 mm) to ensure that size is evenly distributed across study arms.

Patients and endoscopists will not be blinded since the physician needs to know if nCLE images must be acquired during bronchoscopy. Pathologists will be blinded to procedure type and raters who will analyze the nCLE videos after the procedure will be blinded to the patient history and histopathological outcome of the tissue samples.

Study population

Consecutive patients will be recruited by their treating physician at pulmonology outpatient clinics of participating centers. Patients are eligible for inclusion if they meet the following inclusion criteria:

1. ≥18 years of age
2. Suspected malignant peripheral lung lesion with an indication for a bronchoscopic diagnostic work-up as determined by the attending physician or tumor board. Peripheral pulmonary lesions are defined as lesions located beyond the visible segmental bronchi, not detectable by regular flexible bronchoscopy.
3. Solid part of the lesion must be >10 mm
4. Largest dimension of lesion size on CT ≤30 mm (long-axis)
5. Positive bronchus sign on pre-procedural CT-scan: in case no airway leads to the target lesion, our expectations are that the chance of reaching the lesion with a conventional bronchoscope are very low. As nCLE is still dependent on bronchoscopic navigation to bring the needle in the proximity of the target nodule, the added value of nCLE-imaging is estimated to be redundant in the absence of a bronchus sign.
6. Ability to understand and willingness to sign a written informed consent

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Inability or non-willingness to provide informed consent
2. Endobronchial visible malignancy on bronchoscopic inspection
3. Target lesion within reach of the linear EBUS scope
4. Failure to comply with the study protocol
5. Known allergy or risk factors for an allergic reaction to fluorescein
6. Pregnancy or breastfeeding

7. Hemodynamic instability
8. Refractory hypoxemia
9. Therapeutic anticoagulant use that cannot be withheld for an appropriate interval before the procedure
10. Unable to tolerate general anesthesia according to the anesthesiologist
11. Undergoing chemotherapy as several chemotherapies have fluorescent properties at the same wavelength (e.g., doxorubicin)

Primary outcome measure

Diagnostic yield (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis (either malignant, specific benign or non-specific benign confirmed as benign in follow-up), relative to the total number of patients that underwent the diagnostic bronchoscopic procedure). If patients with multiple lesions are included, the diagnostic yield will be computed per nodule.

Secondary outcome measures

1. Diagnostic sensitivity for malignancy (defined as the proportion of patients in whom the bronchoscopic procedure diagnoses malignancy relative to the total number of patients with a final diagnosis of malignancy as determined by the reference standard).
2. Diagnostic yield according to the strict definition by Vachani et al.(19) (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant or specific benign diagnosis], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure).
3. Procedure duration (from bronchoscope insertion until removal).
4. Percentage of patients in which the needle was fine-tuned (defined as moving the needle within the same distal airway) or repositioned (defined as the selection of a different distal airway for tissue sampling) based on nCLE feedback (defined as the number of patients the needle was fine-tuned/repositioned divided by the total number of patients in which nCLE imaging was used).
5. Radiation dose.
6. Diagnostic yield of ROSE (defined as the proportion of patients in whom ROSE resulted in a classifying diagnosis [malignant or specific benign diagnosis], relative to the total number of patients).

7. Proportion of patients in which ROSE provided tool-in-lesion confirmation, meaning that the acquired tissue shows signs of a malignant or non-malignant diagnosis and was not related to airway/lung parenchyma sampling such as bronchus epithelium/blood contamination, and tissue not suitable for a specific diagnosis such as atypical cells.
8. Complication rate (defined as any complication or complication categories occurring during or directly after the bronchoscopic procedure or any procedure-related complication within one week after the procedure).
9. Requirement of additional diagnostic procedures (CT-guided transthoracic biopsies, surgical diagnostics and/or additional bronchoscopy) during the 6-month follow-up period.

Exploratory endpoints

As an exploratory endpoint, we aim to identify potential new nCLE image characteristics for malignant and benign pathologies. We will also create an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods.

Outcome parameters

Table 1 shows the baseline patient characteristics and corresponding procedural information that will be collected at the time of study inclusion, during the procedure and 6-month follow-up period.

Investigational product

The Cellvizio® confocal laser endomicroscopy system with the corresponding AQ-Flex™ 19 miniprobe (Mauna Kea technologies, Paris, France) is the investigational medical device of this study. The probe has a compatible operating diameter of 0.91 mm, a resolution of 3,5 µm, a penetration depth of 40 to 50 µm and a maximum field of view of 325 µm. The device and corresponding probes are CE-marked and will be used within the intended purpose.(20)

The technique uses a laser beam (488 nm) focused by an objective lens to illuminate the tissue, with the illumination focus at a pre-defined depth. The light strikes the tissue resulting in fluorescent light emission back from autofluorescent structures such as elastin in the airways or an exogenous fluorescent dye such as fluorescein, a contrast dye used for nCLE imaging in the lung. Light originating from the focal layer will be focused by the objective lens at the opening of a pinhole and detected, while light from out-of-focus layers is rejected by the pinhole. This results in high-resolution imaging of individual cells and structures at a specific point with limited influence of (scattered) light from out-

of-focus areas.(20) The advantage of needle-based CLE is that it has the capability to provide real-time high-resolution information on a microscopic level at the tip of the biopsy needle.

Table 1: Data to be collected

Patient characteristics
Age
Sex
BMI
Smoking history
Patient cancer history
Family history of lung cancer
Pre-procedural (PET)CT scan lesion characteristics
Size (largest diameter)
Localization (segmental level)
Lesion appearance/nodule type (Solid, non-solid/ground glass, partially solid)
Spiculation sign (present/absent)
Emphysema (present/absent)
PET uptake (not performed/no uptake/faint *(SUV < 1)*/moderate *(SUV 1 – 2.5)* /intense *(SUV >2.5))*
Intra-procedural information
r-EBUS sign (eccentric, concentric, absent)
Location of tissue sampling (lung segment)
nCLE image observations (for every needle pass)
Needle fine-tuning & repositioning done (for every needle pass)
Sampling techniques used (TBNA, biopsy, brush)
ROSE results of tissue sample (if available)
Bronchoscopy start and end time
Fluoroscopy duration
Additional procedures performed (e.g., EBUS/EUS-B/etc.)
(Serious) complications
Post-procedural information
(Serious) complications (up to 1 week after the procedure)
Final pathological diagnosis (cytology and/or histology)
(Additional) Diagnostic follow-up procedures needed (e.g., transthoracic needle biopsies, surgery, additional bronchoscopy, follow-up imaging) including (altered) diagnosis and/or results of follow-up CT-scans of the lesion(s)

RUL: right upper lobe; RML: right middle lobe; RLL; right lower lobe; LUP: left upper lobe; LLL: left lower lobe; CT: computed tomography; ROSE: rapid on-site evaluation; r-EBUS: radial endobronchial ultrasound; TBNA: transbronchial needle aspiration; SUV: standard uptake value

Study procedures

Conventional diagnostic bronchoscopy (control group & intervention group)

The following procedure will be performed routinely (regardless of study participation): Bronchoscopic procedures will be performed at the pulmonology department by experienced pulmonologists specifically trained in navigational bronchoscopy and nCLE-imaging. Bronchoscopists are aware of the study randomization arm. All procedures are performed according to institutional practice, usually on an outpatient basis. Patient preparation and sedation will be done according to institutional practice and might include deep propofol sedation or midazolam sedation. Topical anesthesia will usually be applied to the pharynx, larynx, trachea, and bronchi. Vital parameters will be monitored during and after the procedure.

Systematic bronchoscopic inspection of the airways will be performed, followed by r-EBUS imaging (guide sheath optional) to select the distal airway with the highest probability of reaching the lesion. The use of fluoroscopy, EMN, VB or ultrathin bronchoscope is optional if regularly used at that institution. CBCT navigation will not be used in patients included in this trial. Transbronchial needle aspirations (TBNA) using the 18G FleXNeedle® (Broncus Medical Inc., San Jose, United States of America) and biopsies will be performed to acquire tissue for pathological evaluation (a recommended minimum of 3 TBNA and 3 biopsies). During the bronchoscopic work up, some of the cytological aspirations will be evaluated on site (Rapid onsite evaluation, ROSE) by a member of the pathological department and the representativeness of the samples will be reported back to the bronchoscopist. ROSE will always be performed for the first TBNA pass. For the following passes, the bronchoscopist decides if it is indicated.

Addition of nCLE imaging (intervention group)

The same procedure will be performed as described above for the patients randomized to the intervention arm, except for the addition of fluorescein administration and nCLE imaging before TBNA. Prior to the procedure, an 18G needle is preloaded with the CLE probe (AQ-Flex™ 19 Miniprobe, Mauna Kea Technologies, Paris, France) using a locking device. The CLE probe is advanced through the needle until the probe is positioned approximately 4 mm past the needle tip. The locking device ensures the probe cannot protrude more than the specified 4 mm during the procedure.

After determining the sample location based on lesion identification with r-EBUS and/or fluoroscopy, fluorescein (2.5 mL of 10% fluoresceindinatrium solution) is administered intravenously. Then, the

preloaded 18G needle punctures the target area, followed by the insertion of the CLE probe through the biopsy needle for real-time microscopic feedback. In case nCLE visualizes airway or lung parenchyma, indicating a near-miss, the biopsy needle is fine-tuned (i.e., the needle is moved within the same distal airway) or repositioned (i.e., a different distal airway is chosen). If nCLE demonstrates that the biopsy needle is placed within the lesion, the CLE probe is removed from the biopsy needle while holding the needle in position, followed by tissue sampling at the same location (repeated for at least 3 TBNAs). A flowchart of the procedure steps for both the conventional bronchoscopy and the nCLE-guided bronchoscopy is shown in Figure 2.

Pathological examination

The cytological and histological examination will be done according to standard hospital procedure. In case the bronchoscopic procedure is considered non-diagnostic, additional procedures (transthoracic needle aspiration, surgical procedure, etc.) could follow to obtain a definite diagnosis. Results of the nCLE imaging do not influence the indication for additional diagnostic procedures. If a surgical procedure is indicated, the histological images will be collected to compare this with the nCLE imaging.

In this study, the final pathological diagnosis will be subdivided into four categories as described by Vachani et al.,(19) namely [1] malignant, [2] non-malignant, which is divided into specific benign (including granulomatous, infectious and lymphocyte-predominant patterns) or nonspecific benign (e.g. inflammation), and [3] non-diagnostic (i.e., insufficient material for classifying diagnosis or in case atypical cells could not be classified further).

Reference standard

For the primary outcome, diagnostic yield will be calculated using the intermediate method described by Vachani et al.(19) The abovementioned final pathological diagnosis categories will be used regardless of the results of the reference standard, except for initial non-specific benign diagnoses. In these cases, results from the reference standard will be considered. If the initial benign diagnosis is confirmed benign in follow-up, the bronchoscopic procedure will be considered diagnostic.

For the calculation of diagnostic sensitivity, malignant cases identified by the procedures under investigation will be considered as true positive since false positive results (almost) never occur. Benign (either specific or non-specific) and non-diagnostic samples will undergo a reference standard, which can be a subsequent sampling method such as transthoracic needle biopsy or surgery. Alternatively, if no subsequent sampling method is performed, clinical and radiological follow-up at 6 months is considered the reference standard. If follow-up CT imaging shows regression or resolution

of the nodule or in case a nodule remains stable, it will be considered as a confirmation of non-malignant diagnosis (i.e., true negative). Cases that are benign (either specific or non-specific) or non-diagnostic at the index bronchoscopy will be considered false negative if a malignancy diagnosis is established by the reference standard or if therapeutic procedures are done without confirmation of diagnosis. Figure 3 gives a schematic overview of the calculation methods of diagnostic yield and sensitivity for malignancy.

Informed consent procedure

Patients will be recruited by their treating physician. If the patient is willing to receive more information about study participation, information will be provided by the local investigator. The eligible participants will have sufficient time until the scheduled procedure to consider their consent. Written informed consent must be provided before any study-related procedures take place. After informed consent, patients will be randomized using Castor EDC software and assigned to the control or intervention group. The bronchoscopy will then be performed according to the study protocol. In case patients decline participation in the study, they will be treated to the usual local clinical practices and guidelines.

Quality assurance

Only experienced pulmonologists will perform the procedures to ensure high-quality bronchoscopic procedures. Additionally, all participating centers will be trained in the use of the CLE Cellvizio device and to maintain homogeneous quality of the nCLE image acquisition and interpretation over all centers.

Sample size justification

Based on previous studies and meta-analyses, we expect the diagnostic yield in patients with a lesion <30 mm in the conventional bronchoscopy arm to be 62%.(21, 22) We hypothesize that additional nCLE guidance in the intervention arm will result in a diagnostic yield of 80%. In total, 198 patients are needed to show that nCLE guidance results in a diagnostic yield that is 18 percent point higher than the conventional bronchoscopy arm (alpha=0.05 and power=0.80). Taking into account a 5% study drop-out, a total of 208 patients will be included. We believe an increase in the diagnostic yield (from 62% to 80%) demonstrates a clinically relevant improvement in lung cancer diagnosis.

Data analysis

Results for continuous variables will be expressed as means and standard deviations or medians with interquartile ranges. Categorical variables will be expressed as frequencies and percentages. The Chi-squared test will be used to compare diagnostic yield (or other comparisons between categorical variables) between the two randomization groups. Continuous variables will be compared using Student's t-test, or Mann-Whitney-U tests. A two-tailed P-value <0.05 will be considered statistically significant. All analyses are done based on an intention-to-treat approach, meaning that patients are analyzed as part of the intervention group they were assigned to, even if nCLE imaging was not executed in a patient in the intervention arm due to unforeseen circumstances. These specific cases will be reported in the manuscript. Patients not undergoing the planned bronchoscopy procedure after randomization are excluded from the analysis. Patients with missing outcome data will be excluded from analysis. Patients with incomplete essential follow-up information will also be excluded from the calculation of diagnostic sensitivity. However, we will also calculate diagnostic sensitivity based on a 'worst-case scenario', in which these patients are considered false negatives. For the primary outcome, subgroup analysis will be performed for several lesions and procedural characteristics (lesion size (<20 mm vs ≥20 mm), radial EBUS image (eccentric vs concentric vs absent), location (upper lobe (without lingual) vs middle lobe/lingual vs lower lobe), pre-test probability that the nodule is cancerous (<10%, 10 – 35%, 36-70% and >70%) based on the Brock score.(23)

Ethics and dissemination

The CLEVER study will be conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the Medical Research Involving Human Subjects Act (WMO, The Netherlands) principles. The Medical Ethical Committee of the Amsterdam UMC has approved the study (NL83257.018.22). Written informed consent will be obtained prior to randomization and any study-related procedures. In case of major changes to the protocol, the ethical review board will be notified, and it will be communicated with all participating centers and registered on clinicaltrials.gov.

Data management and safety

After informed consent, the patient will be given a code. This code will be used on all (pseudonymized) data, including CLE images and electronic Case Report Form (eCRF) data. Castor Electronic Data Capture ecosystem (International Organization of Standardization (ISO) 27001 and 9001 certified) will be used to collect key patient information described in outcome parameters. The key to the code is

safeguarded by the local principal investigator and access to all records is limited to directly involved researchers. The coordinating investigator will centralize patients' data, and principal investigators will have direct access to their own site's data sets and to other sites' data upon reasonable request. All principal investigators will maintain records, including signed patient informed consent forms and information on adverse events.

Data management of all data (collection, storage, and analysis) will be done according to the local data management plan. All records will be stored for a period of 15 years following the completion or termination of the study. Monitoring will be done according to a monitoring plan with specific attention paid to informed consent, completion of the eCRF, and storage of CLE video data.

Patient safety and adverse events

The study was deemed a negligible risk study (according to the Nederlandse Federatie van Universitaire Medisch Centra (NFU) descriptions) by the ethical committee of the Amsterdam UMC. Previous study publications showed that nCLE-imaging and intravenous fluorescein administration are safe.(24) Fluorescein adverse reactions are rare and mostly mild in nature. No study related adverse events occurred in the prior bronchoscopic nCLE studies for peripheral lung nodule analysis in Amsterdam UMC.(16, 17) Estimated prolonged endoscopy time due to study participation is approximately 10 minutes. Patients will not be aware of this as they will already be sedated for the bronchoscopic procedure.

In case any (serious) adverse event ((S)AE) occur during the procedure or up to one week after the procedure, the sponsor will register SAEs through the web portal *Toetsingonline* to the accredited METC that has approved the protocol. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the trial procedure. The severity and possible relatedness to the investigational product or the procedure will be documented. Investigators of the participating centers will report all serious adverse events to the coordinating and principal investigator of the initiating site. Reporting of SAEs that result in death or are life-threatening will be done within 7 days after initial identification, followed by a period of a maximum of 8 days to complete the preliminary report. All other SAEs will be reported within 15 days after first knowledge of the SAE.

Annual progress report

The sponsor will ensure that a progress report is submitted to the medical ethics committee once a year. Information on the start date of inclusion, number of subjects included to date, number of subjects that have completed participation, serious adverse events, and amendments.

Dissemination

We aim to publish the study results in a peer-reviewed journal. Reporting will be in line with CONSORT and STARD 2015 reporting guidelines.(25, 26)

Discussion

In this multicenter, investigator-initiated, randomized controlled trial, we aim to determine if the addition of nCLE-imaging to bronchoscopic peripheral lung lesion analysis results in an improved diagnostic yield.

Since there still is a gap between the success rate of navigating the tissue sampling instrument toward the target lesion and the final diagnostic yield, there is a need for real-time tool-in-lesion confirmation. The addition of high-resolution microscopic nCLE imaging at the tip of the needle could potentially lead to a decrease in mispositioning rates and an improved diagnostic yield. As a result, fewer patients would need additional diagnostic procedures such as transthoracic needle biopsy or surgery, which are more invasive and have higher incidences of complications such as pneumothorax and hemorrhage.(27) Previous smaller studies have already shown that nCLE is safe, and raters can distinguish different image characteristics with high accuracy. On top of that, it has also been demonstrated that fine-tuning the needle based on these image characteristics is often done, even when navigation to the lesion was successful.(16-18)

Current literature on nCLE imaging for this purpose remains limited to smaller patient groups and the clinical benefit remains to be demonstrated. The results of the CLEVER study provide a formal comparison between conventional image-guided diagnostic bronchoscopy and conventional bronchoscopy with the addition of nCLE in a large randomized patient group. The results of this trial will clarify the added benefit of nCLE for bronchoscopic diagnosis of peripheral lung nodules and identify which patients could benefit from the use of this technique.

