Title:	EBUS-Miniforceps biopsy specimen acquisition for PD-L1 testing in nonsmall cell lung cancer: A feasibility study
NCT:	NCT04159025
Version Date:	05/26/2021

EBUS-Miniforceps biopsy specimen acquisition for PD-L1 testing in nonsmall cell lung cancer: A feasibility study

Protocol Date: 9/20/2018

Version 2 Date: 05/26/21

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OBJECTIVES

The objective of this study is to assess the feasibility of utilizing endobronchial ultrasound guided miniforceps to acquire adequate amounts of tissue to perform PD-L1 testing on in patients with nonsmall cell lung cancer. Adequacy will be defined as sufficient core biopsy material to perform the PD-L1 assay specific for nivolumab.

SPECIFIC AIMS:

To evaluate the technique of EBUS-MFB in obtaining adequate tissue to perform PD-L1 testing.

I. BACKGROUND

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) has significantly changed the landscape for the diagnosis and staging of lung cancer and intrathoracic malignancy. Previously, patients with suspected lung cancer required invasive surgical techniques to adequately stage disease prior to therapy. Presently, EBUS-TBNA is recommended by the American College of Chest Physicians for the diagnosis of suspected lung cancer based on the body of literature supporting the efficacy of this technique as well as its favorable safety profile compared to more invasive surgical techniques such as mediastinoscopy or thoracoscopy (Silvestri GA et al. *Chest* 2013;143:e211S-e250S).

The development of molecular profiling for lung cancer has significantly altered the treatment for this disease (NCCN Task Force Report. JNCCN 2011;9(5)). The discovery of molecular mutations such as the epidermal growth factor receptor (EGFR) have allowed targeted therapy to be given to patients with certain molecular profiles, improving quality of life as well as periods of disease free progression for certain patients with advanced stages of lung cancer.

Immune checkpoint inhibition targeting PD-L1 is therapy for patients with advanced nonsmall cell lung cancer that has become standard of care. Currently, the assay for detection of PD-L1 has only been approved for use with core biopsy specimens, and not cytologic, needle based preparations. As EBUS-TBNA is recommended as a first line procedure over surgical techniques, many patients undergoing this staging procedure will not have tissue specimens which are adequate for PD-L1 testing, and may require additional procedures to obtain core material for PD-L1 testing.

Recently, new instrumentation has become available for endobronchial ultrasound which propose to provide core biopsy specimens using this bronchoscopic approach. Endobronchial ultrasound guided miniforceps biopsies (EBUS-MFB) have been safely used to obtain larger amounts of tissue than traditionally provided by transbronchial needle aspiration. In this technique, small, 1mm forceps are placed through the working channel of the EBUS bronchoscope and are passed directly into mediastinal lymph nodes or centrally located mediastinal or hilar lesions and are used to obtain “core” biopsies of

targeted areas. EBUS-MFB has shown efficacy and safety for the diagnosis of granulomatous inflammation and lung cancer (Chen A, et al. Ann Thorac Surg 2011;92:284-289), though has not been specifically evaluated for the use of collecting material for PD-L1 testing, which is relatively new.

RATIONALE:

The incorporation of PD-L1 testing into clinical practice has progressed at a rapid pace, and now offers an additional line of therapy for eligible patients with nonsmall cell lung cancer. The assay used to detect circulating levels of PD-L1 currently requires core biopsies, and is not approved to be used for specimens collected through a needle based cytological technique. Though EBUS-TBNA has markedly improved the manner in which patients are diagnosed and staged for lung cancer, alternative means of tissue collection may be mandatory to offer patients access to newer lines of therapy such as PD-L1 inhibition. EBUS-miniforceps biopsy may allow bronchoscopists to obtain core biopsy specimens through the technique of endobronchial ultrasound, so that more invasive approaches such as surgery may be avoided. Feasibility using this approach would indicate that all patients being staged with endobronchial ultrasound procedures would be candidates for PD-L1 testing and potential therapy.

This study is proposed to evaluate the feasibility of using EBUS-MFB to acquire tissue that is adequate for PD-L1 testing. Feasibility in this study is defined as the ability to obtain adequate material during EBUS procedures to perform PD-L1 testing.

II. RESEARCH DESIGN AND METHODS

This is a single center prospective feasibility study to assess the adequacy of specimens collected using EBUS-miniforceps for performing PD-L1 testing using the nivolomab assay.