Author contributions

SvH, TK, DAK, DMdB, PIB and JTA were involved in conception and trial design. SvH, TK, DAK, PIB and JTA were involved in drafting of the study protocol. DAK provided statistical expertise. All authors were involved in editing and final approval of the protocol. SvH, DG, CB, JH, MH, VP, CR, AS, GS, EK, NA, JV, ZS, MAH, JMAD, PIB and JTA will be involved in the conduct of the study and data acquisition. SvH, DAK, DMdB, PIB and JTA will be involved in the data analysis and interpretation. SvH, DAK, PIB and JTA will be involved in drafting of the final manuscript for dissemination. All authors will provide editing and approval of the final manuscript for publication.

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Declaration of interests

JTA declares material and financial support from Mauna Kea Technologies for this study.

Data sharing statement

The final trial dataset will be available to study investigators on completion of the trial, and after publication of the primary manuscript. Data requests can be submitted to the researchers at the Amsterdam UMC, Amsterdam, The Netherlands.

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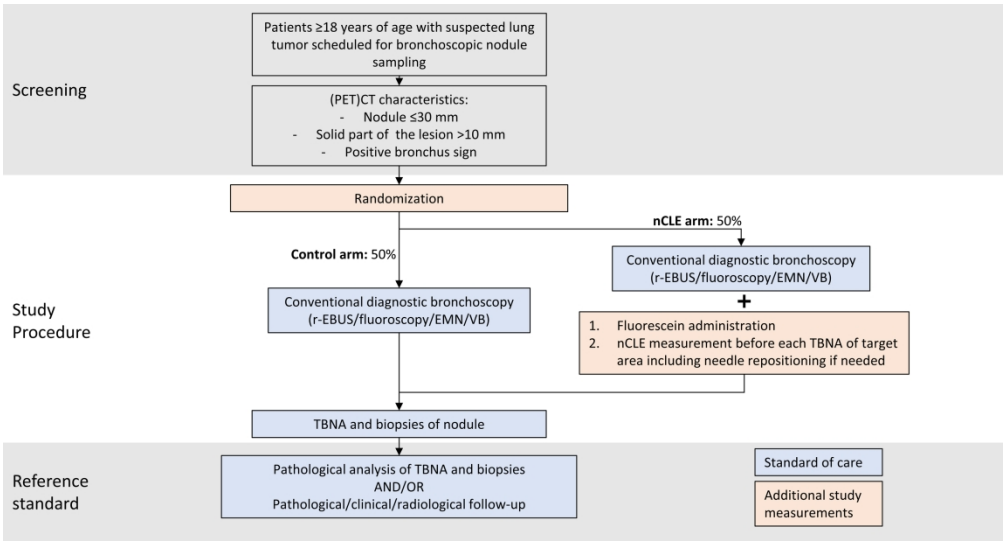
Figure headings

Figure 1: Study flowchart. r-EBUS: radial endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: neelde-based confocal laser endomicroscopy; TBNA: transbronchial needle aspiration; PET: positron emission tomography; CT: computed tomography

Figure 2: Procedure flowchart for control and interventional group (without and with nCLE). Note: fluorescein administration is only done once before the first puncture. TBNA: transbronchial needle aspiration; ROSE: rapid on-site evaluation; EBUS: endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: needle-based confocal laser endomicroscopy

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography



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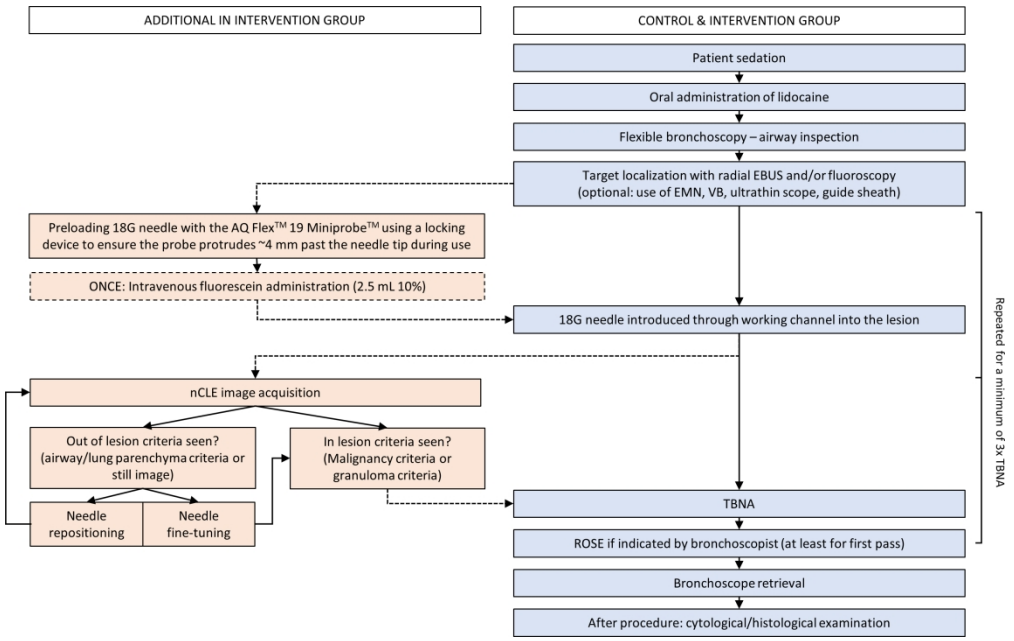

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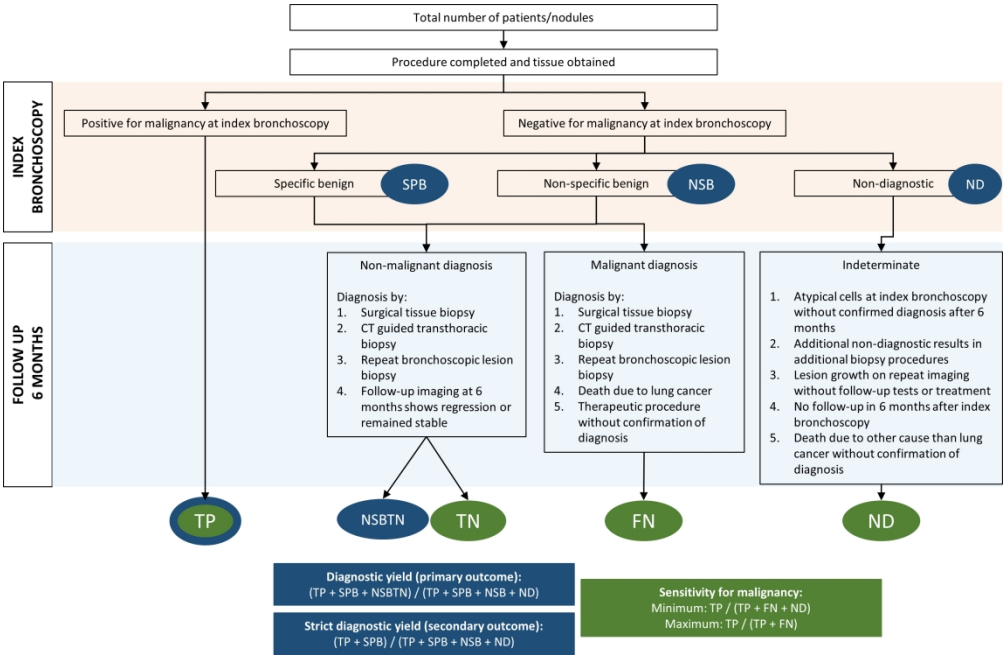

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1 and 14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1, 14, 15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	4

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9,10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9,10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4,5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4,5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4,5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4,5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	7, 11

		Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12,13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10,11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10,11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events	13

		and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the	14

		public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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	of Biomedical Engineering and Physics; Cancer Centre Amsterdam, Imaging and Biomarkers Bonta, Peter; Amsterdam Universitair Medische Centra, Department of Respiratory Medicine Annema, Jouke; Amsterdam Universitair Medische Centra, Department of Respiratory Medicine
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Secondary Subject Heading:	Oncology, Pathology, Diagnostics
Keywords:	Biopsy, Clinical Trial, Diagnostic Imaging, Respiratory tract tumours < ONCOLOGY, Cytopathology < PATHOLOGY

Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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Abstract

Introduction: Despite many technological advances, the diagnostic yield of bronchoscopic peripheral lung nodule analysis remains limited due to frequent mispositioning. Needle-based confocal laser endomicroscopy (nCLE) enables real-time microscopic feedback on needle positioning, potentially improving the sampling location and diagnostic yield. Previous studies have defined and validated nCLE criteria for malignancy, airway, and lung parenchyma. Larger studies demonstrating the effect of nCLE on diagnostic yield are lacking. We aim to investigate if nCLE-imaging integrated with

conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE.

Methods and analysis: This is a parallel-group randomized controlled trial. Recruitment is performed at pulmonology outpatient clinics in university and general hospitals in six different European countries and one hospital in the United States. Consecutive patients with a for malignancy suspected peripheral lung nodule (10-30 mm) with an indication for diagnostic bronchoscopy will be screened, and 208 patients will be included. Web-based randomization (1:1) between the two procedures will be performed. The primary outcome is diagnostic yield. Secondary outcomes include diagnostic sensitivity for malignancy, needle repositionings, procedure and fluoroscopy duration, and complications. Pathologists will be blinded to procedure type; patients and endoscopists will not.

Discussion: Results of the CLEVER trial will inform on the added value of nCLE for the bronchoscopic diagnosis of peripheral lung nodules.

Ethics and dissemination: Primary approval by the Ethics Committee of the Amsterdam University Medical Center. Dissemination involves publication in a peer-reviewed journal.

Support: Financial and material support from Mauna Kea Technologies.

Trial registration: ClinicalTrials.gov NCT06079970.

Keywords: Respiratory tract neoplasms, bronchoscopy, confocal laser scanning microscopy, nCLE, rapid-on-site evaluation (ROSE), histology/cytology, lung cancer, peripheral lung nodule

Manuscript word count: 4053

Article summary

Strengths and limitations

- This is the first (international multicenter) randomized controlled trial on needle-based confocal laser endomicoscopy (nCLE) for bronchoscopic diagnosis of peripheral lung nodules.
- This study provides the opportunity to evaluate the added benefit of the nCLE technique to conventional diagnostic bronchoscopy including radial EBUS in multiple centers and countries.
- The definition of diagnostic yield is under debate. In this study, the diagnostic yield will be reported based on two different definitions for better comparison with existing and future studies.
- Each participating center uses their own methods for conventional bronchoscopic diagnosis of peripheral lung nodules and will therefore not be completely uniform across all centers. Each

center will keep conventional methods uniform in both the control and intervention group to ensure differences can be attributed to the nCLE technique.

- In this study only peripheral pulmonary nodules between 1 – 3 cm are included.

Introduction

Lung cancer remains the leading cause of cancer-related deaths, with 2.09 million new diagnoses and 1.76 million deaths worldwide per year.(1, 2) The increased use of chest computed tomography (CT) and the future implementation of low-dose CT lung cancer screening result in an increased detection of lung nodules.(3, 4) Consequently, more early-stage lung cancer is detected, which is most often located in the periphery of the lung.(5, 6) Depending on lesion characteristics and associated risk factors, tissue sampling is needed to establish a definitive diagnosis and determine the appropriate treatment.

Bronchoscopic analysis of peripheral lung nodules remains challenging despite many technological innovations. The procedure comprises three essential pillars needed for a diagnostic success: navigation to the lesion, confirmation of tool location within the lesion (i.e., tool-in-lesion confirmation) and successful tissue sampling. In the past years, fluoroscopy, radial probe endobronchial ultrasound (r-EBUS), electromagnetic navigation (EMN), virtual bronchoscopy (VB) or cone beam computed tomography (CBCT) combined with augmented fluoroscopy have improved navigation with or without tool-in-lesion confirmation.(7) Additionally, rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%,(8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern, pre-test probability of malignancy and sampling tools used.(9-12) The arrival of robotic bronchoscopy platforms combined with existing techniques have shown promising results with high navigation success rates. However, diagnostic yield remains behind due to substantial mispositioning rates, retaining a large gap between navigation success and diagnostic yield.(13-15) The persistently low diagnostic yield calls for complementary techniques providing real-time information for fine-tuning the needle position.

Confocal laser endomicroscopy (CLE) is a high-resolution microscopic technique that visualizes individual cells in real-time. It has proven useful in the gastroenterology field, where it has been demonstrated that CLE could be used for rapid diagnosis, targeting of biopsies, and prediction of neoplasms.(16) CLE has been recently introduced in the respiratory tract, including for the peripheral lung nodule analysis.(17-19) CLE probes are thin enough to fit through 18G biopsy needles to provide microscopic feedback at the tip of the needle (needle-based CLE (nCLE)). Fluorescein dye is used as a contrast agent and binds to the extracellular matrix, resulting in a highly fluorescent background in which individual cells can be seen. Previous studies have identified three nCLE image characteristics

for malignancy in the lung,(19) and criteria for airway and lung parenchyma.(18) The identification of malignancy and distinction from airway and lung parenchyma were accurate based on these criteria.(18, 19)

A recent study demonstrated a high needle mispositioning rate, as nCLE-imaging resulted in a repositioning of the biopsy needle in 9 out of 20 patients.(20) nCLE could therefore potentially bridge the gap between navigation success and diagnostic yield.

To date, larger studies investigating the effect of the addition of nCLE to bronchoscopic peripheral lung nodule analysis are lacking. The improved diagnostic yield could reduce the necessity further or more invasive diagnostic interventions such as CT-guided transthoracic biopsies or diagnostic surgery. In this multicenter randomized controlled trial, we aim to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules.

Methods and analysis

Study design

This study is an investigator-initiated, international, multicenter, parallel-group randomized controlled trial comparing two bronchoscopy procedures (with or without nCLE) for the diagnosis of suspected peripheral lung nodules. The study flowchart is shown in Figure 1.

Participating centers

The study is executed in university or general hospitals in six countries in Europe and one hospital in the United States. Study inclusion started on 18 October 2023. Other centers will start including in 2024 and the estimated duration of the study is 24 months including follow up.

Randomization

After the participant has given written informed consent, patient data is entered into a digital database (CASTOR Electronic Data Capture (EDC) electronic case report form (eCRF)). We will use a web-based block-randomization module in Castor to randomize participants into the control and interventional group (1:1). Randomization will be stratified by participating center to ensure that the nCLE and non-nCLE group is of the same size in each center. As nodule size has significant impact on

diagnostic yield,(8) we will stratify for nodule size (≤20 mm and >20 mm) to ensure that size is evenly distributed across study arms.

Patients and endoscopists will not be blinded since the physician needs to know if nCLE images must be acquired during bronchoscopy. Pathologists will be blinded to procedure type and raters who will analyze the nCLE videos after the procedure will be blinded to the patient history and histopathological outcome of the tissue samples.

Study population

Consecutive patients will be recruited by their treating physician at pulmonology outpatient clinics of participating centers. Patients are eligible for inclusion if they meet the following inclusion criteria:

1. ≥18 years of age
2. Suspected malignant peripheral lung lesion with an indication for a bronchoscopic diagnostic work-up as determined by the attending physician or tumor board. Peripheral pulmonary lesions are defined as lesions located beyond the visible segmental bronchi, not detectable by regular flexible bronchoscopy
3. Bronchus sign on pre-procedural CT or estimated confidence for successful navigation to the nodule resulting in a r-EBUS signal
4. Solid part of the lesion must be ≧10 mm
5. Largest dimension of lesion size on CT ≦30 mm (long-axis)
6. Ability to understand and willingness to sign a written informed consent

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Inability or non-willingness to provide informed consent
2. Endobronchial visible malignancy on bronchoscopic inspection
3. Target lesion within reach of the linear EBUS scope
4. Failure to comply with the study protocol
5. Known allergy or risk factors for an allergic reaction to fluorescein
6. Pregnancy or breastfeeding
7. Hemodynamic instability
8. Refractory hypoxemia
9. Therapeutic anticoagulant use that cannot be withheld for an appropriate interval before the procedure

10. Unable to tolerate general anesthesia according to the anesthesiologist
11. Undergoing chemotherapy as several chemotherapies have fluorescent properties at the same wavelength (e.g., doxorubicin)

Primary outcome measure

Diagnostic yield (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant, specific benign or non-specific benign confirmed as benign in follow-up], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure). If patients with multiple lesions are included, the diagnostic yield will be computed per nodule.

Secondary outcome measures

1. Diagnostic sensitivity for malignancy (defined as the proportion of patients in whom the bronchoscopic procedure diagnoses malignancy relative to the total number of patients with a final diagnosis of malignancy as determined by the reference standard).
2. Diagnostic yield according to the strict definition by Vachani et al.(21) (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant or specific benign diagnosis], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure).
3. Procedure duration (from bronchoscope insertion until removal).
4. Percentage of patients in which the needle was fine-tuned (defined as moving the needle within the same distal airway) or repositioned (defined as the selection of a different distal airway for tissue sampling) based on nCLE feedback (defined as the number of patients the needle was fine-tuned/repositioned divided by the total number of patients in which nCLE imaging was used).
5. Fluoroscopy radiation time and dose.
6. Diagnostic yield of ROSE (defined as the proportion of patients in whom ROSE resulted in a classifying diagnosis [malignant or specific benign diagnosis], relative to the total number of patients).
7. Proportion of patients in which ROSE provided tool-in-lesion confirmation, meaning that the acquired tissue shows signs of a malignant or non-malignant diagnosis and was not related to airway/lung parenchyma sampling such as bronchus epithelium/blood contamination, and tissue not suitable for a specific diagnosis such as atypical cells.

8. Complication rate (defined as any complication or complication categories occurring during or directly after the bronchoscopic procedure or any procedure-related complication within one week after the procedure).
9. Requirement of additional diagnostic procedures (CT-guided transthoracic biopsies, surgical diagnostics and/or additional bronchoscopy) during the 6-month follow-up period.

Exploratory endpoints

As an exploratory endpoint, we aim to identify potential new nCLE image characteristics for malignant and benign pathologies. We will also create an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods.

Outcome parameters

Table 1 shows the baseline patient characteristics and corresponding procedural information that will be collected at the time of study inclusion, during the procedure and 6-month follow-up period.

Investigational product

The Cellvizio® confocal laser endomicroscopy system with the corresponding AQ-Flex™ 19 miniprobe (Mauna Kea technologies (MKT), Paris, France) is the investigational medical device of this study. The probe has a compatible operating diameter of 0.91 mm, a resolution of 3,5 µm, a penetration depth of 40 to 50 µm and a maximum field of view of 325 µm. The device and corresponding probes are CE-marked and will be used within the intended purpose.(22)

The technique uses a laser beam (488 nm) focused by an objective lens to illuminate the tissue, with the illumination focus at a pre-defined depth. The light strikes the tissue resulting in fluorescent light emission back from autofluorescent structures such as elastin in the airways or an exogenous fluorescent dye such as fluorescein, a contrast dye used for nCLE imaging in the lung. Light originating from the focal layer will be focused by the objective lens at the opening of a pinhole and detected, while light from out-of-focus layers is rejected by the pinhole. This results in high-resolution imaging of individual cells and structures at a specific point with limited influence of (scattered) light from out-of-focus areas.(22)

Table 1: Data to be collected

Patient characteristics
Age
Sex
BMI
Smoking history
Patient cancer history
Family history of lung cancer
Pre-procedural (PET)CT scan lesion characteristics
CT scan quality (slice thickness)
Size (largest diameter)
Localization (segmental level)
Lesion appearance/nodule type (Solid, non-solid/ground glass, partially solid)
Bronchus sign (present(concentric/eccentric)/absent/insufficient CT scan quality)
Spiculation sign (present/absent)
Emphysema (present/absent)
PET uptake (not performed/no uptake/faint *(SUV < 1)*/moderate *(SUV 1 – 2.5)* /intense *(SUV >2.5))*
Intra-procedural information
r-EBUS sign (eccentric, concentric, absent)
Location of tissue sampling (lung segment)
nCLE image observations (for every needle pass)
Needle fine-tuning & repositioning done (for every needle pass)
Sampling techniques used (TBNA, (cryo)biopsy, brush)
ROSE results of tissue sample (if available)
Bronchoscopy start and end time
Fluoroscopy duration
Additional procedures performed (e.g., EBUS/EUS-B/etc.)
(Serious) complications
Post-procedural information
(Serious) complications (up to 1 week after the procedure)
Final pathological diagnosis (cytology and/or histology)
(Additional) Diagnostic follow-up procedures needed (e.g., transthoracic needle biopsies, surgery, additional bronchoscopy, follow-up imaging) including (altered) diagnosis and/or results of follow-up CT-scans of the lesion(s)

RUL: right upper lobe; RML: right middle lobe; RLL; right lower lobe; LUP: left upper lobe; LLL: left lower lobe; CT: computed tomography; ROSE: rapid on-site evaluation; r-EBUS: radial endobronchial ultrasound; TBNA: transbronchial needle aspiration; SUV: standard uptake value

Study procedures

Conventional diagnostic bronchoscopy (control group & intervention group)

The following procedure will be performed routinely (regardless of study participation): Bronchoscopic procedures will be performed by experienced pulmonologists specifically trained in navigational bronchoscopy and nCLE-imaging. All procedures are performed according to institutional practice, usually on an outpatient basis. Patient preparation and sedation will be done according to institutional practice and might include propofol or midazolam sedation and the use of topical anesthesia. Vital parameters will be monitored during and after the procedure.

Systematic bronchoscopic inspection of the airways will be performed, followed by r-EBUS imaging (guide sheath optional) to select the distal airway with the highest probability of reaching the lesion. The use of fluoroscopy, EMN, VB or ultrathin bronchoscope is optional if regularly used at that institution. CBCT navigation with or without augmented fluoroscopy and robotic bronchoscopy will not be used in patients included in this trial. Bronchoscopist may use these techniques after following all actions related to this protocol while ensuring tissue samples are processed separately. Transbronchial needle aspirations (TBNA) using the 18G FleXNeedle® (Broncus Medical Inc., San Jose, United States of America) and (cryo)biopsies will be performed to acquire tissue for pathological evaluation (a recommended minimum of 3 TBNA and 3 biopsies). During the bronchoscopic work up, some of the cytological aspirations will be evaluated on site (Rapid onsite evaluation, ROSE) and the representativeness of the samples will be reported to the bronchoscopist. ROSE will always be performed for the first TBNA pass. For the following passes, the bronchoscopist decides if it is indicated.

Addition of nCLE imaging (intervention group)

The same procedure will be performed as described above for the patients randomized to the intervention arm, except for the addition of fluorescein administration and nCLE imaging before TBNA. Prior to the procedure, an 18G needle is preloaded with the CLE probe (AQ-Flex™ 19 Miniprobe, Mauna Kea Technologies, Paris, France). The CLE probe is advanced through the needle until the probe is positioned approximately 4 mm past the needle tip and secured using a locking device to maintain the probe position relative to the needle tip.

After determining the sample location based on r-EBUS and/or fluoroscopy, fluorescein (2.5 mL of 10% fluoresceindinatrium solution) is administered intravenously. Then, the preloaded 18G needle punctures the target area, followed by the insertion of the CLE probe through the biopsy needle for real-time microscopic feedback. In case nCLE visualizes airway or lung parenchyma, indicating a near-miss, the biopsy needle is fine-tuned (i.e., the needle is moved within the same distal airway) or repositioned (i.e., a different distal airway is chosen). If nCLE demonstrates that the biopsy needle is placed within the lesion, the CLE probe is removed from the biopsy needle while holding the needle in position, followed by tissue sampling at the same location (repeated for at least 3 TBNAs). A flowchart of the procedure steps for both the conventional bronchoscopy and the nCLE-guided bronchoscopy is shown in Figure 2.

nCLE image interpretation

The airway and lung parenchyma nCLE criteria as described by Kramer et al.(18) will be considered as "out-of-lesion" criteria indicating mispositioning of the needle. Currently known criteria for "tool-in-lesion" are malignancy criteria and granuloma criteria.(18, 19, 23) nCLE images will be interpreted during the procedure by the performing bronchoscopist and their team. Additionally, all videos are rated post-procedure by blinded raters of the initiating center to establish a ground truth interpretation of the images.

Pathological examination

The cytological and histological examination will be done according to standard hospital procedure. In case the bronchoscopic procedure is considered non-diagnostic, additional procedures (transthoracic needle aspiration, surgical procedure, etc.) could follow to obtain a definite diagnosis. Results of the nCLE imaging do not influence the indication for additional diagnostic procedures. If a surgical procedure is indicated, the histological images will be collected to compare this with the nCLE imaging.

In this study, the final pathological diagnosis will be subdivided into four categories as described by Vachani et al.,(21) namely [1] malignant, [2] non-malignant, which is divided into specific benign (including granulomatous, infectious and lymphocyte-predominant patterns) or nonspecific benign (e.g. inflammation), and [3] non-diagnostic (i.e., insufficient material for classifying diagnosis or in case atypical cells could not be classified further).

Reference standard

For the primary outcome, diagnostic yield will be calculated using the intermediate method described by Vachani et al.(21) The abovementioned final pathological diagnosis categories will be used regardless of the results of the reference standard, except for initial non-specific benign diagnoses. In these cases, results from the reference standard will be considered. If the initial benign diagnosis is confirmed benign in follow-up, the bronchoscopic procedure will be considered diagnostic.

For the calculation of diagnostic sensitivity, malignant cases identified by the procedures under investigation will be considered as true positive since false positive results (almost) never occur. Benign (either specific or non-specific) and non-diagnostic samples will undergo a reference standard, which can be a subsequent sampling method such as transthoracic needle biopsy or surgery. Alternatively, if no subsequent sampling method is performed, clinical and radiological follow-up at 6 months is considered the reference standard. If follow-up CT imaging shows regression or resolution of the nodule or in case a nodule remains stable, it will be considered as a confirmation of non-malignant diagnosis (i.e., true negative). Cases that are benign (either specific or non-specific) or non-diagnostic at the index bronchoscopy will be considered false negative if a malignancy diagnosis is established by the reference standard or if therapeutic procedures are done without confirmation of diagnosis. Figure 3 gives a schematic overview of the calculation methods of diagnostic yield and sensitivity for malignancy.

Informed consent procedure

Patients will be recruited by their treating physician. If the patient is willing to receive more information about study participation, information will be provided by the local investigator. The eligible participants will have sufficient time to consider their consent. Written informed consent must be provided before any study-related procedures take place. After informed consent, patients will be randomized using Castor EDC software and assigned to the control or intervention group. The bronchoscopy will then be performed according to the study protocol. In case patients decline participation in the study, they will be treated to the usual local clinical practices and guidelines.

Quality assurance

Only experienced pulmonologists will perform the procedures to ensure high-quality bronchoscopic procedures. Additionally, all participating centers will be trained in the use of the CLE Cellvizio device and to maintain homogeneous quality of the nCLE image acquisition and interpretation over all centers. Training entails theoretic and practical training by the initiating center with extensive nCLE experience and MKT representatives.

Sample size justification

Based on previous studies and meta-analyses, we expect the diagnostic yield in patients with a lesion <30 mm in the conventional bronchoscopy arm to be 62%.(24, 25) We hypothesize that additional nCLE guidance in the intervention arm will result in a diagnostic yield of 80%. In total, 198 patients are needed to show that nCLE guidance results in a diagnostic yield that is 18 percent point higher than the conventional bronchoscopy arm (alpha=0.05 and power=0.80). Taking into account a 5% study drop-out, a total of 208 patients will be included. We believe an increase in the diagnostic yield (from 62% to 80%) demonstrates a clinically relevant improvement in lung cancer diagnosis.

Data analysis

Results for continuous variables will be expressed as means and standard deviations or medians with interquartile ranges. Categorical variables will be expressed as frequencies and percentages. The Chi-squared test will be used to compare diagnostic yield (or other comparisons between categorical variables) between the two randomization groups. Continuous variables will be compared using Student's t-test, or Mann-Whitney-U tests. A two-tailed P-value <0.05 will be considered statistically significant. All analyses are done based on an intention-to-treat approach, meaning that patients are analyzed as part of the intervention group they were assigned to, even if nCLE imaging was not executed in a patient in the intervention arm due to unforeseen circumstances. These specific cases will be reported in the manuscript. Patients not undergoing the planned bronchoscopy procedure after randomization are excluded from the analysis. Patients with missing outcome data will be excluded from analysis. Patients with incomplete essential follow-up information will also be excluded from the calculation of diagnostic sensitivity. However, we will also calculate diagnostic sensitivity based on a 'worst-case scenario', in which these patients are considered false negatives. For the primary outcome, subgroup analysis will be performed for several lesions and procedural characteristics (lesion size (≤20 mm vs >20 mm), radial EBUS image (eccentric vs concentric vs absent), location (upper lobe (without lingual) vs middle lobe/lingual vs lower lobe), pre-test probability that the nodule is cancerous (<10%, 10 – 35%, 36-70% and >70%) based on the Brock score.(26)

Patient and Public Involvement statement

There was no patients or public involvement in the design of this study. An original research manuscript will be prepared to present the study results.

Ethics and dissemination

The CLEVER study will be conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the Medical Research Involving Human Subjects Act (WMO, The Netherlands) principles. To date, the Medical Ethical Committee of the Amsterdam UMC (NL83257.018.22), Athens Chest Hospital (21583/25-08-23) and General University Hospital in Prague (č.j. 143/23 S) have approved the study. All participating sites will obtain local ethical approval prior to starting inclusions. Written informed consent will be obtained prior to randomization and any study-related procedures. In case of major changes to the protocol, the ethical review board will be notified, and it will be communicated with all participating centers and registered on clinicaltrials.gov.

Data management and safety

After informed consent, the patient will be given a code. This code will be used on all (pseudonymized) data, including CLE images and electronic Case Report Form (eCRF) data. Castor Electronic Data Capture ecosystem (International Organization of Standardization (ISO) 27001 and 9001 certified) will be used to collect key patient information described in outcome parameters. The key to the code is safeguarded by the local principal investigator and access to all records is limited to directly involved researchers. The coordinating investigator will centralize patients' data, and principal investigators will have direct access to their own site's data sets and to other sites' data upon reasonable request. All principal investigators will maintain records, including signed patient informed consent forms and information on adverse events.

Data management of all data (collection, storage, and analysis) will be done according to the local data management plan. All records will be stored for a period of 15 years following the completion or termination of the study. Monitoring will be done according to a monitoring plan with specific attention paid to informed consent, completion of the eCRF, and storage of CLE video data.

Patient safety and adverse events

The study was deemed a negligible risk study (according to the Nederlandse Federatie van Universitaire Medisch Centra (NFU) descriptions) by the ethical committee of the Amsterdam UMC. Previous study publications showed that nCLE-imaging and intravenous fluorescein administration are safe.(27) Fluorescein adverse reactions are rare and mostly mild in nature. No study related adverse

events occurred in the prior bronchoscopic nCLE studies in the Amsterdam UMC.(18, 19) Estimated prolonged endoscopy time due to study participation is approximately 10 minutes. Patients will not be aware of this as they will already be sedated for the bronchoscopic procedure.

In case any (serious) adverse event ((S)AE) occur during the procedure or up to one week after the procedure, the sponsor will register SAEs through the web portal *Toetsingonline* to the accredited METC that has approved the protocol. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the trial procedure. The severity and possible relatedness to the investigational product or the procedure will be documented. Investigators of the participating centers will report all serious adverse events to the coordinating and principal investigator of the initiating site. Reporting of SAEs that result in death or are life-threatening will be done within 7 days after initial identification, followed by a period of a maximum of 8 days to complete the preliminary report. All other SAEs will be reported within 15 days after first knowledge of the SAE.

Annual progress report

The sponsor will ensure that a progress report is submitted to the medical ethics committee once a year. Information on the start date of inclusion, number of subjects included to date, number of subjects that have completed participation, serious adverse events, and amendments.

Dissemination

We aim to publish the study results in a peer-reviewed journal. Reporting will be in line with CONSORT and STARD 2015 reporting guidelines.(28, 29)

Discussion

In this multicenter, investigator-initiated, randomized controlled trial, we aim to determine if the addition of nCLE-imaging to bronchoscopic peripheral lung lesion analysis results in an improved diagnostic yield.

Since there still is a gap between the success rate of navigating the tissue sampling instrument toward the target lesion and the final diagnostic yield, there is a need for real-time tool-in-lesion confirmation. The addition of high-resolution microscopic nCLE imaging at the tip of the needle could potentially lead to a decrease in mispositioning rates and an improved diagnostic yield. As a result, fewer patients

would need additional diagnostic procedures such as transthoracic needle biopsy or surgery, which are more invasive and have higher incidences of complications such as pneumothorax and hemorrhage.(30) Previous smaller studies have already shown that nCLE is safe, and raters can distinguish different image characteristics with high accuracy. On top of that, it has also been demonstrated that fine-tuning the needle based on these image characteristics is often done, even when navigation to the lesion was successful.(18-20) However, nCLE image interpretation remains subjective and challenging, especially when interpreting images live in the bronchoscopy suite. As described by Tian et al.(31), the role of artificial intelligence might be important to make the technique routinely implementable in clinical practice. An exploratory endpoint of this study is to develop a deep-learning network for automated image interpretation. This is the first step towards easier, quicker and reproducible image interpretation.

Current literature on nCLE imaging for this purpose remains limited to smaller patient groups and the clinical benefit remains to be demonstrated. The results of the CLEVER study provide a formal comparison between conventional image-guided diagnostic bronchoscopy and conventional bronchoscopy with the addition of nCLE in a large randomized patient group. The results of this trial will clarify the added benefit of nCLE for bronchoscopic diagnosis of peripheral lung nodules and identify which patients could benefit from the use of this technique.

Author contributions

SvH, TK, DAK, DMdB, PIB and JTA were involved in conception and trial design. SvH, TK, DAK, PIB and JTA were involved in drafting of the study protocol. DAK provided statistical expertise. All authors were involved in editing and final approval of the protocol. SvH, DG, CB, JH, MH, VP, CR, AS, GS, EK, NA, JV, ZS, MAH, JMAD, PIB and JTA will be involved in the conduct of the study and data acquisition. SvH, DAK, DMdB, PIB and JTA will be involved in the data analysis and interpretation. SvH, DAK, PIB and JTA will be involved in drafting of the final manuscript for dissemination. All authors will provide editing and approval of the final manuscript for publication.

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Declaration of interests

JTA declares material and financial support to the sponsor's institution from Mauna Kea Technologies for this study.

Data sharing statement

The final trial dataset will be available to study investigators on completion of the trial, and after publication of the primary manuscript. All data relevant to the study will be included in the article or uploaded as supplementary information. Data are available upon reasonable request.