The patient population in this study is patients with advanced stage lung cancer. The protocol outlines our standard of care approach, which is to perform a full staging EBUS examination of the mediastinum and hila, starting with N3 nodes, then moving to N2 nodes and finally to N1 nodes as needed. Rapid on-site evaluation (ROSE) is utilized to give on-site feedback to the bronchoscopist so that we know if the lymph node being sampled is diagnostic on-site. If, for example, an N3 lymph node is diagnostic, then we do not need to subject the patient to additional procedural or anesthesia related risks of sampling additional lymph nodes. The information provided from the original lymph node is diagnostic and pathologically stages the patient. This is standard of care.

Primary outcomes: Adequacy of collected material to perform PD-L1 testing on specimens collected using EBUS-MFB using the nivolomab assay.

Secondary outcomes:

1. Adverse event rates and safety profile of EBUS-MFB.

DEFINITIONS:

Central lung lesion: lesions immediately adjacent to the tracheobronchial tree accessible by biopsy using convex-probe endobronchial ultrasound and inclusive of, but not limited to, mediastinal and hilar lymphadenopathy.

Feasibility: feasibility will be defined as the ability to provide adequate specimens to perform PD-L1 testing. Any specimen in which the requested assay returns as "insufficient material to perform testing" will be deemed an "inadequate" specimen.

SUBJECT ENROLLMENT:

Subjects presenting to the Interventional Pulmonology service at Washington University School of Medicine in need of a bronchoscopic biopsy of their central lung lesion as part of their standard medical care, and who meet study inclusion/exclusion criteria, will be invited to participate. A total of 20 patients will be enrolled in this feasibility study. The PI will explain the study to qualified subjects prior to obtaining consent. The subject will also be given an opportunity to review and sign documentation of HIPAA compliance and authorization.

Inclusion criteria:
Patients to be included for participation in this study are:

1. Patients with central lung lesion 1cm in size or larger identified on chest CT with the intention to undergo bronchoscopic evaluation and biopsy. The decision to pursue biopsy will be made by the treating physician and agreed upon by the patient.
2. Are at least 18 years old
3. Are able to provide informed consent
4. Are not pregnant as confirmed by bHCG testing prior to procedure

Exclusion criteria:
Patients to be excluded from participation in this study are:

1. Patients who refuse to participate
2. Are less than 18 years of age
3. Are pregnant
4. Are physically unable to tolerate flexible bronchoscopy or moderate sedation as determined by the bronchoscopist
5. Are unable to provide informed consent
6. Are on anticoagulant medications and who cannot safely discontinue their medication prior to their procedure at the recommendation of their treating physician

INFORMED CONSENT:

Informed consent will take place in a private environment (e.g. patient exam room), free from distractions. The PI (or another member of the study team with consenting duty) will approach the subject at their standard of care clinic appointment or prior to their scheduled bronchoscopy and will explain the study to qualified subjects prior to obtaining consent. Interviews to obtain consent will not follow any stressful situation (e.g. patient being informed he/she may have cancer) and will not be conducted if patient has received any mind-altering medications or anesthesia. Patients will be assessed for their capacity to consent by the ability to show comprehension of the procedure, ask appropriate

questions, and appear properly oriented. A signed copy of all consents and the HIPAA authorization document will also be given to consenting subjects.

PROCEDURES:

All procedures will be performed in an outpatient bronchoscopy suite. All procedures will be performed under moderate sedation.

Bronchoscopy:

1. *Sedation*: Moderate sedation will be administered by the bronchoscopy team in a dedicated bronchoscopy suite. Patients will be continuously monitored throughout the procedure as per standards of care at Barnes-Jewish Hospital.
2. *Airway inspection*: When adequate sedation has been given, the bronchoscope (BF-P190, Olympus, Tokyo, Japan) will be inserted transorally into the tracheobronchial tree and a standard airway inspection will be performed.
3. *Convex-probe endobronchial ultrasound and transbronchial needle aspiration:* Following standard airway inspection, the convex-probe EBUS bronchoscope (BF-UC180F, Olympus, Tokyo, Japan) will be inserted transorally into the tracheobronchial tree and will be advanced to the targeted centrally located lesion. Transbronchial needle aspiration from the targeted lesion will be performed using a 22 gauge aspiration needle.
4. *Rapid on-site evaluation (ROSE)*: Rapid on-site evaluation will be performed as per standard clinical care by a cytology team.
    a. ROSE diagnostic: in cases in which ROSE yields a specific diagnosis of nonsmall cell lung cancer, EBUS-MFB will subsequently be performed.
    b. ROSE non-diagnostic: in cases in which ROSE does not yield a specific diagnosis, additional lymph nodes that are pathologically enlarged as defined above will be targeted for EBUS-TBNA
        i. If no additional lymph nodes are pathologically enlarged, the procedure will be terminated and EBUS-MFB will not be performed
5. *EBUS-miniforceps biopsy*: following the diagnosis of nonsmall cell lung cancer using ROSE, EBUS-MFB will be performed in the following manner:
    a. With the EBUS bronchoscope positioned at the targeted lymph node station, 6 needle punctures will be made into the targeted lymph node using the 22 gauge aspiration needle through the EBUS bronchoscope using continuous endobronchial ultrasound guidance.
    b. The 22 gauge aspiration needle will then be removed and 1mm miniforceps (Boston Scientific), will be passed through the working channel of the EBUS-bronchoscope into the targeted lymph node through the puncture site made using the 22 gauge needle using continuous endobronchial ultrasound guidance.
    c. The miniforceps will be used to obtain a core biopsy of the targeted lymph node using continuous endobronchial ultrasound guidance and will be withdrawn from the EBUS bronchoscope.