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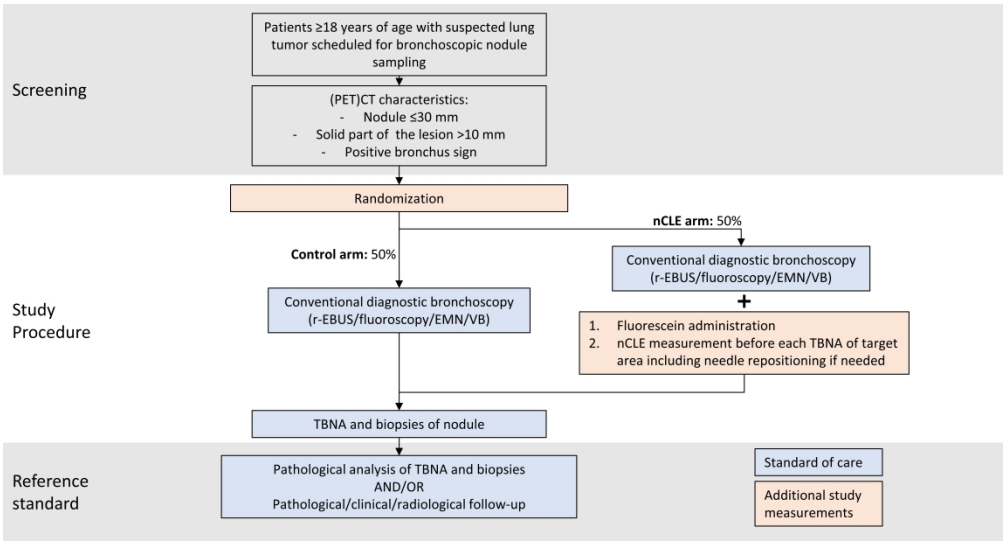
Figure headings

Figure 1: Study flowchart. r-EBUS: radial endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: neelde-based confocal laser endomicroscopy; TBNA: transbronchial needle aspiration; PET: positron emission tomography; CT: computed tomography

Figure 2: Procedure flowchart for control and interventional group (without and with nCLE). Note: fluorescein administration is only done once before the first puncture. TBNA: transbronchial needle aspiration; ROSE: rapid on-site evaluation; EBUS: endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: needle-based confocal laser endomicroscopy

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography



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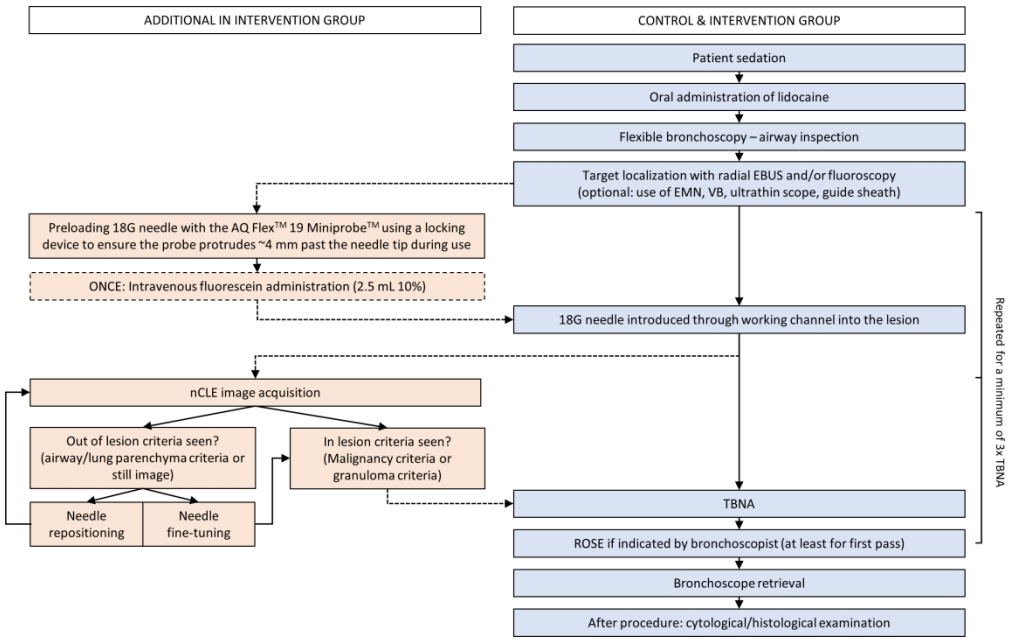

Figure 2: Procedure flowchart for control and interventional group (without and with nCLE). Note: fluorescein administration is only done once before the first puncture. TBNA: transbronchial needle aspiration; ROSE: rapid on-site evaluation; EBUS: endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: needle-based confocal laser endomicroscopy

246x155mm (300 x 300 DPI)

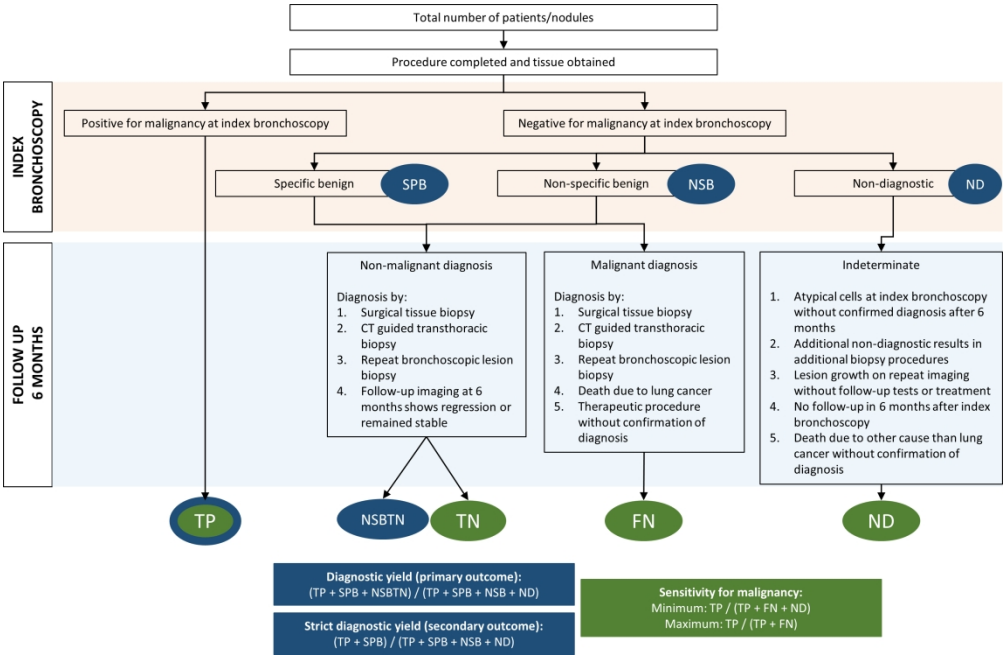

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography

262x170mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1 and 14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1, 14, 15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9,10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9,10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	11

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4,5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4,5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4,5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4,5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7, 11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12,13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10,11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10,11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to	13

		where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary information
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Oncology, Pathology, Diagnostics
Keywords:	Biopsy, Clinical Trial, Diagnostic Imaging, Respiratory tract tumours < ONCOLOGY, Cytopathology < PATHOLOGY

Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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Abstract

Introduction: Despite many technological advances, the diagnostic yield of bronchoscopic peripheral lung nodule analysis remains limited due to frequent mispositioning. Needle-based confocal laser endomicroscopy (nCLE) enables real-time microscopic feedback on needle positioning, potentially improving the sampling location and diagnostic yield. Previous studies have defined and validated nCLE criteria for malignancy, airway, and lung parenchyma. Larger studies demonstrating the effect of nCLE on diagnostic yield are lacking. We aim to investigate if nCLE-imaging integrated with

conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE.

Methods and analysis: This is a parallel-group randomized controlled trial. Recruitment is performed at pulmonology outpatient clinics in university and general hospitals in six different European countries and one hospital in the United States. Consecutive patients with a for malignancy suspected peripheral lung nodule (10-30 mm) with an indication for diagnostic bronchoscopy will be screened, and 208 patients will be included. Web-based randomization (1:1) between the two procedures will be performed. The primary outcome is diagnostic yield. Secondary outcomes include diagnostic sensitivity for malignancy, needle repositionings, procedure and fluoroscopy duration, and complications. Pathologists will be blinded to procedure type; patients and endoscopists will not.

Discussion: Results of the CLEVER trial will inform on the added value of nCLE for the bronchoscopic diagnosis of peripheral lung nodules.

Ethics and dissemination: Primary approval by the Ethics Committee of the Amsterdam University Medical Center. Dissemination involves publication in a peer-reviewed journal.

Support: Financial and material support from Mauna Kea Technologies.

Trial registration: ClinicalTrials.gov NCT06079970.

Keywords: Respiratory tract neoplasms, bronchoscopy, confocal laser scanning microscopy, nCLE, rapid-on-site evaluation (ROSE), histology/cytology, lung cancer, peripheral lung nodule

Manuscript word count: 4187

Article summary

Strengths and limitations

- This is the first (international multicenter) randomized controlled trial on needle-based confocal laser endomicoscopy (nCLE) for bronchoscopic diagnosis of peripheral lung nodules.
- This study provides the opportunity to evaluate the added benefit of the nCLE technique to conventional diagnostic bronchoscopy including radial EBUS in multiple centers and countries.
- The definition of diagnostic yield is under debate. In this study, the diagnostic yield will be reported based on two different definitions for better comparison with existing and future studies.
- Each participating center uses their own methods for conventional bronchoscopic diagnosis of peripheral lung nodules and will therefore not be completely uniform across all centers. Each

center will keep conventional methods uniform in both the control and intervention group to ensure differences can be attributed to the nCLE technique.

- In this study only peripheral pulmonary nodules between 1 – 3 cm are included.

Introduction

Lung cancer remains the leading cause of cancer-related deaths, with 2.09 million new diagnoses and 1.76 million deaths worldwide per year.(1, 2) The increased use of chest computed tomography (CT) and the future implementation of low-dose CT lung cancer screening result in an increased detection of lung nodules.(3, 4) Consequently, more early-stage lung cancer is detected, which is most often located in the periphery of the lung.(5, 6) Depending on lesion characteristics and associated risk factors, tissue sampling is needed to establish a definitive diagnosis and determine the appropriate treatment.

Bronchoscopic analysis of peripheral lung nodules remains challenging despite many technological innovations. The procedure comprises three essential pillars needed for a diagnostic success: navigation to the lesion, confirmation of tool location within the lesion (i.e., tool-in-lesion confirmation) and successful tissue sampling. In the past years, fluoroscopy, radial probe endobronchial ultrasound (r-EBUS), electromagnetic navigation (EMN), virtual bronchoscopy (VB) or cone beam computed tomography (CBCT) combined with augmented fluoroscopy have improved navigation with or without tool-in-lesion confirmation.(7) Additionally, rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%,(8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern, pre-test probability of malignancy and sampling tools used.(9-12) The arrival of robotic bronchoscopy platforms combined with existing techniques have shown promising results with high navigation success rates. However, diagnostic yield remains behind due to substantial mispositioning rates, retaining a large gap between navigation success and diagnostic yield.(13-15) The persistently low diagnostic yield calls for complementary techniques providing real-time information for fine-tuning the needle position.

Confocal laser endomicroscopy (CLE) is a high-resolution microscopic technique that visualizes individual cells in real-time. It has proven useful in the gastroenterology field, where it has been demonstrated that CLE could be used for rapid diagnosis, targeting of biopsies, and prediction of neoplasms.(16) CLE has been recently introduced in the respiratory tract, including for the peripheral lung nodule analysis.(17-19) CLE probes are thin enough to fit through 18G biopsy needles to provide microscopic feedback at the tip of the needle (needle-based CLE (nCLE)). Fluorescein dye is used as a contrast agent and binds to the extracellular matrix, resulting in a highly fluorescent background in which individual cells can be seen. Previous studies have identified three nCLE image characteristics

for malignancy in the lung,(19) and criteria for airway and lung parenchyma.(18) The identification of malignancy and distinction from airway and lung parenchyma were accurate based on these criteria.(18, 19)

A recent study demonstrated a high needle mispositioning rate, as nCLE-imaging resulted in a repositioning of the biopsy needle in 9 out of 20 patients.(20) nCLE could therefore potentially bridge the gap between navigation success and diagnostic yield.

To date, larger studies investigating the effect of the addition of nCLE to bronchoscopic peripheral lung nodule analysis are lacking. The improved diagnostic yield could reduce the necessity further or more invasive diagnostic interventions such as CT-guided transthoracic biopsies or diagnostic surgery. In this multicenter randomized controlled trial, we aim to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules.

Methods and analysis

Study design

This study is an investigator-initiated, international, multicenter, parallel-group randomized controlled trial comparing two bronchoscopy procedures (with or without nCLE) for the diagnosis of suspected peripheral lung nodules. The study flowchart is shown in Figure 1.

Participating centers

The study is executed in university or general hospitals in six countries in Europe and one hospital in the United States. Study inclusion started on 18 October 2023. Other centers will start including in 2024 and the estimated duration of the study is 24 months including follow up.

Randomization

After the participant has given written informed consent, patient data is entered into a digital database (CASTOR Electronic Data Capture (EDC) electronic case report form (eCRF)). We will use a web-based block-randomization module in Castor to randomize participants into the control and interventional group (1:1). Randomization will be stratified by participating center to ensure that the nCLE and non-nCLE group is of the same size in each center. As nodule size has significant impact on

diagnostic yield,(8) we will stratify for nodule size (≤20 mm and >20 mm) to ensure that size is evenly distributed across study arms.

Patients and endoscopists will not be blinded since the physician needs to know if nCLE images must be acquired during bronchoscopy. Pathologists will be blinded to procedure type and raters who will analyze the nCLE videos after the procedure will be blinded to the patient history and histopathological outcome of the tissue samples.

Study population

Consecutive patients will be recruited by their treating physician at pulmonology outpatient clinics of participating centers. Patients are eligible for inclusion if they meet the following inclusion criteria:

1. ≥18 years of age
2. Suspected malignant peripheral lung lesion with an indication for a bronchoscopic diagnostic work-up as determined by the attending physician or tumor board. Peripheral pulmonary lesions are defined as lesions located beyond the visible segmental bronchi, not detectable by regular flexible bronchoscopy
3. Bronchus sign on pre-procedural CT or estimated confidence for successful navigation to the nodule resulting in a r-EBUS signal
4. Solid part of the lesion must be ≧10 mm
5. Largest dimension of lesion size on CT ≦30 mm (long-axis)
6. Ability to understand and willingness to sign a written informed consent

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Inability or non-willingness to provide informed consent
2. Endobronchial visible malignancy on bronchoscopic inspection
3. Target lesion within reach of the linear EBUS scope
4. Failure to comply with the study protocol
5. Known allergy or risk factors for an allergic reaction to fluorescein
6. Pregnancy or breastfeeding
7. Hemodynamic instability
8. Refractory hypoxemia
9. Therapeutic anticoagulant use that cannot be withheld for an appropriate interval before the procedure

10. Unable to tolerate general anesthesia according to the anesthesiologist
11. Undergoing chemotherapy as several chemotherapies have fluorescent properties at the same wavelength (e.g., doxorubicin)

Primary outcome measure

Diagnostic yield (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant, specific benign or non-specific benign confirmed as benign in follow-up], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure). If patients with multiple lesions are included, the diagnostic yield will be computed per nodule.

Secondary outcome measures

1. Diagnostic sensitivity for malignancy (defined as the proportion of patients in whom the bronchoscopic procedure diagnoses malignancy relative to the total number of patients with a final diagnosis of malignancy as determined by the reference standard).
2. Diagnostic yield according to the strict definition by Vachani et al.(21) (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant or specific benign diagnosis], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure).
3. Procedure duration (from bronchoscope insertion until removal).
4. Percentage of patients in which the needle was fine-tuned (defined as moving the needle within the same distal airway) or repositioned (defined as the selection of a different distal airway for tissue sampling) based on nCLE feedback (defined as the number of patients the needle was fine-tuned/repositioned divided by the total number of patients in which nCLE imaging was used).
5. Fluoroscopy radiation time and dose.
6. Diagnostic yield of ROSE (defined as the proportion of patients in whom ROSE resulted in a classifying diagnosis [malignant or specific benign diagnosis], relative to the total number of patients).
7. Proportion of patients in which ROSE provided tool-in-lesion confirmation, meaning that the acquired tissue shows signs of a malignant or non-malignant diagnosis and was not related to airway/lung parenchyma sampling such as bronchus epithelium/blood contamination, and tissue not suitable for a specific diagnosis such as atypical cells.

8. Complication rate (defined as any complication or complication categories occurring during or directly after the bronchoscopic procedure or any procedure-related complication within one week after the procedure).
9. Requirement of additional diagnostic procedures (CT-guided transthoracic biopsies, surgical diagnostics and/or additional bronchoscopy) during the 6-month follow-up period.

Exploratory endpoints

As an exploratory endpoint, we aim to identify potential new nCLE image characteristics for malignant and benign pathologies. We will also create an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods.

Outcome parameters

Table 1 shows the baseline patient characteristics and corresponding procedural information that will be collected at the time of study inclusion, during the procedure and 6-month follow-up period.

Investigational product

The Cellvizio® confocal laser endomicroscopy system with the corresponding AQ-Flex™ 19 miniprobe (Mauna Kea technologies (MKT), Paris, France) is the investigational medical device of this study. The probe has a compatible operating diameter of 0.91 mm, a resolution of 3,5 µm, a penetration depth of 40 to 50 µm and a maximum field of view of 325 µm. The device and corresponding probes are CE-marked and will be used within the intended purpose.(22)

The technique uses a laser beam (488 nm) focused by an objective lens to illuminate the tissue, with the illumination focus at a pre-defined depth. The light strikes the tissue resulting in fluorescent light emission back from autofluorescent structures such as elastin in the airways or an exogenous fluorescent dye such as fluorescein, a contrast dye used for nCLE imaging in the lung. Light originating from the focal layer will be focused by the objective lens at the opening of a pinhole and detected, while light from out-of-focus layers is rejected by the pinhole. This results in high-resolution imaging of individual cells and structures at a specific point with limited influence of (scattered) light from out-of-focus areas.(22)

Table 1: Data to be collected

Patient characteristics
Age
Sex
BMI
Smoking history
Patient cancer history
Family history of lung cancer
Pre-procedural (PET)CT scan lesion characteristics
CT scan quality (slice thickness)
Size (largest diameter)
Localization (segmental level)
Lesion appearance/nodule type (Solid, non-solid/ground glass, partially solid)
Bronchus sign (present(concentric/eccentric)/absent/insufficient CT scan quality)
Spiculation sign (present/absent)
Emphysema (present/absent)
PET uptake (not performed/no uptake/faint *(SUV < 1)*/moderate *(SUV 1 – 2.5)* /intense *(SUV >2.5))*
Intra-procedural information
r-EBUS sign (eccentric, concentric, absent)
Location of tissue sampling (lung segment)
nCLE image observations (for every needle pass)
Needle fine-tuning & repositioning done (for every needle pass)
Sampling techniques used (TBNA, (cryo)biopsy, brush)
ROSE results of tissue sample (if available)
Bronchoscopy start and end time
Fluoroscopy duration
Additional procedures performed (e.g., EBUS/EUS-B/etc.)
(Serious) complications
Post-procedural information
(Serious) complications (up to 1 week after the procedure)
Final pathological diagnosis (cytology and/or histology)
(Additional) Diagnostic follow-up procedures needed (e.g., transthoracic needle biopsies, surgery, additional bronchoscopy, follow-up imaging) including (altered) diagnosis and/or results of follow-up CT-scans of the lesion(s)

RUL: right upper lobe; RML: right middle lobe; RLL; right lower lobe; LUP: left upper lobe; LLL: left lower lobe; CT: computed tomography; ROSE: rapid on-site evaluation; r-EBUS: radial endobronchial ultrasound; TBNA: transbronchial needle aspiration; SUV: standard uptake value

Study procedures

Conventional diagnostic bronchoscopy (control group & intervention group)

The following procedure will be performed routinely (regardless of study participation): Bronchoscopic procedures will be performed by experienced pulmonologists specifically trained in navigational bronchoscopy and nCLE-imaging. All procedures are performed according to institutional practice, usually on an outpatient basis. Patient preparation and sedation will be done according to institutional practice and might include propofol or midazolam sedation and the use of topical anesthesia. Vital parameters will be monitored during and after the procedure.

Systematic bronchoscopic inspection of the airways will be performed, followed by r-EBUS imaging (guide sheath optional) to select the distal airway with the highest probability of reaching the lesion. The use of fluoroscopy, EMN, VB or ultrathin bronchoscope is optional if regularly used at that institution. CBCT navigation with or without augmented fluoroscopy and robotic bronchoscopy will not be used in patients included in this trial. Bronchoscopist may use these techniques after following all actions related to this protocol while ensuring tissue samples are processed separately. Transbronchial needle aspirations (TBNA) using the 18G FleXNeedle® (Broncus Medical Inc., San Jose, United States of America) and (cryo)biopsies will be performed to acquire tissue for pathological evaluation (a recommended minimum of 3 TBNA and 3 biopsies). During the bronchoscopic work up, some of the cytological aspirations will be evaluated on site (Rapid onsite evaluation, ROSE) and the representativeness of the samples will be reported to the bronchoscopist. ROSE will always be performed for the first TBNA pass. For the following passes, the bronchoscopist decides if it is indicated.

Addition of nCLE imaging (intervention group)

The same procedure will be performed as described above for the patients randomized to the intervention arm, except for the addition of fluorescein administration and nCLE imaging before TBNA. Prior to the procedure, an 18G needle is preloaded with the CLE probe (AQ-Flex™ 19 Miniprobe, Mauna Kea Technologies, Paris, France). The CLE probe is advanced through the needle until the probe is positioned approximately 4 mm past the needle tip and secured using a locking device to maintain the probe position relative to the needle tip.

After determining the sample location based on r-EBUS and/or fluoroscopy, fluorescein (2.5 mL of 10% fluoresceindinatrium solution) is administered intravenously. Then, the preloaded 18G needle punctures the target area, followed by the insertion of the CLE probe through the biopsy needle for real-time microscopic feedback. In case nCLE visualizes airway or lung parenchyma, indicating a near-miss, the biopsy needle is fine-tuned (i.e., the needle is moved within the same distal airway) or repositioned (i.e., a different distal airway is chosen). If nCLE demonstrates that the biopsy needle is placed within the lesion, the CLE probe is removed from the biopsy needle while holding the needle in position, followed by tissue sampling at the same location (repeated for at least 3 TBNAs). A flowchart of the procedure steps for both the conventional bronchoscopy and the nCLE-guided bronchoscopy is shown in Figure 2.

nCLE image interpretation

The airway and lung parenchyma nCLE criteria as described by Kramer et al.(18) will be considered as "out-of-lesion" criteria indicating mispositioning of the needle. Currently known criteria for "tool-in-lesion" are malignancy criteria and granuloma criteria.(18, 19, 23) nCLE images will be interpreted during the procedure by the performing bronchoscopist and their team. Additionally, all videos are rated post-procedure by blinded raters of the initiating center to establish a ground truth interpretation of the images.

Pathological examination

The cytological and histological examination will be done according to standard hospital procedure. In case the bronchoscopic procedure is considered non-diagnostic, additional procedures (transthoracic needle aspiration, surgical procedure, etc.) could follow to obtain a definite diagnosis. Results of the nCLE imaging do not influence the indication for additional diagnostic procedures. If a surgical procedure is indicated, the histological images will be collected to compare this with the nCLE imaging.

In this study, the final pathological diagnosis will be subdivided into four categories as described by Vachani et al.,(21) namely [1] malignant, [2] non-malignant, which is divided into specific benign (including granulomatous, infectious and lymphocyte-predominant patterns) or nonspecific benign (e.g. inflammation), and [3] non-diagnostic (i.e., insufficient material for classifying diagnosis or in case atypical cells could not be classified further).

Reference standard

For the primary outcome, diagnostic yield will be calculated using the intermediate method described by Vachani et al.(21) The abovementioned final pathological diagnosis categories will be used regardless of the results of the reference standard, except for initial non-specific benign diagnoses. In these cases, results from the reference standard will be considered. If the initial benign diagnosis is confirmed benign in follow-up, the bronchoscopic procedure will be considered diagnostic.

For the calculation of diagnostic sensitivity, malignant cases identified by the procedures under investigation will be considered as true positive since false positive results (almost) never occur. Benign (either specific or non-specific) and non-diagnostic samples will undergo a reference standard, which can be a subsequent sampling method such as transthoracic needle biopsy or surgery. Alternatively, if no subsequent sampling method is performed, clinical and radiological follow-up at 6 months is considered the reference standard. If follow-up CT imaging shows regression or resolution of the nodule or in case a nodule remains stable, it will be considered as a confirmation of non-malignant diagnosis (i.e., true negative). Cases that are benign (either specific or non-specific) or non-diagnostic at the index bronchoscopy will be considered false negative if a malignancy diagnosis is established by the reference standard or if therapeutic procedures are done without confirmation of diagnosis. Figure 3 gives a schematic overview of the calculation methods of diagnostic yield and sensitivity for malignancy.

Informed consent procedure

Patients will be recruited by their treating physician. If the patient is willing to receive more information about study participation, information will be provided by the local investigator. The eligible participants will have sufficient time to consider their consent. Written informed consent must be provided before any study-related procedures take place. After informed consent, patients will be randomized using Castor EDC software and assigned to the control or intervention group. The bronchoscopy will then be performed according to the study protocol. In case patients decline participation in the study, they will be treated to the usual local clinical practices and guidelines.

Quality assurance

Only experienced pulmonologists will perform the procedures to ensure high-quality bronchoscopic procedures. Additionally, all participating centers will be trained in the use of the CLE Cellvizio device and to maintain homogeneous quality of the nCLE image acquisition and interpretation over all centers. Training entails theoretic and practical training by the initiating center with extensive nCLE experience and MKT representatives.

Sample size justification

Based on previous studies and meta-analyses, we expect the diagnostic yield in patients with a lesion <30 mm in the conventional bronchoscopy arm to be 62%.(24, 25) We hypothesize that additional nCLE guidance in the intervention arm will result in a diagnostic yield of 80%. In total, 198 patients are needed to show that nCLE guidance results in a diagnostic yield that is 18 percent point higher than the conventional bronchoscopy arm (alpha=0.05 and power=0.80). Taking into account a 5% study drop-out, a total of 208 patients will be included. We believe an increase in the diagnostic yield (from 62% to 80%) demonstrates a clinically relevant improvement in lung cancer diagnosis.

Data analysis

Results for continuous variables will be expressed as means and standard deviations or medians with interquartile ranges. Categorical variables will be expressed as frequencies and percentages. The Chi-squared test will be used to compare diagnostic yield (or other comparisons between categorical variables) between the two randomization groups. Continuous variables will be compared using Student's t-test, or Mann-Whitney-U tests. A two-tailed P-value <0.05 will be considered statistically significant. All analyses are done based on an intention-to-treat approach, meaning that patients are analyzed as part of the intervention group they were assigned to, even if nCLE imaging was not executed in a patient in the intervention arm due to unforeseen circumstances. These specific cases will be reported in the manuscript. Patients not undergoing the planned bronchoscopy procedure after randomization are excluded from the analysis. Patients with missing outcome data will be excluded from analysis. Patients with incomplete essential follow-up information will also be excluded from the calculation of diagnostic sensitivity. However, we will also calculate diagnostic sensitivity based on a 'worst-case scenario', in which these patients are considered false negatives. For the primary outcome, subgroup analysis will be performed for several lesions and procedural characteristics (lesion size (≤20 mm vs >20 mm), radial EBUS image (eccentric vs concentric vs absent), location (upper lobe (without lingual) vs middle lobe/lingual vs lower lobe), pre-test probability that the nodule is cancerous (<10%, 10 – 35%, 36-70% and >70%) based on the Brock score.(26)

Patient and Public Involvement statement

There was no patients or public involvement in the design of this study. An original research manuscript will be prepared to present the study results.

Ethics, amendments and dissemination

The CLEVER study will be conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the Medical Research Involving Human Subjects Act (WMO, The Netherlands) principles. To date, the Medical Ethical Committee of the Amsterdam UMC (NL83257.018.22), Athens Chest Hospital (21583/25-08-23) and General University Hospital in Prague (č.j. 143/23 S) have approved the study. All participating sites will obtain local ethical approval prior to starting inclusions. Written informed consent will be obtained prior to randomization and any study-related procedures. In case of major changes to the protocol, the ethical review board will be notified, and it will be communicated with all participating centers and registered on clinicaltrials.gov.

Data management and safety

After informed consent, the patient will be given a code. This code will be used on all (pseudonymized) data, including CLE images and electronic Case Report Form (eCRF) data. Castor Electronic Data Capture ecosystem (International Organization of Standardization (ISO) 27001 and 9001 certified) will be used to collect key patient information described in outcome parameters. The key to the code is safeguarded by the local principal investigator and access to all records is limited to directly involved researchers. The coordinating investigator will centralize patients' data, and principal investigators will have direct access to their own site's data sets and to other sites' data upon reasonable request. All principal investigators will maintain records, including signed patient informed consent forms and information on adverse events.

Data management of all data (collection, storage, and analysis) will be done according to the local data management plan. All records will be stored for a period of 15 years following the completion or termination of the study. Monitoring will be done according to a monitoring plan with specific attention paid to informed consent, completion of the eCRF, and storage of CLE video data.

Patient safety and adverse events

The study was deemed a negligible risk study (according to the Nederlandse Federatie van Universitaire Medisch Centra (NFU) descriptions) by the ethical committee of the Amsterdam UMC. Previous study publications showed that nCLE-imaging and intravenous fluorescein administration are safe.(27) Fluorescein adverse reactions are rare and mostly mild in nature. No study related adverse

events occurred in the prior bronchoscopic nCLE studies in the Amsterdam UMC.(18, 19) Estimated prolonged endoscopy time due to study participation is approximately 10 minutes. Patients will not be aware of this as they will already be sedated for the bronchoscopic procedure.

In case any (serious) adverse event ((S)AE) occur during the procedure or up to one week after the procedure, the sponsor will register SAEs through the web portal *Toetsingonline* to the accredited METC that has approved the protocol. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the trial procedure. The severity and possible relatedness to the investigational product or the procedure will be documented. Investigators of the participating centers will report all serious adverse events to the coordinating and principal investigator of the initiating site. Reporting of SAEs that result in death or are life-threatening will be done within 7 days after initial identification, followed by a period of a maximum of 8 days to complete the preliminary report. All other SAEs will be reported within 15 days after first knowledge of the SAE.

Annual progress report

The sponsor will ensure that a progress report is submitted to the medical ethics committee once a year. Information on the start date of inclusion, number of subjects included to date, number of subjects that have completed participation, serious adverse events, and amendments.

Amendments

Substantive protocol amendments will be assessed by the METC Amsterdam UMC. A substantial amendment is already incorporated in this publication. In the course of subject screening, it was observed that certain patients, integral to the population that could potentially benefit from nCLE, were excluded. Initially presence of a positive bronchus sign was obligatory. After inclusion of 12 patients, we also include patients if the bronchoscopist has estimated confidence for successful navigation to the nodule resulting in a r-EBUS signal without a clear bronchus sign on chest CT. As only 5% of patients were included at a single center at the moment of the change, effects on the outcomes are negligible. In the event of other substantial amendments, all changes with a rationale will be reported in future publications arising from this protocol.

Dissemination

We aim to publish the study results in a peer-reviewed journal. Reporting will be in line with CONSORT and STARD 2015 reporting guidelines.(28, 29)

Discussion

In this multicenter, investigator-initiated, randomized controlled trial, we aim to determine if the addition of nCLE-imaging to bronchoscopic peripheral lung lesion analysis results in an improved diagnostic yield.

Since there still is a gap between the success rate of navigating the tissue sampling instrument toward the target lesion and the final diagnostic yield, there is a need for real-time tool-in-lesion confirmation. The addition of high-resolution microscopic nCLE imaging at the tip of the needle could potentially lead to a decrease in mispositioning rates and an improved diagnostic yield. As a result, fewer patients would need additional diagnostic procedures such as transthoracic needle biopsy or surgery, which are more invasive and have higher incidences of complications such as pneumothorax and hemorrhage.(30) Previous smaller studies have already shown that nCLE is safe, and raters can distinguish different image characteristics with high accuracy. On top of that, it has also been demonstrated that fine-tuning the needle based on these image characteristics is often done, even when navigation to the lesion was successful.(18-20) However, nCLE image interpretation remains subjective and challenging, especially when interpreting images live in the bronchoscopy suite. As described by Tian et al.(31), the role of artificial intelligence might be important to make the technique routinely implementable in clinical practice. An exploratory endpoint of this study is to develop a deep-learning network for automated image interpretation. This is the first step towards easier, quicker and reproducible image interpretation.

Current literature on nCLE imaging for this purpose remains limited to smaller patient groups and the clinical benefit remains to be demonstrated. The results of the CLEVER study provide a formal comparison between conventional image-guided diagnostic bronchoscopy and conventional bronchoscopy with the addition of nCLE in a large randomized patient group. The results of this trial will clarify the added benefit of nCLE for bronchoscopic diagnosis of peripheral lung nodules and identify which patients could benefit from the use of this technique.

Author contributions

SvH, TK, DAK, DMdB, PIB and JTA were involved in conception and trial design. SvH, TK, DAK, PIB and JTA were involved in drafting of the study protocol. DAK provided statistical expertise. All authors were involved in editing and final approval of the protocol. SvH, DG, CB, JH, MH, VP, CR, AS, GS, EK, NA, JV, ZS, MAH, JMAD, PIB and JTA will be involved in the conduct of the study and data acquisition. SvH, DAK, DMdB, PIB and JTA will be involved in the data analysis and interpretation. SvH, DAK, PIB and JTA will be involved in drafting of the final manuscript for dissemination. All authors will provide editing and approval of the final manuscript for publication.

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This is an investigator-initiated study with financial and material support from Mauna Kea Technologies (9 Rue d'Enghien, 75010 Paris, France). The funder has no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of interests

JTA declares material and financial support to the sponsor's institution from Mauna Kea Technologies for this study.

Data sharing statement

The final trial dataset will be available to study investigators on completion of the trial, and after publication of the primary manuscript. All data relevant to the study will be included in the article or uploaded as supplementary information. Data are available upon reasonable request.

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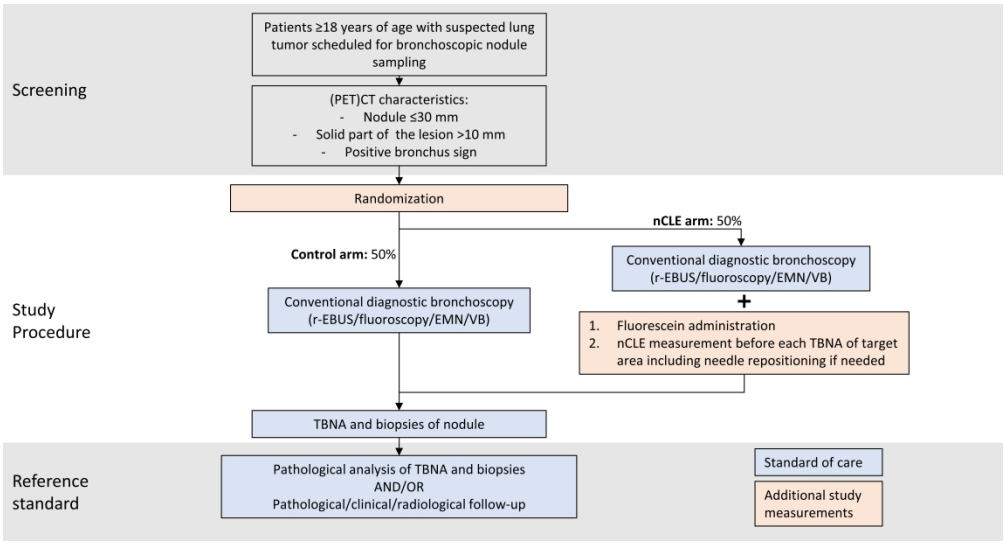
Figure headings

Figure 1: Study flowchart. r-EBUS: radial endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: neelde-based confocal laser endomicroscopy; TBNA: transbronchial needle aspiration; PET: positron emission tomography; CT: computed tomography

Figure 2: Procedure flowchart for control and interventional group (without and with nCLE). Note: fluorescein administration is only done once before the first puncture. TBNA: transbronchial needle aspiration; ROSE: rapid on-site evaluation; EBUS: endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: needle-based confocal laser endomicroscopy

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography



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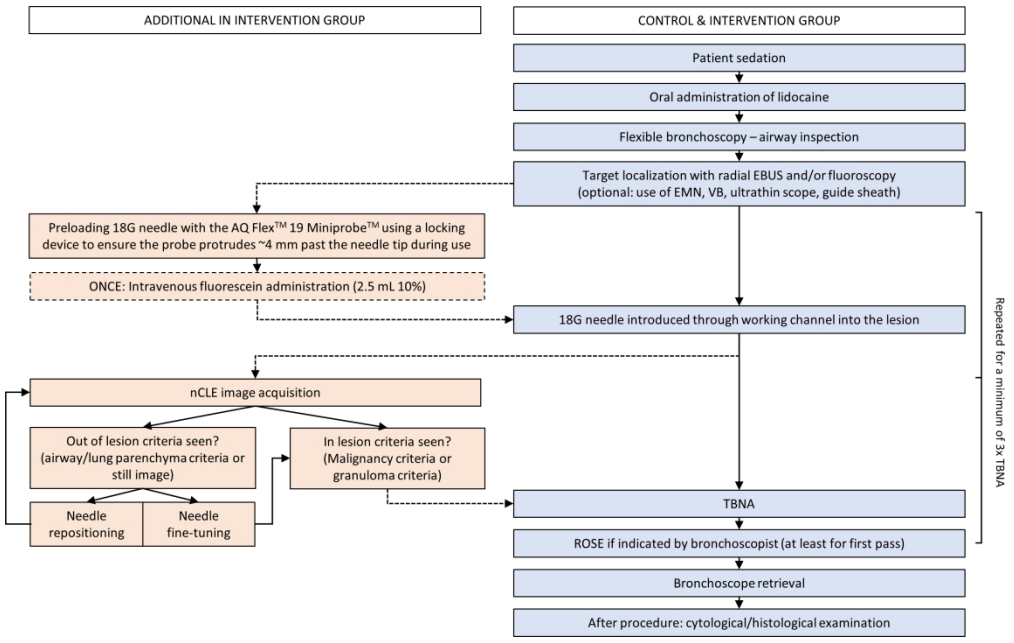

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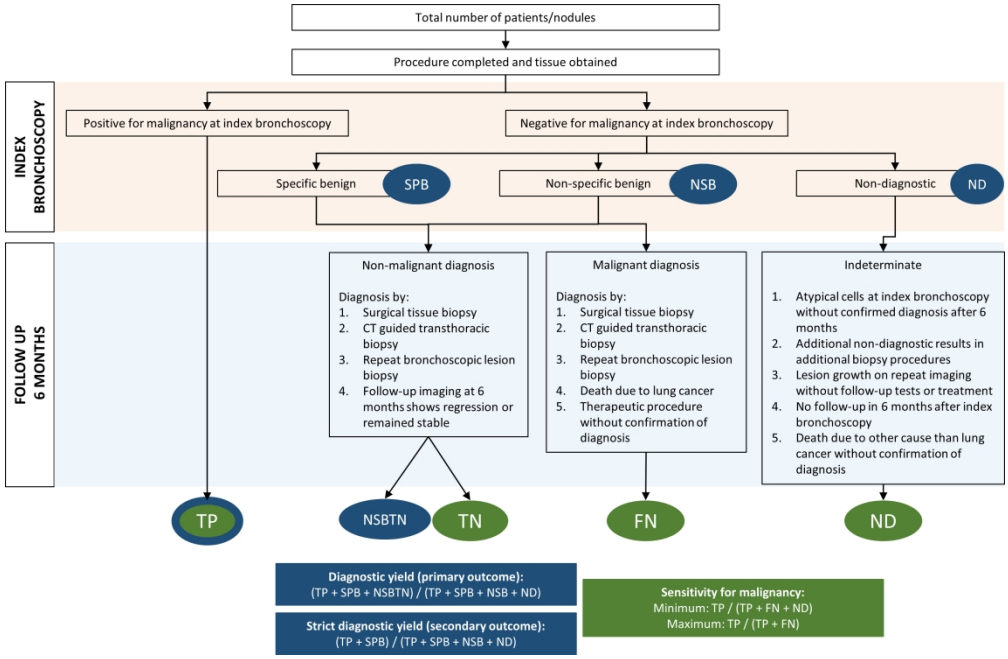

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262x170mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1 and 14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1, 14, 15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9,10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9,10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	11

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4,5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4,5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4,5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4,5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7, 11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12,13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10,11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10,11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to	13

		where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary information
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

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Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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Bronchoscopy with and without needle-based confocal laser endomicroscopy for peripheral lung nodule diagnosis: protocol for a multicenter randomized controlled trial (CLEVER trial)

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Abstract

Introduction: Despite many technological advances, the diagnostic yield of bronchoscopic peripheral lung nodule analysis remains limited due to frequent mispositioning. Needle-based confocal laser endomicroscopy (nCLE) enables real-time microscopic feedback on needle positioning, potentially improving the sampling location and diagnostic yield. Previous studies have defined and validated nCLE criteria for malignancy, airway, and lung parenchyma. Larger studies demonstrating the effect of nCLE on diagnostic yield are lacking. We aim to investigate if nCLE-imaging integrated with

conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE.