   d. 8 core biopsies will be obtained from each targeted lymph node using this technique, with each core biopsy specimen placed in formalin for PD-L1 testing.

6. Management of complications: the major complications of bronchoscopic biopsy include pneumothorax and bleeding:
   a. Pneumothorax: a post procedure chest x ray will be performed following the bronchoscopy. Should a pneumothorax be present, it will be managed by either observation and follow up chest x-ray or by tube thoracostomy at the discretion of the bronchoscopist.
   b. Bleeding: intra procedural bleeding will be managed using local tamponade, iced saline lavage or mechanical occlusion at the discretion of the bronchoscopist.

Post-bronchoscopy:

1. *Chest x-ray*: A routine portable chest x-ray will be performed on all patients following bronchoscopy as per standard clinical practice at Barnes-Jewish Hospital/Washington University School of Medicine to assess for pneumothorax.
2. *Recovery*: Patients will be recovered in a dedicated post-procedure area as per standard care at Barnes-Jewish Hospital/Washington University School of Medicine

Laboratory Analysis:

1. Formalin placed specimens will be sent for PD-L1 testing using the assay specific for nivolumab.

RISKS OF PROCEDURES:

The greatest risks from bronchoscopy are related to moderate or deep sedation and the very act of passing a bronchoscope into the airway. To mitigate risks of bronchoscopy all patients will be monitored by a nurse and a respiratory therapist during the procedure. In addition, oxygen saturation (using pulse oximetry) respiratory rate, blood pressure, ECG, and heart rate will be continuously monitored. Patients will not be released until they are fully awake and medically stable.

The risks associated with bronchoscopy are collapsed lung, breathing difficulty, vocal cord spasm, vomiting, dizziness, bronchial spasm, infection, low blood oxygen, heart attack and bleeding from biopsied site. The occurrence of these risks is extremely low. The measures to mitigate the risks are the same as for all bronchoscopy. To assess for pneumothorax following the bronchoscopy, patients will get a portable chest X-ray. To minimize the chance of bleeding, all patients will be questioned about tendency to bleed prior to bronchoscopy as per standard care. Anticoagulation with antiplatelet agents will be held according to guideline recommendations for that drug. If bleeding occurs, patients will be treated with direct pressure, local instillation of epinephrine or electrocautery. Patients with lung disease may receive bronchodilators prior to bronchoscopy at the discretion of the performing bronchoscopist. There is no way to reduce the minimal risk of infection due to bronchoscopy since the scope must go through the oropharynx. Patients are given supplemental oxygen and their oxygen level is continuously monitored using pulse oximetry. Patients ECG, heart rate, blood pressure, and blood oxygen are continuously monitored by a nurse, respiratory therapist and physician during the

procedure. If there is any sign of a worsening in status, such as elevated heart rate, low oxygen, or ECG changes, the procedure will be aborted.

RESEARCH ONLY RISKS:

Risk of breach in confidentiality
Every effort will be made to protect the privacy of the research subjects. All information and data related to this study will be maintained in secured, protected space, and access will be restricted to study personnel only.