Methods and analysis: This is a parallel-group randomized controlled trial. Recruitment is performed at pulmonology outpatient clinics in university and general hospitals in six different European countries and one hospital in the United States. Consecutive patients with a for malignancy suspected peripheral lung nodule (10-30 mm) with an indication for diagnostic bronchoscopy will be screened, and 208 patients will be included. Web-based randomization (1:1) between the two procedures will be performed. The primary outcome is diagnostic yield. Secondary outcomes include diagnostic sensitivity for malignancy, needle repositionings, procedure and fluoroscopy duration, and complications. Pathologists will be blinded to procedure type; patients and endoscopists will not.

Ethics and dissemination: Primary approval by the Ethics Committee of the Amsterdam University Medical Center. Dissemination involves publication in a peer-reviewed journal.

Support: Financial and material support from Mauna Kea Technologies.

Trial registration: ClinicalTrials.gov NCT06079970.

Keywords: Respiratory tract neoplasms, bronchoscopy, confocal laser scanning microscopy, nCLE, rapid-on-site evaluation (ROSE), histology/cytology, lung cancer, peripheral lung nodule

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Article summary

Strengths and limitations

- This is a (international multicenter) randomized controlled trial evaluating a novel sampling technique needle-based confocal laser endomicoscopy (nCLE) with the current standard for bronchoscopic diagnosis of peripheral lung nodules.
- The definition of diagnostic yield is under debate. In this study, the diagnostic yield will be reported based on two different definitions for better comparison with existing and future studies.
- Each participating center uses their own methods for conventional bronchoscopic diagnosis of peripheral lung nodules and will therefore not be completely uniform across all centers. Each center will keep conventional methods uniform in both the control and intervention group to ensure differences can be attributed to the nCLE technique.
- In this study only peripheral pulmonary nodules between 1 – 3 cm are included.

Introduction

Lung cancer remains the leading cause of cancer-related deaths, with 2.09 million new diagnoses and 1.76 million deaths worldwide per year.(1, 2) The increased use of chest computed tomography (CT) and the future implementation of low-dose CT lung cancer screening result in an increased detection of lung nodules.(3, 4) Consequently, more early-stage lung cancer is detected, which is most often located in the periphery of the lung.(5, 6) Depending on lesion characteristics and associated risk factors, tissue sampling is needed to establish a definitive diagnosis and determine the appropriate treatment.

Bronchoscopic analysis of peripheral lung nodules remains challenging despite many technological innovations. The procedure comprises three essential pillars needed for a diagnostic success: navigation to the lesion, confirmation of tool location within the lesion (i.e., tool-in-lesion confirmation) and successful tissue sampling. In the past years, fluoroscopy, radial probe endobronchial ultrasound (r-EBUS), electromagnetic navigation (EMN), virtual bronchoscopy (VB) or cone beam computed tomography (CBCT) combined with augmented fluoroscopy have improved navigation with or without tool-in-lesion confirmation.(7) Additionally, rapid on-site evaluation (ROSE) is sometimes used for direct feedback on representativeness of the sample and forming a preliminary diagnosis. Nevertheless, diagnostic yield rarely exceeds 71%,(8) as it depends highly on factors such as nodule size, bronchus sign on pre-procedural CT, eccentric vs. concentric r-EBUS pattern, pre-test probability of malignancy and sampling tools used.(9-12) The arrival of robotic bronchoscopy platforms combined with existing techniques have shown promising results with high navigation success rates. However, diagnostic yield remains behind due to substantial mispositioning rates, retaining a large gap between navigation success and diagnostic yield.(13-15) The persistently low diagnostic yield calls for complementary techniques providing real-time information for fine-tuning the needle position.

Confocal laser endomicroscopy (CLE) is a high-resolution microscopic technique that visualizes individual cells in real-time. It has proven useful in the gastroenterology field, where it has been demonstrated that CLE could be used for rapid diagnosis, targeting of biopsies, and prediction of neoplasms.(16) CLE has been recently introduced in the respiratory tract, including for the peripheral lung nodule analysis.(17-19) CLE probes are thin enough to fit through 18G biopsy needles to provide microscopic feedback at the tip of the needle (needle-based CLE (nCLE)). Fluorescein dye is used as a contrast agent and binds to the extracellular matrix, resulting in a highly fluorescent background in which individual cells can be seen. Previous studies have identified three nCLE image characteristics

for malignancy in the lung,(19) and criteria for airway and lung parenchyma.(18) The identification of malignancy and distinction from airway and lung parenchyma were accurate based on these criteria.(18, 19)

A recent study demonstrated a high needle mispositioning rate, as nCLE-imaging resulted in a repositioning of the biopsy needle in 9 out of 20 patients.(20) nCLE could therefore potentially bridge the gap between navigation success and diagnostic yield.

To date, larger studies investigating the effect of the addition of nCLE to bronchoscopic peripheral lung nodule analysis are lacking. The improved diagnostic yield could reduce the necessity further or more invasive diagnostic interventions such as CT-guided transthoracic biopsies or diagnostic surgery. In this multicenter randomized controlled trial, we aim to investigate if nCLE-imaging integrated with conventional bronchoscopy results in a higher diagnostic yield compared to conventional bronchoscopy without nCLE in diagnosing peripheral lung nodules.

Methods and analysis

Study design

This study is an investigator-initiated, international, multicenter, parallel-group randomized controlled trial comparing two bronchoscopy procedures (with or without nCLE) for the diagnosis of suspected peripheral lung nodules. The study flowchart is shown in Figure 1.

Participating centers

The study is executed in university or general hospitals in six countries in Europe and one hospital in the United States. Study inclusion started on 18 October 2023. Other centers will start including in 2024 and the estimated duration of the study is 24 months including follow up.

Randomization

After the participant has given written informed consent, patient data is entered into a digital database (CASTOR Electronic Data Capture (EDC) electronic case report form (eCRF)). We will use a web-based block-randomization module in Castor to randomize participants into the control and interventional group (1:1). Randomization will be stratified by participating center to ensure that the nCLE and non-nCLE group is of the same size in each center. As nodule size has significant impact on

diagnostic yield,(8) we will stratify for nodule size (≤20 mm and >20 mm) to ensure that size is evenly distributed across study arms.

Patients and endoscopists will not be blinded since the physician needs to know if nCLE images must be acquired during bronchoscopy. Pathologists will be blinded to procedure type and raters who will analyze the nCLE videos after the procedure will be blinded to the patient history and histopathological outcome of the tissue samples.

Study population

Consecutive patients will be recruited by their treating physician at pulmonology outpatient clinics of participating centers. Patients are eligible for inclusion if they meet the following inclusion criteria:

1. ≥18 years of age
2. Suspected malignant peripheral lung lesion with an indication for a bronchoscopic diagnostic work-up as determined by the attending physician or tumor board. Peripheral pulmonary lesions are defined as lesions located beyond the visible segmental bronchi, not detectable by regular flexible bronchoscopy
3. Bronchus sign on pre-procedural CT or estimated confidence for successful navigation to the nodule resulting in a r-EBUS signal
4. Solid part of the lesion must be ≧10 mm
5. Largest dimension of lesion size on CT ≦30 mm (long-axis)
6. Ability to understand and willingness to sign a written informed consent

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Inability or non-willingness to provide informed consent
2. Endobronchial visible malignancy on bronchoscopic inspection
3. Target lesion within reach of the linear EBUS scope
4. Failure to comply with the study protocol
5. Known allergy or risk factors for an allergic reaction to fluorescein
6. Pregnancy or breastfeeding
7. Hemodynamic instability
8. Refractory hypoxemia
9. Therapeutic anticoagulant use that cannot be withheld for an appropriate interval before the procedure

10. Unable to tolerate general anesthesia according to the anesthesiologist
11. Undergoing chemotherapy as several chemotherapies have fluorescent properties at the same wavelength (e.g., doxorubicin)

Primary outcome measure

Diagnostic yield (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant, specific benign or non-specific benign confirmed as benign in follow-up], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure). If patients with multiple lesions are included, the diagnostic yield will be computed per nodule.

Secondary outcome measures

1. Diagnostic sensitivity for malignancy (defined as the proportion of patients in whom the bronchoscopic procedure diagnoses malignancy relative to the total number of patients with a final diagnosis of malignancy as determined by the reference standard).
2. Diagnostic yield according to the strict definition by Vachani et al.(21) (defined as the proportion of patients in whom the bronchoscopic procedure results in a definitive diagnosis [either malignant or specific benign diagnosis], relative to the total number of patients that underwent the diagnostic bronchoscopic procedure).
3. Procedure duration (from bronchoscope insertion until removal).
4. Percentage of patients in which the needle was fine-tuned (defined as moving the needle within the same distal airway) or repositioned (defined as the selection of a different distal airway for tissue sampling) based on nCLE feedback (defined as the number of patients the needle was fine-tuned/repositioned divided by the total number of patients in which nCLE imaging was used).
5. Fluoroscopy radiation time and dose.
6. Diagnostic yield of ROSE (defined as the proportion of patients in whom ROSE resulted in a classifying diagnosis [malignant or specific benign diagnosis], relative to the total number of patients).
7. Proportion of patients in which ROSE provided tool-in-lesion confirmation, meaning that the acquired tissue shows signs of a malignant or non-malignant diagnosis and was not related to airway/lung parenchyma sampling such as bronchus epithelium/blood contamination, and tissue not suitable for a specific diagnosis such as atypical cells.

8. Complication rate (defined as any complication or complication categories occurring during or directly after the bronchoscopic procedure or any procedure-related complication within one week after the procedure).
9. Requirement of additional diagnostic procedures (CT-guided transthoracic biopsies, surgical diagnostics and/or additional bronchoscopy) during the 6-month follow-up period.

Exploratory endpoints

As an exploratory endpoint, we aim to identify potential new nCLE image characteristics for malignant and benign pathologies. We will also create an algorithm for automated nCLE criteria recognition using machine- or deep-learning methods.

Outcome parameters

Table 1 shows the baseline patient characteristics and corresponding procedural information that will be collected at the time of study inclusion, during the procedure and 6-month follow-up period.

Investigational product

The Cellvizio® confocal laser endomicroscopy system with the corresponding AQ-Flex™ 19 miniprobe (Mauna Kea technologies (MKT), Paris, France) is the investigational medical device of this study. The probe has a compatible operating diameter of 0.91 mm, a resolution of 3,5 µm, a penetration depth of 40 to 50 µm and a maximum field of view of 325 µm. The device and corresponding probes are CE-marked and will be used within the intended purpose.(22)

The technique uses a laser beam (488 nm) focused by an objective lens to illuminate the tissue, with the illumination focus at a pre-defined depth. The light strikes the tissue resulting in fluorescent light emission back from autofluorescent structures such as elastin in the airways or an exogenous fluorescent dye such as fluorescein, a contrast dye used for nCLE imaging in the lung. Light originating from the focal layer will be focused by the objective lens at the opening of a pinhole and detected, while light from out-of-focus layers is rejected by the pinhole. This results in high-resolution imaging of individual cells and structures at a specific point with limited influence of (scattered) light from out-of-focus areas.(22)

Table 1: Data to be collected

Patient characteristics
Age
Sex
BMI
Smoking history
Patient cancer history
Family history of lung cancer
Pre-procedural (PET)CT scan lesion characteristics
CT scan quality (slice thickness)
Size (largest diameter)
Localization (segmental level)
Lesion appearance/nodule type (Solid, non-solid/ground glass, partially solid)
Bronchus sign (present(concentric/eccentric)/absent/insufficient CT scan quality)
Spiculation sign (present/absent)
Emphysema (present/absent)
PET uptake (not performed/no uptake/faint *(SUV < 1)*/moderate *(SUV 1 – 2.5)* /intense *(SUV >2.5))*
Intra-procedural information
r-EBUS sign (eccentric, concentric, absent)
Location of tissue sampling (lung segment)
nCLE image observations (for every needle pass)
Needle fine-tuning & repositioning done (for every needle pass)
Sampling techniques used (TBNA, (cryo)biopsy, brush)
ROSE results of tissue sample (if available)
Bronchoscopy start and end time
Fluoroscopy duration
Additional procedures performed (e.g., EBUS/EUS-B/etc.)
(Serious) complications
Post-procedural information
(Serious) complications (up to 1 week after the procedure)
Final pathological diagnosis (cytology and/or histology)
(Additional) Diagnostic follow-up procedures needed (e.g., transthoracic needle biopsies, surgery, additional bronchoscopy, follow-up imaging) including (altered) diagnosis and/or results of follow-up CT-scans of the lesion(s)

RUL: right upper lobe; RML: right middle lobe; RLL; right lower lobe; LUP: left upper lobe; LLL: left lower lobe; CT: computed tomography; ROSE: rapid on-site evaluation; r-EBUS: radial endobronchial ultrasound; TBNA: transbronchial needle aspiration; SUV: standard uptake value

Study procedures

Conventional diagnostic bronchoscopy (control group & intervention group)

The following procedure will be performed routinely (regardless of study participation): Bronchoscopic procedures will be performed by experienced pulmonologists specifically trained in navigational bronchoscopy and nCLE-imaging. All procedures are performed according to institutional practice, usually on an outpatient basis. Patient preparation and sedation will be done according to institutional practice and might include propofol or midazolam sedation and the use of topical anesthesia. Vital parameters will be monitored during and after the procedure.

Systematic bronchoscopic inspection of the airways will be performed, followed by r-EBUS imaging (guide sheath optional) to select the distal airway with the highest probability of reaching the lesion. The use of fluoroscopy, EMN, VB or ultrathin bronchoscope is optional if regularly used at that institution. CBCT navigation with or without augmented fluoroscopy and robotic bronchoscopy will not be used in patients included in this trial. Bronchoscopist may use these techniques after following all actions related to this protocol while ensuring tissue samples are processed separately. Transbronchial needle aspirations (TBNA) using the 18G FleXNeedle® (Broncus Medical Inc., San Jose, United States of America) and (cryo)biopsies will be performed to acquire tissue for pathological evaluation (a recommended minimum of 3 TBNA and 3 biopsies). During the bronchoscopic work up, some of the cytological aspirations will be evaluated on site (Rapid onsite evaluation, ROSE) and the representativeness of the samples will be reported to the bronchoscopist. ROSE will always be performed for the first TBNA pass. For the following passes, the bronchoscopist decides if it is indicated.

Addition of nCLE imaging (intervention group)

The same procedure will be performed as described above for the patients randomized to the intervention arm, except for the addition of fluorescein administration and nCLE imaging before TBNA. Prior to the procedure, an 18G needle is preloaded with the CLE probe (AQ-Flex™ 19 Miniprobe, Mauna Kea Technologies, Paris, France). The CLE probe is advanced through the needle until the probe is positioned approximately 4 mm past the needle tip and secured using a locking device to maintain the probe position relative to the needle tip.

After determining the sample location based on r-EBUS and/or fluoroscopy, fluorescein (2.5 mL of 10% fluoresceindinatrium solution) is administered intravenously. Then, the preloaded 18G needle punctures the target area, followed by the insertion of the CLE probe through the biopsy needle for real-time microscopic feedback. In case nCLE visualizes airway or lung parenchyma, indicating a near-miss, the biopsy needle is fine-tuned (i.e., the needle is moved within the same distal airway) or repositioned (i.e., a different distal airway is chosen). If nCLE demonstrates that the biopsy needle is placed within the lesion, the CLE probe is removed from the biopsy needle while holding the needle in position, followed by tissue sampling at the same location (repeated for at least 3 TBNAs). A flowchart of the procedure steps for both the conventional bronchoscopy and the nCLE-guided bronchoscopy is shown in Figure 2.

nCLE image interpretation

The airway and lung parenchyma nCLE criteria as described by Kramer et al.(18) will be considered as "out-of-lesion" criteria indicating mispositioning of the needle. Currently known criteria for "tool-in-lesion" are malignancy criteria and granuloma criteria.(18, 19, 23) nCLE images will be interpreted during the procedure by the performing bronchoscopist and their team. Additionally, all videos are rated post-procedure by blinded raters of the initiating center to establish a ground truth interpretation of the images.

Pathological examination

The cytological and histological examination will be done according to standard hospital procedure. In case the bronchoscopic procedure is considered non-diagnostic, additional procedures (transthoracic needle aspiration, surgical procedure, etc.) could follow to obtain a definite diagnosis. Results of the nCLE imaging do not influence the indication for additional diagnostic procedures. If a surgical procedure is indicated, the histological images will be collected to compare this with the nCLE imaging.

In this study, the final pathological diagnosis will be subdivided into four categories as described by Vachani et al.,(21) namely [1] malignant, [2] non-malignant, which is divided into specific benign (including granulomatous, infectious and lymphocyte-predominant patterns) or nonspecific benign (e.g. inflammation), and [3] non-diagnostic (i.e., insufficient material for classifying diagnosis or in case atypical cells could not be classified further).

Reference standard

For the primary outcome, diagnostic yield will be calculated using the intermediate method described by Vachani et al.(21) The abovementioned final pathological diagnosis categories will be used regardless of the results of the reference standard, except for initial non-specific benign diagnoses. In these cases, results from the reference standard will be considered. If the initial benign diagnosis is confirmed benign in follow-up, the bronchoscopic procedure will be considered diagnostic.

For the calculation of diagnostic sensitivity, malignant cases identified by the procedures under investigation will be considered as true positive since false positive results (almost) never occur. Benign (either specific or non-specific) and non-diagnostic samples will undergo a reference standard, which can be a subsequent sampling method such as transthoracic needle biopsy or surgery. Alternatively, if no subsequent sampling method is performed, clinical and radiological follow-up at 6 months is considered the reference standard. If follow-up CT imaging shows regression or resolution of the nodule or in case a nodule remains stable, it will be considered as a confirmation of non-malignant diagnosis (i.e., true negative). Cases that are benign (either specific or non-specific) or non-diagnostic at the index bronchoscopy will be considered false negative if a malignancy diagnosis is established by the reference standard or if therapeutic procedures are done without confirmation of diagnosis. Figure 3 gives a schematic overview of the calculation methods of diagnostic yield and sensitivity for malignancy.

Informed consent procedure

Patients will be recruited by their treating physician. If the patient is willing to receive more information about study participation, information will be provided by the local investigator. The eligible participants will have sufficient time to consider their consent. Written informed consent must be provided before any study-related procedures take place. The English template of the informed consent is provided as a supplementary file. After informed consent, patients will be randomized using Castor EDC software and assigned to the control or intervention group. The bronchoscopy will then be performed according to the study protocol. In case patients decline participation in the study, they will be treated to the usual local clinical practices and guidelines.

Quality assurance

Only experienced pulmonologists will perform the procedures to ensure high-quality bronchoscopic procedures. Additionally, all participating centers will be trained in the use of the CLE Cellvizio device and to maintain homogeneous quality of the nCLE image acquisition and interpretation over all centers. Training entails theoretic and practical training by the initiating center with extensive nCLE experience and MKT representatives.

Sample size justification

Based on previous studies and meta-analyses, we expect the diagnostic yield in patients with a lesion <30 mm in the conventional bronchoscopy arm to be 62%.(24, 25) We hypothesize that additional nCLE guidance in the intervention arm will result in a diagnostic yield of 80%. In total, 198 patients are needed to show that nCLE guidance results in a diagnostic yield that is 18 percent point higher than the conventional bronchoscopy arm (alpha=0.05 and power=0.80). Taking into account a 5% study drop-out, a total of 208 patients will be included. We believe an increase in the diagnostic yield (from 62% to 80%) demonstrates a clinically relevant improvement in lung cancer diagnosis.

Data analysis

Results for continuous variables will be expressed as means and standard deviations or medians with interquartile ranges. Categorical variables will be expressed as frequencies and percentages. The Chi-squared test will be used to compare diagnostic yield (or other comparisons between categorical variables) between the two randomization groups. Continuous variables will be compared using Student's t-test, or Mann-Whitney-U tests. A two-tailed P-value <0.05 will be considered statistically significant. All analyses are done based on an intention-to-treat approach, meaning that patients are analyzed as part of the intervention group they were assigned to, even if nCLE imaging was not executed in a patient in the intervention arm due to unforeseen circumstances. These specific cases will be reported in the manuscript. Patients not undergoing the planned bronchoscopy procedure after randomization are excluded from the analysis. Patients with missing outcome data will be excluded from analysis. Patients with incomplete essential follow-up information will also be excluded from the calculation of diagnostic sensitivity. However, we will also calculate diagnostic sensitivity based on a 'worst-case scenario', in which these patients are considered false negatives. For the primary outcome, subgroup analysis will be performed for several lesions and procedural characteristics (lesion size (≤20 mm vs >20 mm), radial EBUS image (eccentric vs concentric vs absent), location (upper lobe (without lingual) vs middle lobe/lingual vs lower lobe), pre-test probability that the nodule is cancerous (<10%, 10 – 35%, 36-70% and >70%) based on the Brock score.(26)

Protocol amendments

Substantive protocol amendments will be assessed by the METC Amsterdam UMC. A substantial amendment is already incorporated in this publication. In the course of subject screening, it was observed that certain patients, integral to the population that could potentially benefit from nCLE,

were excluded. Initially presence of a positive bronchus sign was obligatory. After inclusion of 12 patients, we also include patients if the bronchoscopist has estimated confidence for successful navigation to the nodule resulting in a r-EBUS signal without a clear bronchus sign on chest CT. As only 5% of patients were included at a single center at the moment of the change, effects on the outcomes are negligible. In the event of other substantial amendments, all changes with a rationale will be reported in future publications arising from this protocol.

Patient and Public Involvement statement

There was no patients or public involvement in the design of this study. An original research manuscript will be prepared to present the study results.

Ethics and dissemination

The CLEVER study will be conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the Medical Research Involving Human Subjects Act (WMO, The Netherlands) principles. To date, the Medical Ethical Committee of the Amsterdam UMC (NL83257.018.22), Athens Chest Hospital (21583/25-08-23) and General University Hospital in Prague (č.j. 143/23 S) have approved the study. All participating sites will obtain local ethical approval prior to starting inclusions. Written informed consent will be obtained prior to randomization and any study-related procedures. In case of major changes to the protocol, the ethical review board will be notified, and it will be communicated with all participating centers and registered on clinicaltrials.gov.

Data management and safety

After informed consent, the patient will be given a code. This code will be used on all (pseudonymized) data, including CLE images and electronic Case Report Form (eCRF) data. Castor Electronic Data Capture ecosystem (International Organization of Standardization (ISO) 27001 and 9001 certified) will be used to collect key patient information described in outcome parameters. The key to the code is safeguarded by the local principal investigator and access to all records is limited to directly involved researchers. The coordinating investigator will centralize patients' data, and principal investigators will have direct access to their own site's data sets and to other sites' data upon reasonable request. All principal investigators will maintain records, including signed patient informed consent forms and information on adverse events.

Data management of all data (collection, storage, and analysis) will be done according to the local data management plan. All records will be stored for a period of 15 years following the completion or termination of the study. Monitoring will be done according to a monitoring plan with specific attention paid to informed consent, completion of the eCRF, and storage of CLE video data.

Patient safety and adverse events

The study was deemed a negligible risk study (according to the Nederlandse Federatie van Universitaire Medisch Centra (NFU) descriptions) by the ethical committee of the Amsterdam UMC. Previous study publications showed that nCLE-imaging and intravenous fluorescein administration are safe.(27) Fluorescein adverse reactions are rare and mostly mild in nature. No study related adverse events occurred in the prior bronchoscopic nCLE studies in the Amsterdam UMC.(18, 19) Estimated prolonged endoscopy time due to study participation is approximately 10 minutes. Patients will not be aware of this as they will already be sedated for the bronchoscopic procedure.

In case any (serious) adverse event ((S)AE) occur during the procedure or up to one week after the procedure, the sponsor will register SAEs through the web portal *Toetsingonline* to the accredited METC that has approved the protocol. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the trial procedure. The severity and possible relatedness to the investigational product or the procedure will be documented. Investigators of the participating centers will report all serious adverse events to the coordinating and principal investigator of the initiating site. Reporting of SAEs that result in death or are life-threatening will be done within 7 days after initial identification, followed by a period of a maximum of 8 days to complete the preliminary report. All other SAEs will be reported within 15 days after first knowledge of the SAE.

Annual progress report

The sponsor will ensure that a progress report is submitted to the medical ethics committee once a year. Information on the start date of inclusion, number of subjects included to date, number of subjects that have completed participation, serious adverse events, and amendments.

Dissemination

We aim to publish the study results in a peer-reviewed journal. Reporting will be in line with CONSORT and STARD 2015 reporting guidelines.(28, 29)

Discussion

In this multicenter, investigator-initiated, randomized controlled trial, we aim to determine if the addition of nCLE-imaging to bronchoscopic peripheral lung lesion analysis results in an improved diagnostic yield.

Since there still is a gap between the success rate of navigating the tissue sampling instrument toward the target lesion and the final diagnostic yield, there is a need for real-time tool-in-lesion confirmation. The addition of high-resolution microscopic nCLE imaging at the tip of the needle could potentially lead to a decrease in mispositioning rates and an improved diagnostic yield. As a result, fewer patients would need additional diagnostic procedures such as transthoracic needle biopsy or surgery, which are more invasive and have higher incidences of complications such as pneumothorax and hemorrhage.(30) Previous smaller studies have already shown that nCLE is safe, and raters can distinguish different image characteristics with high accuracy. On top of that, it has also been demonstrated that fine-tuning the needle based on these image characteristics is often done, even when navigation to the lesion was successful.(18-20) However, nCLE image interpretation remains subjective and challenging, especially when interpreting images live in the bronchoscopy suite. As described by Tian et al.(31), the role of artificial intelligence might be important to make the technique routinely implementable in clinical practice. An exploratory endpoint of this study is to develop a deep-learning network for automated image interpretation. This is the first step towards easier, quicker and reproducible image interpretation.

Current literature on nCLE imaging for this purpose remains limited to smaller patient groups and the clinical benefit remains to be demonstrated. The results of the CLEVER study provide a formal comparison between conventional image-guided diagnostic bronchoscopy and conventional bronchoscopy with the addition of nCLE in a large randomized patient group. The results of this trial will clarify the added benefit of nCLE for bronchoscopic diagnosis of peripheral lung nodules and identify which patients could benefit from the use of this technique.

Author contributions

SvH, TK, DAK, DMdB, PIB and JTA were involved in conception and trial design. SvH, TK, DAK, PIB and JTA were involved in drafting of the study protocol. DAK provided statistical expertise. All authors were involved in editing and final approval of the protocol. SvH, DG, CB, JH, KJ, VP, CR, AS, GS, KB, EK, NA, JV, ZS, MAH, JMAD, PIB and JTA will be involved in the conduct of the study and data acquisition. SvH,

DAK, DMdB, PIB and JTA will be involved in the data analysis and interpretation. SvH, DAK, PIB and JTA will be involved in drafting of the final manuscript for dissemination. All authors will provide editing and approval of the final manuscript for publication.

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Declaration of interests

JTA declares material and financial support to the sponsor's institution from Mauna Kea Technologies for this study. All Cellvizio equipment needed for the conduct of the study is provided in-kind to participating centers. All other authors declare no other conflicts of interest.

Data sharing statement

The final trial dataset will be available to study investigators on completion of the trial, and after publication of the primary manuscript. All data relevant to the study will be included in the article or uploaded as supplementary information. Data are available upon reasonable request.

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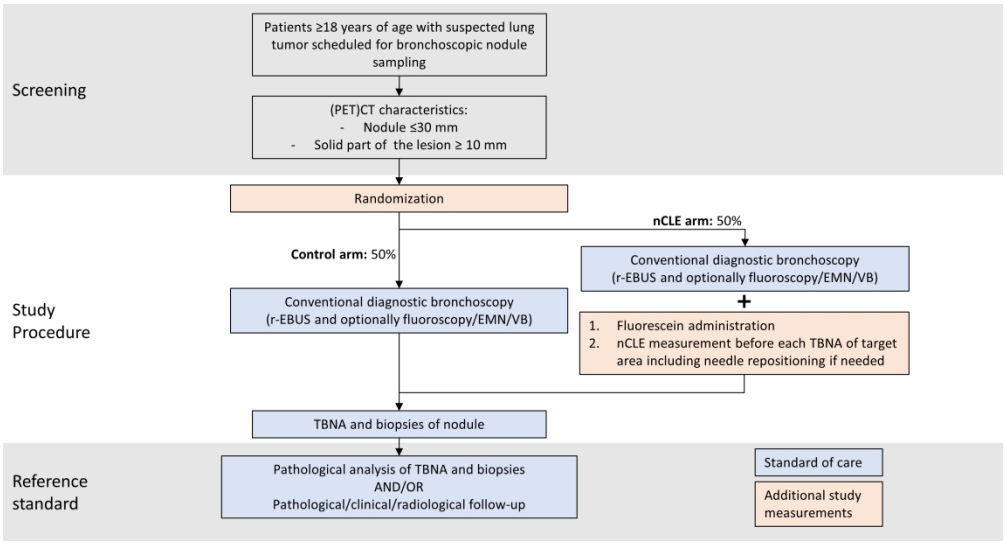
Figure headings

Figure 1: Study flowchart. r-EBUS: radial endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: neelde-based confocal laser endomicroscopy; TBNA: transbronchial needle aspiration; PET: positron emission tomography; CT: computed tomography

Figure 2: Procedure flowchart for control and interventional group (without and with nCLE). Note: fluorescein administration is only done once before the first puncture. TBNA: transbronchial needle aspiration; ROSE: rapid on-site evaluation; EBUS: endobronchial ultrasound; EMN: electromagnetic navigation; VB: virtual bronchoscopy; nCLE: needle-based confocal laser endomicroscopy

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography



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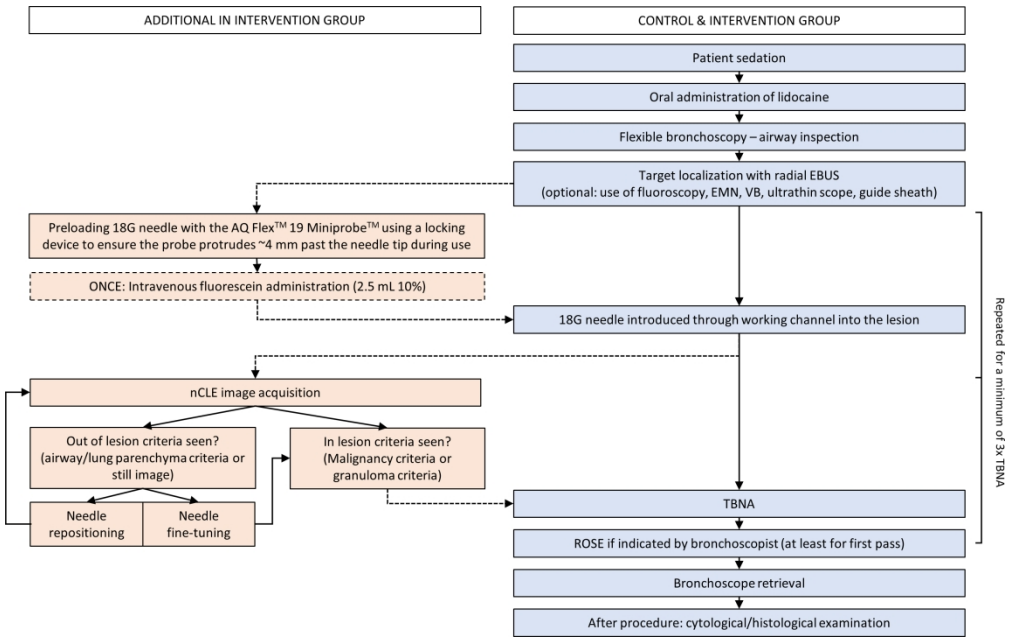

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246x155mm (300 x 300 DPI)

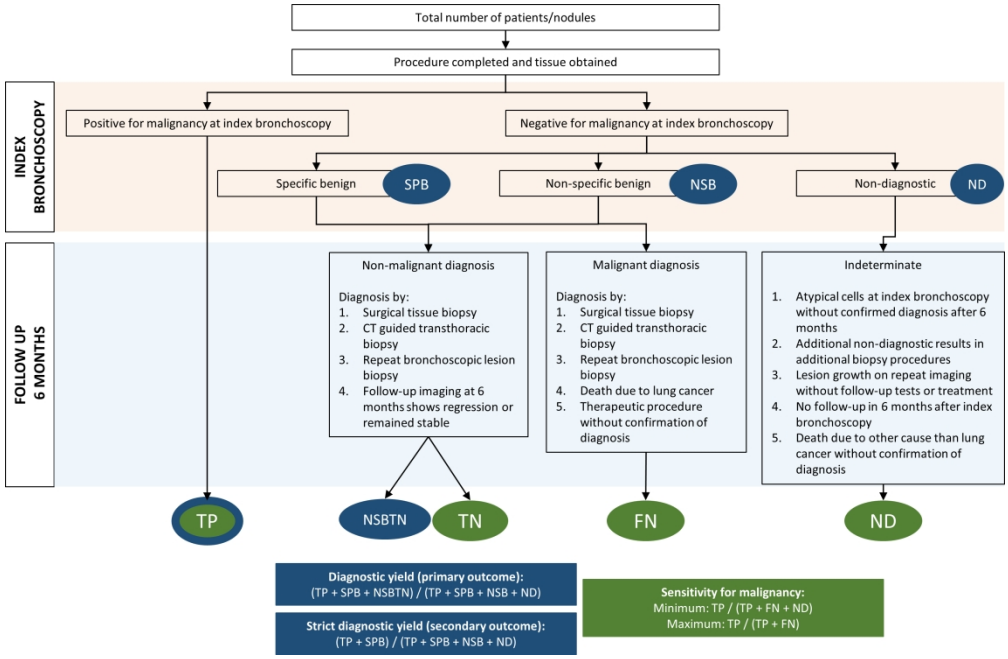

Figure 3: Flowchart explaining calculation methods of diagnostic yield and sensitivity of malignancy. SPB: specific benign; NSB: non-specific benign; ND: non-diagnostic; TP: true positive; TN: true negative; FN: false negative; CT: computed tomography

262x170mm (300 x 300 DPI)

Subject information for participation in medical research

nCLE in lung tumors

Official tittle: nCLE-guided bronchoscopy for peripheral lung cancer diagnosis; a randomized controlled trial

Introduction

Dear Sir/Madam,

With this letter, we would like to ask you to take part in a medical study. Participation is voluntary. You have received this letter because a scan showed an abnormality in the lung. You are scheduled for an examination of the lungs (bronchoscopy) to establish a diagnosis. You can read about the medical study in this information sheet, what it means for you, and what the pros and cons are. It is a lot of information. Can you please read the information and decide if you want to take part? If you want to take part, complete the form in Appendix D.

Ask your questions

You can take your decision based on the information in this information sheet. We also suggest that you do this:

- Put your questions to the investigator who gave you this information.
- Talk to your partner, family or friends about this study.

1. General information

The Amsterdam University Medical Center (Amsterdam UMC), location AMC in The Netherlands has set up this study and is funded by Mauna Kea Technologies, a company specialized in making instruments used during bronchoscopy. Below, we always call the Amsterdam UMC the 'sponsor'.

Investigators, these can be doctors/research nurses, conduct the study in different hospitals.

Participants in medical research are often referred to as subjects.

This study needs 208 subjects from different countries. In [country], it is expected that 30 - 40 subjects will take part.

The Medical Ethics Review Committee Amsterdam UMC has initially approved this study in The Netherlands. The Medical Ethics Review Committee [X] has also approved the start of this study in [country].