DATA COLLECTION

(1) *Feasibility*: feasibility will be determined as the ability to provide adequate specimens to perform PD-L1 testing. Any specimen in which the requested assay returns as "insufficient material to perform testing" will be deemed an "inadequate" specimen. Adequacy for PD-L1 testing will be recorded.
(2) *Radiographic Information*: Size and location of the central pulmonary lesion will be recorded. Size will be recorded as the longest axis diameter.
(3) *Procedural Characteristics*: Procedural information to be recorded will include procedure time, amount of sedation given, and number of biopsies performed
(4) *Diagnostic yield*: Diagnostic yield based on final cytology and/or histopathology will be determined from the results of the bronchoscopy. A biopsy that results in a specific diagnosis, either malignant or benign, that adequately explains the clinical scenario as determined by the treating physician, will be considered truly positive. Plan for further diagnostic management for patients who have a non-diagnostic procedure will be determined by the treating physician as clinical indicated.
(5) *Complications:* Adverse event rates will be documented. Pneumothorax will be documented by post-biopsy CXR or chest ultrasonography, and the number requiring intervention, such as chest tube placement, will be recorded. Significant hemoptysis will be defined as bleeding noted at the time of procedure that requires a change in the level of care (e.g. outpatient to inpatient or inpatient to ICU) or a blood transfusion. Other adverse events that are common to bronchoscopy described above in "Risks" section will be monitored and reported by the patient or nursing staff.

DATA SAFETY MONITORING PLAN

All subjects enrolled in the trial will undergo routine standard of care bronchoscopy that is indicated as medically necessary by their physicians. Prior to the bronchoscopic procedure, PI will explain the risks associated with bronchoscopy, and verify that the research only risks have been explained, and that the subject has signed an informed consent form. There are no anticipated risks, outside of the risk for the standard of care procedures for this clinical trial. All subjects are required to read, understand, and sign the informed consent form associated with the research study. Each subject will be informed of post procedure symptoms of which to be aware that may represent adverse reactions to the bronchoscopy. Symptoms that the patient will be informed to look out for include:

- Pain or difficulty with swallowing

- Difficulty breathing
- Vomiting
- Dizziness
- Abdominal or chest pain
- Continuous bright red blood in your sputum
- Fever above 100.0 degrees Fahrenheit

If the subject notes any of these symptoms, he/she is instructed to contact his/her physician, who will determine the course of action. All adverse events (AEs) will be reviewed by the medical monitor within one month of their occurrence and summarized for review. The PI will be responsible for submitting this report to the IRB at his/her institution as required. AEs and other reportable events are defined below.

Reportable Events

(1) AEs

AEs will be defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Regarding AEs and SAEs, "temporally" will be defined as occurring within 24 hours post-procedure. Serious Adverse Events (SAEs) will be defined as any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

* results in death;
* is life-threatening;
* requires inpatient hospitalization or prolongation of existing hospitalization;
* results in a persistent or significant disability/incapacity;
* results in a congenital anomaly/birth defect; or
* any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

If the subject notes any of these symptoms, he/she is instructed to contact his/her physician, who will determine the course of action. PI will be responsible for identifying adverse events during the procedure and during the standard of care follow-up period. Site PIs will review adverse events experienced by subjects treated at their site during the procedure standard of care follow-up period and will record them in the medical record. The study team will review the participant's medical record up to 24 hours after the procedure to monitor if adverse events have occurred. The PI will review all adverse events, expected or unexpected, per standard medical care. PI will classify AEs as expected or unexpected, and report AEs directly to the IRB per WU IRB reporting policy.

Unanticipated problems and noncompliance events will be reported to the WU IRB according to IRB definition and reporting timeframes. Study participants will be followed for 24 hours post-procedure for AEs.

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

DATA STORAGE AND MAINTENANCE:

The PI will review the results, and the results will become part of the subject's medical record and research record. Any clinical follow-up or repeat procedures will be dictated by the patient's physician based on clinically relevant data and will not be influenced by enrollment into this study. To protect subject confidentiality, all patient health information will be de-identified through use of 6 digit patient codes. All information and data related to this study will be stored in a secured, locked cabinet in the PI's office which is only accessible by the PI. Electronic data will be stored on the PI's password protected computer located in the PI's locked office. Study participants will be added to Oncore within 1 day of being enrolled into the study.

REGISTRATION PROCEDURES:

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database at Washington University.

STATISTICAL ANALYSIS

The primary objective of this study is to determine the success rate of performing PD-L1 testing using the nivolumab specific assay on specimens collected using an EBUS-miniforceps core biopsy technique.

This is a feasibility study with a target enrollment of 20 patients.

Descriptive statistics will be calculated to characterize study subjects and procedure, which include the mean±SD for continuous variables and percentages for categorical variables, such as subject's age at procedure performed, race, gender, the number of biopsy passes, location of lymph node, the time length of procedure, type and amount of sedation administered, and adverse events.