2. What is the purpose of the study?

With this study we investigate the addition of confocal laser endomicroscopy (CLE) to the regular bronchoscopic examination. We investigate whether the addition of CLE contributes to the accurate diagnosis of suspected lung abnormalities compared to normal diagnostic bronchoscopy without the addition of CLE.

3. What is the background of the study?

Abnormalities in the lungs can be a results of many different conditions. With current techniques used to visualize these abnormalities (PET-CT scan, CT scan and ultrasound) it is not always possible to say with certainty what causes the abnormality. Therefore, it is often decided to perform a bronchoscopy. During the examination, the suspected lung abnormality is localized in the lungs and some tissue is extracted with a thin needle of for further examination. The current tissue examination has limitations because tissue is not always obtained from the optimal site. As a result, the cause of the abnormality cannot always be diagnosed with certainty.

The newly available technique confocal laser endomicroscopy (CLE) offers possibilities to potentially establish a diagnosis with more certainty. The CLE technique works like a microscope where individual cells can be imaged with safe laser light. The CLE laser beam can be emitted into the tissue via the thin needle used for the standard bronchoscopy exam. By using CLE imaging at the tip of the needle, we expect to be able to extract tissue at the right place in more cases. We also call this the 'smart needle'.

The advantage of this technique is that a lung abnormality can be accurately imaged inside the body. However, it is not yet sufficiently known how and to what extent we can establish a better diagnosis if we use the 'smart needle'. That is why we are investigating whether adding the CLE technique to the existing standard examination leads to a better diagnosis.

4. What happens during the study?

How long will the study take?

Are you taking part in the study? The bronchoscopy will approximately take 10 minutes longer than usual. Because of the sedative that we administer during the bronchoscopy, you will not notice the extended duration of the procedure.

Step 1: are you eligible to take part?

First, we want to know if you are eligible to take part. The investigator will assess whether you are eligible based on your scans (PET-CT or CT scan). Your physician will discuss your potential participation with you.

Step 2: the bronchoscopy

Subject information

As part of standard care, you will undergo a bronchoscopy exam via the trachea. A sedative will be administered through an IV in your arm to ensure you don't notice anything during the examination.
For the study, we will use the CLE-technique as an addition to the bronchoscopy exam in 50% of participants.

For this study, we will have 2 groups:

- Group 1. The participants in this group undergo the regular bronchoscopy exam without the addition of the CLE technique.
- Group 2. The participants in this group undergo the regular bronchoscopy exam with the addition of the CLE technique.

A draw will decide in which group you are in. Before the start of the bronchoscopy, you will not know which group you were assigned to.

First the bronchoscopy will start. The lungs will be inspected and we will search for the lung abnormality located deeper in the lungs. As part of the standard procedure, a needle will be used to puncture the suspected lung lesion to extract tissue samples for diagnosis.
In case you were assigned to group 2 (i.e., implementation of the CLE technique), CLE images will be acquired at the tip of the needle used for tissue sampling. Administration of a contrast dye (fluorescein) is needed for CLE imaging. This dye will be administered via an IV which is also used to administer sedatives.

What is the difference with standard care?
This study is not very different from standard care. Due to participation in the study, the bronchoscopy may approximately take 10 minutes longer. You will not notice this because of the sedative. No additional body material is collected for this study. If you are placed in the group in which CLE is done in addition to the normal procedure, the safe dye fluorescein will also be administered.
After the bronchoscopy, you will stay in the recovery room for approximately 1.5 hours, which is standard protocol after a normal bronchoscopic exam. You do not have to come to the hospital for an extra visit if you participate in this study.

5. What agreements do we make with you?

If you participate in this study, you have to follow the instructions your doctor gave you for the regular bronchosopy. In total, the bronchoscopy will be extended by a maximum of 10 minutes due to the extra study measurements.
To obtain good images with the CLE method, it is essential that the fluorescein dye is administered via the IV. Fluorescein is a commonly used and safe drug. A small proportion of people (1.1%) may experience side effects such as nausea. In case you have a known sensitivity to fluorescein, you should not participate in this study. Consult your doctor if this applies to you.

Is it OK for you to get pregnant during the study?
Women who are pregnant or breastfeeding cannot take part in this study. This is because it is not known what the effects of fluorescein are during pregnancy or breastfeeding.

6. What side effects, adverse events or discomforts could you experience?

Few risks are associated with the CLE technique. Extensive research has been done and the CLE technique has been assessed as safe.
To obtain good image quality with the CLE method, it is essential that fluorescein dye is administered via your IV. Fluorescein is a widely used and safe contrast dye and side effects are rarely reported. About one in hundred people develop side effects of which nausea, vomiting and a rash are most commonly seen. Please let the doctor or researcher know if you experience any of these side effects.

You can find more information about fluorescein in the information leaflet, see Appendix C.

What are the possible discomforts you may experience with checks or measurements during the study?
Due acquisition of the CLE images, the bronchoscopy can take about 10 minutes longer than usual. You will be given sedatives as part of the standard bronchoscopy protocol and will therefore hardly notice the additional 10 minutes during the exam. After administration of fluorescein, you urine can be more yellow than usual for a day, this is normal.

7. What are the pros and cons if you take part in the study?

Taking part in the study can have pros and cons, listed below. Think about this carefully and talk to other people about it.
If you participate in this research you will not have a direct benefit. In case you are assigned to group B in which CLE is used in addition to the normal bronchoscopy, there might be a higher chance of a diagnosis. However, the purpose of this research is to investigate if that is the case. If you take part you will help with the search for better diagnostics of lung abnormalities.

Taking part in the study can have these cons:
- You may experience side effects or adverse events from the fluorescein drug.
- The bronchoscopy will be 10 minutes longer than usual.

You do not wish to participate in the study?
It is up to you to decide if you wish to participate in the study. You do not wish to participate? Then the regular bronchoscopy exam will be done.

8. When does the study end?

Subject information

The investigator will let you know if there is any new information about the study that is important to you. The investigator will then ask you if you want to continue to take part.

In these situations, the study will stop for you:

- All checks are finished and you are discharged from the hospital.
- You want to stop participating in the study yourself. You can stop at any time. Report this to the investigator immediately. You do not have to explain why you want to stop. You will then get the standard bronchoscopy exam.
- The investigator thinks it is better for you to stop.
- One of the following authorities decides that the study should stop:
    - The government, or
    - The Medical Ethics Review Committee assessing the study, or
    - The Sponsor,

What happens if you stop participating in the study?
The investigators use the data that have been collected up to the moment that you decide to stop participating in the study.

9. What happens after the study has ended?

Will you get the results of the study?
If there is new information about the study that is important for you , the investigator will let you know what the main findings are. The researcher can also tell you which group you were in. Do you prefer not to know? Please tell the invesitagor. He/she will not tell you in that case.

10. What will be done with your data?

Are you taking part in the study? Then you also give your consent to collect, use and store your data.

What data do we store?
We store these data:
- your gender
- your date of birth
- information about your health
- (medical) information we collect during the study
- CLE videos

Why do we collect, use and store you data?
We collect, use and store your data to answer the questions of this study. And to be able to publish the results. Data can be used by the sponsor to perform analysis of the data. The company that support this research (Mauna Kea Technologies) will receive anonymized CLE videos upon reasonable request. This data will only be shared anonymously and cannot be traced back to you.

Subject information

How do we protect your privacy?

To protect your privacy, we give a code to your data. We only put this code on your data. We keep the key to the code in a safe place in the hospital. When we process your data we always use only that code. Even in reports and publications about the study, nobody will be able to see that it was about you.

Who can see your data?

Some people can see your name and other personal information without a code. This could include data specifically collected for this study, but also data from your medical file. These are people checking whether the investigators are carrying out the study properly and reliably. These persons can access your data:

- An auditor who works for the investigator or sponsor
- National and international supervisory authorities.
- [other]

These people will keep your information confidential. We ask you to give permission for this access.

For how long do we store your data?

We store your data in the hospital for […] years. And for 15 years with the sponsor.

Can we use your data for other research?

Your collected data may also be important for other medical research on suspected lung lesions and diagnostics. For this purpose , your data will be stored in the hospital for X years. Please indicate in the consent form whether you agree with this. Do you not want to give your consent? Then you can still take part in this study. You will get the same healthcare.

Can you take back your consent for the use of your data?

You can take back your consent for the use of your data at any time. Please tell the investigator if you wish to do so. But please note: if you take back your consent, and the investigators have already collected data for research, they are still allowed to use this information.

Do you want to know more about your privacy?

- Do you want to know more about your rights when processing personal data? Visit [URL]
- Do you have questions about your rights? Or do you have a complaint about the processing of your personal data? Please contact the person who is responsible for processing your personal data. For the present, this is:
    - The Amsterdam UMC and [institution] See Appendix A for contact details and website(s).
- If you have any complaints about the processing of your personal data, we recommend that you first discuss them with the research team. You can also contact

the Data Protection Officer of [the institution]. Or you can submit a complaint to the Dutch Data Protection Authority.

Where can you find more information about the study?
You can find more information about the study on the following website (s). www.ClinicalTrials.gov. After the study, the website may show a summary of the results of this study. You can find the study by searching for *"Confocal Laser Endomicroscopy VERification"* (number: NCT06079970).

11. Will you receive compensation if you participate in the study?

Participation in the study will not cost you anything. Neither will you get any compensation if you take part in this study. Because no additional travel expenses are made for participation, you will not be reimbursed for travel expenses.

12. Are you insured during the study?

Insurance has been taken out for everyone who takes part in this study. The insurance pays for damage caused by the study. But not for all damage. You can find more information about this insurance and any exceptions in **Appendix B**. It also says who you can report damage to.

13. Informing other physicians

The pulmonologist who performs the bronchoscopy knows that you are participating in the study. We do not inform your general practitioner or other treating specialists that you are participating, given that the study does not have any complications or additional risks that your general practitioner or other treating physician should be aware of.

14. Do you have any questions?

You can ask questions about the study to the research team.
Do you have a complaint? Discuss it with the investigator or the doctor who is treating you. If you prefer not to do so, please visit [complaints officer/complaints committee of your hospital/institute/other]. Appendix A tells you where to find this.

15. How do you give consent for the study?

You can first think carefully about this study. Then you tell the investigator if you understand the information and if you want to take part or not. If you want to take part, fill in the consent form that you can find with this information sheet. You and the investigator will both get a signed version of this consent form.

Thank you for your attention.

Subject information

16. Appendices tot his information

A. Contact details
B. Information about the insurance
C. Information leaflet fluorescein for patients
D. Consent form

Subject information

Bijlage A: contact details for [name of participating centre]

Principal investigator:

[for principal investigator of centre: name, contact details and accessibility]

< *if applicable*>

Co-investigator:

< *if applicable*>

[Study nurse/study doctor/nurse specialist]:

<if applicable> **Independent expert:**

[name, type of doctor/expert, contact details and accessibility]

Complaints: [service or person with contact details and accessibility]

Data Protection Officer:

Data protection officer of the institution: [contact details]

Data Protection Officer of the Sponsor: privacy@amsterdamumc.nl

For more information about your rights visit: [Contact details [including website]

Subject information

Appendix B: information about the insurance

The [Institution] has taken out insurance for everyone who takes part in the study. The insurance pays for the damage you have suffered because you participated in the study. This concerns damage you suffer during the study or within 4 years after you participated in the study. You must report damage to the insurer within 4 years.

Have you suffered damage as a result of the study? Please report this to this insurer: *<also indicate here how subject should act/report in the event of damage: telephone/mail/post, other instructions?*

The insurer of the study is: Name insurer: … Address: … Telephone number: … Email: … (Policy number: …)

< include only if there is a claims representative – this is compulsory if the insurer is established outside the Netherlands> The claims representative of the study is: Name: … Address: … Email: Telephone number: …

The insurance pays a maximum of *<amount to be copied from policy, this must be at least €650,000 >* per person and *<amount to be copied from policy, this must be at least €5,000,000>* for the entire study (and *<amount to be copied from policy, this must be at least € 7,500,000>* per year for all studies by the same sponsor).

Please note that the insurance does **not** cover the following damage:

- Damage due to a risk about which we have given you information in this sheet. But this does not apply if the risk turned out to be greater than we previously thought. Or if the risk was very unlikely.
- Damage to your health that would also have happened if you had not taken part in the study.
- Damage that happens because you did not follow directions or instructions or did not follow them properly.
- Damage caused by a treatment method that already exists. Or by research into a treatment method that already exists.

Subject information

These provisions can be found in the 'Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen 2015' ('Medical Research (Human Subjects) Compulsory Insurance Decree 2015'). This decision can be found in the Government Law Gazette (https://wetten.overheid.nl).

Subject information

Appendix C – Information leaflet fluorescein for patients

The name of this medicine is Fluorescein Sodium 100mg/ml Solution for Injection, which will be referred to as Fluorescein Sodium Injection throughout this leaflet.

Read all of this leaflet carefully before you are given this medicine because it contains important information for you.

- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor, pharmacist or nurse.
- This medicine has been prescribed for you only. Do not pass it on to others. It may harm them, even if their signs of illness are the same as yours.
- If you get any side effects talk to your doctor, pharmacist or nurse. This includes any possible side effects not listed in this leaflet.

IN THIS LEAFLET

1. What Fluorescein Sodium Injection is and what it is used for

Fluorescein Sodium Injection contains the active ingredient fluorescein sodium which works as a diagnostic stain. It is used in a hospital-based procedure on for example the eye called fluorescein angiography of the ocular fundus (part of the eye). This medicinal product is for diagnostic use only.

2. What you need to know before you are given Fluorescein Sodium Injectio

Do not use Fluorescein Sodium Injection if:

- you are allergic (hypersensitive) to fluorescein sodium or any of the other ingredients of this medicine.

If the above applies to you or you are in any doubt you should ask your doctor or pharmacist for advice before being given this medicine.

Warning and Precautions:

Your doctor or other healthcare professional will give you this medicine through an injection into one of your veins. Fluorescein Sodium Injection is for intravenous injection only and MUST NOT be injected into the arteries (arterial route) or into the spinal column (intrathecal route).

You must tell your doctor if:

- You have previously undergone a hospital procedure on the eye called fluorescein angiography of the ocular fundus (a part of the eye)
- You have a history of allergy
- You have a history of heart or pulmonary disease
- You are taking drugs known as Beta-blockers including those applied in eye drops.
- You have kidney disease

Taking other medicines

Tell your doctor or pharmacist if you are taking, have recently taken or might take any other medicines including medicines obtained without a prescription. Fluorescein sodium can sometimes interact with other medicines that you could be taking causing unwanted side effects.

Preferably do not add anything. Fluorescein disodium is incompatible with acids, salts of acids and salts of heavy metals. Please inform your doctor or pharmacist if you are taking or have recently taken any other medicines. This also applies to medicines that you can obtain without a prescription.

If you are due to have other diagnostic tests including blood, urine, and X-Ray investigations

Fluorescein Sodium Injection may interfere with the results of some blood and urine tests within 3 days of having the procedure. If you are having any blood or urine tests taken, you should tell the doctor or nurse that you have been given Fluorescein Sodium Injection.

If an X-ray procedure is conducted within 36 hours of injection, the resulting high visibility of some organs such as the kidneys may lead to misinterpretation of the results.

Pregnancy, breastfeeding and fertility

If you are pregnant or think you may be pregnant prior to using Fluorescein Sodium Injection, tell your doctor who will decide whether to give you this medicine or not.

Fluorescein disodium should not be used during breast-feeding. Ask your doctor or pharmacist for advice before taking any medicine.

Driving or operating machinery

The use of Fluorescein Sodium during certain procedures, your vision may be temporarily impaired. Patients must abstain from driving a vehicle or operating machinery until the eyesight returns to normal.

3. How to use Fluorescein Sodium 100mg/ml?

Fluorescein disodium is injected directly into the bloodstream.

Dosage:

The exact dose, to be determined by the doctor, is up to a maximum dose of Fluorescein sodium 500mg (equivalent to one 5ml ampoule 100 mg/ml) administered by intravenous injection.

4. Possible side effects

Like all medicines, this medicine can cause side effects, although not everybody gets them. If you have undergone a similar examination, please tell your doctor if you have experienced an intolerance reaction regardless of how severe it may or may not have been.

The following side effects may occur during administration:

- nausea and vomiting,
- allergic reactions such as skin rash with intense itching and lump formation (urticaria), increased salivation (hypersalivation), runny nose (rhinorrhoea) and fever
- decreased number of blood platelets (thrombocytopenia)
- the appearance of fluid in the lungs (pulmonary oedema)
- temporary discoloration (yellowing) of the skin and urine
- anaphylactic reaction – anaphylactic shock

If you notice any side effects not listed in this leaflet or which you consider to be serious, please inform your doctor or pharmacist.

5. How to store Fluorescein Sodium 100 mg/ml

Keep this medicine out of reach and sight of children. Keep ampoules in a cardboard box in order to protect from light

Use by date: Do not use this medicine after the expiry date which is stated on the carton and ampoule label after EXP. The expiry date refers to the last day of that month.
Do not use Fluorescein disodium if you notice that the solution is no longer completely clear or the ampoule is damaged.
For single use only. Once opened the ampoule must be used immediately.

Any unused product or waste material should be disposed of in accordance with local requirements.

Subject information

Appendix D: Informed consent form – subject

Belonging to nCLE in lung tumors

- I have read the information sheet. I was able to ask questions. My questions have been answered well enough. I had enough time to decide if I wanted to take part.
- I know that taking part is voluntary. I also know that at any time I can decide not to take part in the study. Or to stop taking part. I do not have to explain why.
- I give consent to collect and use my date. The investigators only do this to answer the question of this study.
- I know that some people will be able to see all of my data to review the study. These people are mentioned in this information sheet. I give consent to let them see my data for this review.
- I know that I cannot be pregnant during the study.
- Please tick yes or no in the table below.

I give consent to store my data to use for other research, as stated in the information sheet.	Yes ☐	No☐
I give consent to ask me after this study if I want to participate in a follow-up study.	Yes ☐	No☐
I give consent to let me know after the study which treatment I received/in which group I was.	Yes ☐	No☐

- I want to take part in this study.

My name is (subject): ……

Signature: …… Date : __/__/__

I declare that I have fully informed this subject about the study mentioned.

If any information becomes known during the study that could influence the subject's consent, I will let this subject know in good time.

Investigator name (or their representative): .....

Signature:…… Date: __/__/__

The study subject will receive a complete information sheet, together with a signed version of the consent form.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1 and 14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1, 14, 15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9,10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6,7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9,10,11
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	11

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	4,5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	4,5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	4,5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	4,5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			

Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7, 11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12,13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11,12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10,11,12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10,11,12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to	13

		where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	13
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12, 13

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary information
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA