Official Title:	Bronchoscopic Cryo-Immunotherapy of Lung Cancer
NCT Number:	NCT04049474
Study Number:	17-00812
Document Type:	Study Protocol and Statistical Analysis Plan
Date of the Document:	• January 5, 2023

Bronchoscopic Cryo-Immunotherapy of Lung Cancer

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NYULMC Study Number:	S17-00812
Funding Sponsor:	NIH Exploratory/Developmental Research Grant (R21CA229714-01A1) Additional funding may be provided by: Division of Pulmonary, Critical Care, and Sleep Medicine NYU School of Medicine 462 First Avenue, OBV 605A New York, NY 10016
IDE Number:	G190098
Study Product:	ERBOKRYO® CA – Cryosurgical Unit with Flexible ERBECRYO™ Probe of 1.9 mm outer diameter
Study Product Provider:	ERBE, Inc., Tubingen, Germany
ClinicalTrials.gov Number	NCT04049474

Initial version: **October 23, 2017**
Amended: **November 10, 2017**
Amended: **April 12, 2019**
Amended: **May 3, 2019**
Amended: **May 16, 2019**
Amended: **August 22, 2019**
Amended: **September 23, 2019**
Amended: **October 7, 2019**
Amended: **April 7, 2021**
Amended: **October 4, 2022**

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation (“ICH”) Guideline for Good Clinical Practice (“GCP”) (sometimes referred to as “ICH-GCP” or “E6”) will be applied only to the extent that it is compatible with FDA and DHHS regulations. The

Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

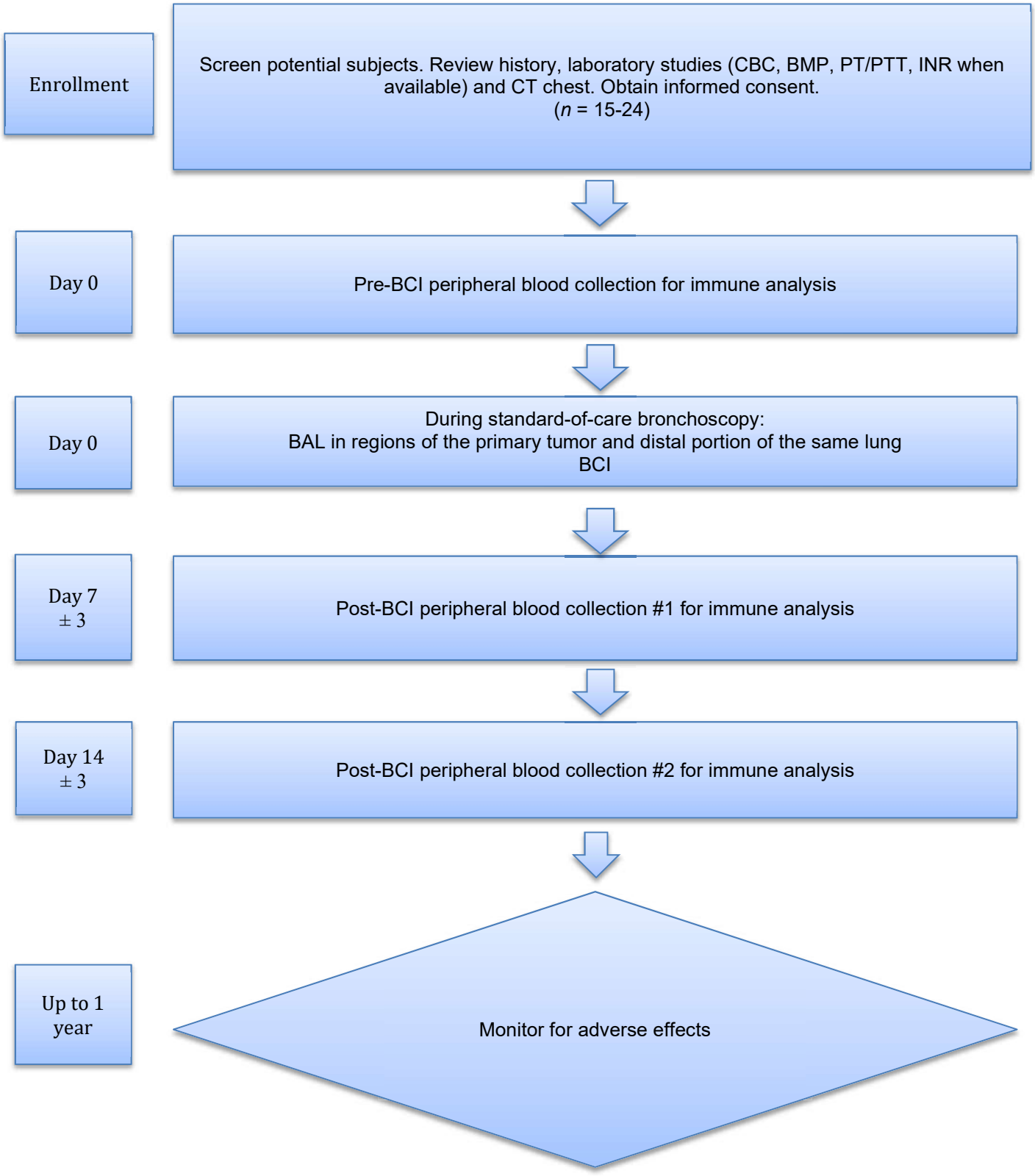
AE	Adverse Event
BAL	Bronchoalveolar Lavage
BCI	Bronchoscopic Cryo-Immunotherapy
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
DLT	Dose-Limiting Toxicity
ECOG	Eastern Cooperative Oncology Group
EBUS	Endobronchial Ultrasound
EGFR	Epidermal Growth Factor Receptor
FACS	Fluorescence-Activated Cell Sorting
ICI	Immune Checkpoint Inhibitor
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NYULMC	New York University Langone Medical Center
NSCLC	Non-Small Cell Lung Cancer
NSAID	Non-Steroidal Anti-Inflammatory Drug
ROSE	Rapid On-Site Cytology Evaluation
SAE	Serious Adverse Event
UP	Unanticipated Problem

Protocol Summary

Title	Bronchoscopic Cryo-Immunotherapy of Lung Cancer
Short Title	BCI Study
Brief Summary	This is a first-in-human, safety and feasibility study of bronchoscopic cryo-immunotherapy (BCI) of peripheral lung tumors in advanced non-small cell lung cancer (NSCLC) and suspected NSCLC. BCI has the potential to stimulate anti-tumor immune responses. Pre- and post-BCI peripheral blood samples will be analyzed to assess for anti-tumor immune responses. Post-BCI peripheral blood will be collected 7 and 14 days after the procedure. Additionally, pre-BCI bronchoalveolar lavage (BAL) specimens will be collected to correlate PD-1 phenotype on pre-BCI BAL lymphocytes by single cell RNA sequencing and BCI-induced anti-tumor immune responses.
Phase	Phase I
Objectives	Primary: • Establish safety and feasibility of BCI and determine the maximum tolerated dose (MTD) (i.e., freeze duration) Secondary: • Assess the extent of BCI-induced anti-tumor immune responses
Methodology	Dose-escalation trial using a 3+3 design
Endpoints	Primary: Feasibility and Safety • Feasibility: successful performance of BCI in at least 80% of participants in whom BCI is attempted • Safety endpoints: Dose-limiting toxicity (DLT), defined as incidence of bleeding complications and pneumothorax, length of time to perform BCI, length of fluoroscopy exposure during BCI, and other adverse events Secondary: Immune Analysis and Tumor Response • Changes in peripheral blood $CD8^+$ T cells expressing a combination of $Ki67^+$,PD-1+, HLA-DR^+, $CD38^+$, and Bcl-2^{low} after BCI • Changes in peripheral blood lymphocyte gene expression after BCI, which will be assessed by the PanCancer OncoImmune™ profile panel, a 770-plex gene expression panel covering innate and adaptive immune responses (NanoString® Technology, Seattle, WA) • Correlation of PD-1 phenotype of pre-BCI BAL lymphocytes by single-cell RNA sequencing and BCI-induced anti-tumor immune responses.
Study Duration	12 months
Participant Duration	14 days
Duration of IP administration	BCI will be performed once at the time of a standard-of-care bronchoscopy for another indication. BCI is anticipated to take approximately 15-25 minutes.

Population	• Patients with known or suspected advanced non-small cell lung cancer (stages IIIA/B/C and IVA/B based on the 8th edition TNM staging), with an accessible peripheral lung tumor, and who have been referred to NYU Interventional Pulmonary for bronchoscopy for diagnostic and/or palliative purposes unrelated to this study • Patients with high risk clinical features (significant smoking history, history of COPD, presence of spiculated lesion with bronchus sign on chest CT, enlargement of mediastinal/hilar lymph nodes and/or evidence of distant metastatic disease) may be candidates for BCI if they meet all other enrollment criteria. • Age: > 22 years of age • Gender: All genders • ECOG performance status ≤ 2 • Location: New York, New York
Study Sites	New York University Langone Medical Center
Number of participants	Up to 24 participants
Description of Intervention	BCI is performed by advancing a flexible cryoprobe through a bronchoscope to reach a target peripheral tumor. Freeze-thaw cycles will be applied to the target peripheral tumor with the intention of inducing anti-tumor immune responses. The number of freeze-thaw cycles will depend on the size of the target tumor. One freeze-thaw cycle will be applied per one centimeter of largest tumor diameter. Providers will be instructed to remain greater than 2 cm away from critical structures. Freeze times will start at 1 second, and will be increased by dose-escalation protocol based on tolerability. The thaw phase will last until the cryoprobe separates from the target tumor.
Reference Therapy	None
Key Procedures	• Bronchoscopic Cryo-Immunotherapy • Bronchoalveolar lavage in the lobe regions of the primary tumor and of the distal portion of the same lung • Pre-BCI (day 0) and Post-BCI (day 7 and day 14 ± 3) blood draws
Statistical Analysis	• A standard 3+3 dose escalation design will be implemented. • Safety endpoints, including the incidence of bleeding and pneumothorax, will be descriptive. • Wilcoxon signed-rank tests will be used to assess paired pre- and post-BCI biomarker levels.

Schematic of Study Design



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2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

I) Cryoablation and Bronchoscopic Cryo-Immunotherapy (BCI)

Cryotherapy is the local application of extremely cold temperatures for tissue destruction, removal, and/or biopsy. Nitrous oxide is the most commonly utilized cryogen, and provides temperatures below -40^0C at the tip of the cryoprobe(1). In cryoablation, repetitive freeze-thaw cycles cause tissue injury and necrosis. Cryoablation causes cell injury leading to necrosis and apoptosis by multiple mechanisms including formation of intracellular ice crystals (damage cell membranes and organelles), cell dehydration (extra-cellular fluid freezes and the osmolarity of the remaining non-frozen extracellular fluid increases leading to water shifts), and vascular compromise (tissue ischemia from micro-vascular thrombosis)(2-4). Additionally, cryoablation may induce an anti-tumor immune response by causing the release of tumor antigens from dying lung cancer cells, and ultimately stimulating anti-tumor specific T cell activation and proliferation. In contrast, in cryobiopsy and cryoadhesion, target tissue is frozen without a follow-up thaw phase. Frozen target tissue becomes adherent to the cryoprobe to facilitate removal from the airway.

Bronchoscopic cryoablation has been utilized for over twenty years for safe and effective palliative treatment and debulking of endobronchial tumors in the central airways (trachea and proximal bronchi)(5, 6). Percutaneous cryoablation has been utilized for more than a decade to treat peripheral primary and secondary lung tumors that are not amenable to surgical resection(7). However, bronchoscopic cryoablation has not yet been applied to the treatment of peripheral lung tumors. Peripheral lung tumors comprise lung nodules and masses, which are completely surrounded by lung parenchyma. These tumors are located in airways distal to sub-segmental bronchi.

Bronchoscopic cryoablation of peripheral lung tumors is anticipated to induce cytotoxicity to tumor cells. Anti-tumor immune responses are hypothesized to be active against any remaining tumor cells locally, as well as at microscopic and macroscopic distant sites of malignancy. In order to achieve anti-tumor responses, bronchoscopic cryoablation may only need to be performed on a portion of the target tumor. The target tumor will be measured in its largest diameter and one freeze-thaw cycle will be performed per centimeter from the distal portion advancing proximally. This will be referred to as bronchoscopic cryo-immunotherapy or BCI. In the context of treating peripheral lung tumors, the safety profile of BCI is currently unknown, but is anticipated to be safe and well tolerated.

II) Safety Profiles of Current Applications of Cryotherapy in Pulmonary Diseases and Anticipated Safety Profile of BCI

Bronchoscopic cryoablation is an accepted, standard-of-care technique for safe and effective treatment of endobronchial tumors in the central airways. The American Thoracic Society and the American College of Chest Physicians have both endorsed the use of cryotherapy for palliation of central airway obstruction due to inoperable malignant tumors(8, 9). In a review of over five hundred consecutive patients with obstructive tracheobronchial malignant tumors, target tissue was freeze-thawed with a cryoprobe and accessible necrotic tumor material was then removed with biopsy forceps. Cryoprobes of 2.2 mm or 5 mm, depending on target tumor size, were able to safely freeze endobronchial tumors using freeze times of 3 minutes. Complication rates were low, including no reported pneumothoraces and only a four percent rate of post-procedure hemoptysis (6). Overall, hemoptysis improved in 76% of symptomatic patients post-endoluminal cryotherapy.(6) Additionally, early case series using smaller 1.1 mm probes in conjuction with robotic bronchoscopy to biopsy peripheral nodules have not reported significant adverse events (37, 38).

Percutaneous cryoablation is also an established approach for the treatment of peripheral primary and secondary lung tumors that are not amenable to surgical resection. In percutaneous cryoablation, rigid cryotherapy probes are inserted via transthoracic CT guidance and the target tumor becomes completely engulfed in an ice ball during freezing. In contrast, in our proposed novel technique of BCI, only a portion of the target tumor will be frozen. Percutaneous cryoablation has been shown to be effective for local tumor control. In percutaneous cryoablation, the target necrotic tumor remains in-situ, and does not undergo any biopsy or tissue removal, which is similar to BCI. In an observational study of over two hundred percutaneous cryoablation procedures of thoracic masses, 86% of masses were reduced or remained stable in size over a six-month period(7). In percutaneous cryoablation of peripheral lung tumors, the cryoprobe must pass through lung parenchyma in order to reach the target peripheral tumor, which carries a significant risk of causing a pneumothorax. In a systematic review of observational studies on percutaneous cryoablation of lung tumors, overall rate of pneumothoraces ranged from 12-62% with up to 17.6% of induced pneumothoraces requiring insertion of thoracostomy tubes(10, 11). In BCI, however, the cryoprobe will reach the target peripheral lung tumor via the airway directly without traversing through the lung parenchyma. Therefore, BCI is anticipated to have a significantly lower risk of pneumothoraces compared to percutaneous cryoablation.

Reports of hemoptysis after percutaneous cryoablation have been small volume and self-limited in the majority of cases (10, 11). For example, in a review of 117 consecutive patients treated with 193 cryoablation sessions of lung tumors, there were no episodes of life-threatening pulmonary hemorrhage (11). However, there have been rare case reports of life-threatening pulmonary hemorrhage previously(12). Post- percutaneous cryoablation hemoptysis may be due to tumor necrosis from freezing or due to lung injury from the cryoprobe traversing through lung parenchyma. In percutaneous cryoablation, cryoprobes with diameters up to 3mm have been used to safely administer freeze times lasting up to 20 minutes 7, 10). In BCI, a smaller cryoprobes of 1.1mm or 1.9mm will administer significantly shorter freeze times based on a dose escalation protocol (see figure). Based on the tolerability of percutaneous cryoablation with non-significant bleeding (if bleeding occurs), significant hemoptysis is not anticipated to occur in BCI. Additionally, the shorter freeze phases, smaller diameter (1.9mm) of the cryoprobe, and avoiding the need for the cryoprobe to traverse through lung parenchyma will further decrease the risk of bleeding in BCI compared to percutaneous cryoablation.

Over the past several years, bronchoscopic cryobiopsy has been studied for the diagnosis of interstitial lung diseases, lung transplant rejection, and peripheral lung lesions, including lung cancer. In contrast to cryoablation, cryobiopsy involves the freezing of target tissue for typically 3-5 seconds without a follow-up thaw phase (13-15). Frozen target tissue becomes adherent to the cryoprobe to facilitate removal via the airway and transport to Pathology for analysis. In observational studies, the rates of pneumothoraces and significant bleeding after trans-bronchial cryobiopsies for the diagnosis of interstitial lung disease and lung transplant rejection are highly variable. For example, in a retrospective review of three hundred consecutive trans-bronchial cryobiopsies for evaluation of interstitial lung disease at a single center, the rates of pneumothoraces and bleeding were 5.0% and 5.2%, respectively(13). In another retrospective review of twenty-five consecutive trans-bronchial cryobiopsies at a single center, the rates of pneumothoraces and significant bleeding (quantified as at least 100cc of hemoptysis post procedure) were 8% and 12%, respectively(14). There are significant differences in technique between our proposed novel procedure of BCI and trans-bronchial cryobiopsy. Most notably, trans-bronchial cryobiopsy does not have a thaw phase and involves the removal of frozen tissue which becomes adherent to the cryoprobe. In BCI, as long as passive thaw phases are of sufficient time to allow for target tissue and cryoprobe to separate, no target tissue will be removed, and significant bleeding and pneumothoraces are not anticipated to occur. For these reasons, BCI of peripheral lung tumors is anticipated to be safe and well-tolerated.

III) Anticipated Feasibility of BCI

The feasibility of performing cryobiopsy of peripheral pulmonary lesions with the use of radial endobronchial ultrasound (rEBUS) guidance has been previously demonstrated (16). In a prospective study of thirty-eight consecutive subjects with peripheral lung lesions of less than four centimeters (excluding one patient with a visible endobronchial tumor), thirty-one peripheral lesions were identified by rEBUS. The average time duration for tumor detection by rEBUS was 7.6 minutes. All thirty-one peripheral lesions underwent transbronchial cryobiopsies successfully. Based upon these experiences, the utilization of rEBUS to guide BCI of peripheral lung tumors is anticipated to be technically feasible (16).

IV) Proposed Mechanism for BCI Inducing Anti-Tumor Immune Responses

BCI is anticipated to induce anti-tumor immune responses. Cryo-immunotherapy involves the killing of tumor cells by freezing, generating an "*in-situ* vaccination" associated with CD8+ T cell activation and proliferation, which has been confirmed in murine models as well as in early-phase human clinical trials(17). A proposed mechanism is that cryoablation may, in part, induce the release of tumor antigens from dying lung cancer cells. These released tumor antigens will be taken up by antigen presenting cells, which may interact with T cells and lead to anti-tumor specific T cell activation and proliferation.

BCI induced anti-tumor immune responses may be further enhanced in combination with other treatments. Cryoablation combined with other therapies have demonstrated enhanced anti-tumor immune responses, slower tumor growth, and have been associated with improved outcomes compared to treatments without cryoablation in pre-clinical and experimental clinical trials of non-small cell lung cancer (18-21). We are particularly interested in the possibility of combining BCI with anti-PD-1 monoclonal antibodies and other checkpoint inhibitors for the treatment of advanced non-small cell lung cancer. Synergistic effects of BCI and immune checkpoint inhibitors (ICI) may enable tumor responses in patients refractory to treatment with ICI alone.

V) BCI Combined with Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICI) have emerged as first-line treatment in select patients with advanced non-small cell lung cancer who have tumor PDL-1 expression of greater than 50% (22). However, only approximately twenty percent of patients with non-small cell lung cancer respond to ICI in the majority of clinical trials (23). Individual response to ICI therapy in advanced NSCLC is affected by PD-1/PD-L1 expression and the presence of T cell infiltration within the tumor microenvironment (24). One strategy to increase ICI response in advanced NSCLC would be to combine ICI with other modalities capable of stimulating tumor-specific T cell responses in the tumor microenvironment such as BCI. BCI may increase the presence of activated, tumor-reactive CD8+ T cells within the tumor microenvironment and peripheral blood, and may ultimately improve clinical responses to ICIs.

The combination cryoablation and ICI has demonstrated enhanced immune responses in the treatment of a murine prostate cancer model and in a phase I clinical trial in breast cancer (25, 26). In a murine prostate cancer model, prostate tumor cryoablation combined with an anti-CTLA-4 monoclonal antibody (Mab) resulted in growth inhibition, greater $CD4^+$ and $CD8^+$ T cell infiltration, and higher T effector to T regulatory cell ratios in distant prostate tumors compared to cryoablation or anti-CTLA-4 MAb alone (25). A phase I clinical trial of neoadjuvant cryo-immunotherapy in breast cancer demonstrated that the combination of percutaneous cryoablation and ipilimumab was safe and associated with increased expression of $CD4^+$ and $CD8^+$ T cell activation markers (e.g. ICOS and Ki67) in peripheral blood mononuclear cells (26). The combination of cryoablation and immune checkpoint inhibitors is currently being investigated for the treatment of non-small cell lung cancer in a clinical trial based at Brown University (ClinicalTrials.gov identifier: NCT02469701).

VI) Biomarkers to Assess for BCI Induced Anti-Tumor Immune Responses

There is no well-established peripheral blood biomarker panel to assess for immune responses after cryo-immunotherapy. After percutaneous cryoablation, previous studies have not demonstrated meaningful changes in total lymphocyte counts in peripheral blood, suggesting that biomarkers of cryoablation-induced immune responses lie within finer subsets of lymphocytes. Consistent with this, Lin, et al. recently reported that IL-2 and IFN-gamma were elevated in serum after cryoablation (25), suggesting that changes in T-cell function may follow the procedure. Assays of secreted cytokines in serum, however, fail to identify the exact cell type producing the cytokines, and these cytokines can be released by non-T-cells. Moreover, tumor-specific T-cells are capable of expressing many cytokines other than IL-2 and IFN-gamma, each of which provides different mechanistic information about the nature of the immune response. Therefore, cellular assays that combine global markers of T-cell activation with cell-by-cell analysis of many different cytokines will be required to determine if cryoablation induces T-cell responses. Additionally, given the rationale and potential for combining cryoablation and checkpoint inhibitor therapies, an analysis of checkpoint molecule expression will also be valuable in this setting.

Recently, a co-investigator on this study (Dr. Pratip Chattopadhyay, Director of Precision Immunology Incubator at NYU Langone Health) developed 30+ parameter flow cytometry technology (which has now been commercialized by BD Biosciences as the Symphony A5). This technology is ideally suited to the analyses described above, because of the bright fluorescent dyes used (Brilliant Dyes, BD Biosciences) and the low electronic noise of the system. (The technology provides better resolution than alternate cytometry technologies, like mass cytometry (CyTOF), with higher throughput, less cell loss, and a more robust hardware platform.) Dr. Chattopadhyay developed a number of antibody staining panels for the system, including a panel that assesses many immune checkpoint molecules at once, and a pan-cytokine/global T-cell activation panel. Table 1 lists the markers studied with each panel. There are three notable advantages of highly multiplexed flow cytometry panels like these. First, they allow broad screening of many markers in order to find the most sensitive biomarkers in a particular disease setting. Second, by finely and precisely characterizing combined expression of every marker studied at the single cell level, a more detailed, mechanistic understanding of responses is possible than with serum, secretion, or bulk population assays. Finally, the technology is sample sparing; it provides maximal information (about protein expression) from a single vial of cells; this will be important given the relatively low numbers of cells collected from BAL. Though a number of institutions now have high-parameter flow cytometry technology, these panels have not been successfully developed elsewhere, and those institutions do not have Dr. Chattopadhyay's expertise in application of this technology.

Panel 1	Panel 2
CD45	CD45
Live/Dead	Live/Dead
CD3	CD3
CD4	CD4
CD8	CD8
CD45RO	CD45RO
CCR7	CD154
CD27	CD107A
CD28	IFNG
CD127	IL2
CD57	TNF
CD25	IL3
CD95	IL4
CD69	IL5
CD103	IL8
CXCR6	IL9
CXCR3	IL10
BTLA	IL13
TIGIT	IL17
CTLA4	IL21
4-1BB	GMCSF
2B4	RANTES
GITR	LTA
TIM3	BCL2
ICOS	KI67
PD1	PD1
TIGIT	CD38
HLA-DR	HLA-DR
OX40	Granzyme B
CD69	CD69

Although this approach is novel in immuno-oncology, there is precedent, particularly from studies of vaccine responses. In healthy individuals, baseline peripheral blood $CD8^+$ T cell $HLA\text{-}DR^+CD38^+$ and $Ki67^+Bcl\text{-}2^{low}$ expression is 0.5-2% and less than 0.5%, respectively (26). After immunization of healthy volunteers with the smallpox vaccine, up to 40% of peripheral blood $CD8^+$ T cells displayed $HLA\text{-}DR^+CD38^+Ki67^+Bcl\text{-}2^{low}$ (26). Similarly, in 29 patients with advanced NSCLC started on PD-1/PDL-1 axis inhibitory antibodies, approximately 70% of patients had a 1.5-fold increase in peripheral blood $Ki67^+CD8^+$ T cells demonstrating high levels of expression of DR^+CD38^+ $Bcl\text{-}2^{low}$ and PD-1 post-treatment (27). Approximately 25-80% of these responding $Ki67^+CD8^+PD\text{-}1^+$T cells expressed intra-cellular granzyme B, consistent with cytotoxic potential (27). Epstein-Barr Virus (EBV) -specific $CD8^+$ T cells had minimal activation and proliferation after PD-1 targeted therapy suggesting these $CD8^+$

T cell responses may be tumor specific (27). Importantly, 80% of patients who developed these $CD8^+$ T cell responses within 4 weeks of starting anti-PD-1 therapy had a clinical benefit.

In addition to assessing for pan-cytokine/global T cell activation and expression of immune checkpoint molecules in peripheral blood mononuclear cells utilizing flow cytometry technology, the NanoString™ can assess for a variety of other immune biomarkers from peripheral blood. The NanoString™ platform offers the convenience of straightforward high-throughput mRNA quantitation using RNA barcodes to probe and count mRNA transcripts of interest (28). The pre-designed OncoImmune™ Profile panel, which measures 770 gene targets most relevant in tumor immunobiology.

2.2 Bronchoscopic Cryo-Immunotherapy (BCI)

BCI is a novel method to cryoablate portions of peripheral lung tumors through a bronchoscope. BCI will be performed using the ERBOKRYO® CA – Cryosurgical Unit with Flexible ERBECRYO™ Probe of 1.9 mm outer diameter or using the ERBECRYO™2 single-use flexible 1.1mm outer diameter cryoprobe for use with robotic bronchoscopy (ERBE, Inc., Tubingen, Germany). This device is FDA approved for tissue destruction of target pulmonary tumors (see attached FDA 510K Approval Form). Current and accepted bronchoscopic procedures using this device are bronchoscopic cryoablation of endobronchial tumors in the central airways and bronchoscopic transbronchial cryobiopsies of lung parenchyma. This device has not yet been used to perform bronchoscopic cryoablation of peripheral lung tumors. However, BCI is only a slight modification of known and accepted current uses of this device.

Pre-BCI Planning:

The clinical team, as per standard protocol, will conduct pre-procedural evaluation and care with no input from the researchers. The bronchoscopy with BCI will be performed by the NYU Interventional Pulmonology team under general anesthesia in the bronchoscopy suite or in the operating room at NYU Langone Medical Center. The pre-procedural chest CT scan will be carefully evaluated to identify the airway path leading to the peripheral lung tumor prior to performing BCI.

If the patient does not have biopsy-proven non-small cell lung cancer prior to the procedure, the clinical team will obtain diagnostic biopsies per standard protocol without input from researchers. Exact location and type of biopsies (i.e. transbronchial needle aspiration of mediastinal or hilar lymph nodes and/or transbronchial biopsies of peripheral tumor) will be determined by the clinical team. The trans-bronchial biopsies will be prepared for frozen section and/or transbronchial needle aspirates for rapid on-site cytology evaluation (ROSE), as indicated. If ROSE is performed a certified cytotechnologist will perform a rapid microscopic analysis of the ROSE specimen to confirm presence or absence of malignant cells. In cases where non-small lung cancer cannot be confirmed, but it is highly suspected based on clinical criteria, ROSE or frozen section, select patients with high-risk features for NSCLC, including significant smoking history, history of COPD, spiculated lesion with bronchus sign on chest CT, enlarged of mediastinal/hilar lymph nodes and or distant metastatic disease on chest CT or PET CT scan, may be enrolled.

Description of BCI:

In BCI, a radial endobronchial ultrasound (EBUS) probe (Olympus Ultrasonic Probe UM-S20-17S or UM-S20-20R, Tokyo, Japan) with a flexible guide sheath will be inserted into the working channel of a flexible bronchoscope, or may be inserted through the working channel of a robotic bronchoscope, and advanced into the target bronchial sub-segment to identify the peripheral tumor. If the peripheral tumor is unable to be identified by radial ultrasound within twenty minutes, then the patient will not be a candidate to undergo BCI. Once the peripheral tumor is identified by radial EBUS, the radial EBUS

probe will be removed, but the guide sheath will remain in position to serve as a position marker and as a conduit from the bronchoscope to the location of peripheral tumor, if robotic bronchoscopy is being performed, the bronchoscope itself serves as the guide sheath. This will be performed under fluoroscopic guidance to confirm stability of guide sheath or robotic bronchoscopy position.

The Flexible ERBECRYO™ Probe of 1.9mm outer diameter (ERBE, Inc., Tubingen, Germany) will then be inserted through the guide sheath or the ERBECRYO™2 single-use flexible 1.1mm outer diameter cryoprobe through the robotic bronchoscope and advanced to reach the peripheral tumor. The cryoprobe will be applied to the target tumor. The cryoprobe will be maintained and calibrated based on manufacturer standards. In this study, freeze time will be selected based on a dose escalation protocol (Please see Section 2.2.3 for further details.) The freeze phase will be followed by a passive thaw phase, which will last until the flexible cryoprobe has separated from the target tissue. Freese-thaw cycles will be performed (with exact number based on tumor size and dose escalation protocol) from distal to proximal within the target tumor. One freeze-thaw cycle will be performed per one cm diameter of the tumor. Only one airway will be used to access the target tumor. Providers will be instructed to remain greater than 2 cm away from critical structures. The objective will not be to completely engulf the peripheral tumor in an “ice ball”, but to cryoablate a portion of the peripheral tumor.

We will evaluate for formation of an ice ball with fluoroscopy as we perform BCI. We will note the presence or absence of discernible ice ball formation for each patient at each dose level after completion of BCI.

2.2.1 Preclinical Data

Cryoablation Induced Anti-Tumor Immune Response in Lewis Cell Lung Cancer Murine Model

The Lewis lung carcinoma model is a syngeneic and reproducible non-small cell cancer line in mice(29). In a syngeneic mouse model, immunologically compatible cancer cells are injected into an immunocompetent mouse. In this study, immunocompetent mice were implanted with Lewis lung cancer tumors in bilateral flanks and treated with cryoablation. In the control group, mice received a subcutaneous administration of LPS alone without undergoing cryoablation. Cryoablation of heterotopic lung tumors in the flanks of mice led to significantly slower growth of contralateral flank tumors consistent with an “abscopal-like” effect of cryoablation. At day 14 post-cryoablation, contralateral tumor volume was approximately five times smaller in the group receiving two cycles of cryoablation compared to the control group (17). Furthermore, the percentage of $CD4^+$ T cells and $CD8^+$ T cells infiltrating the contralateral tumors increased by over five and six times, respectively in the group receiving two cycles of cryoablation compared to the control group. Additionally, the subcutaneous peritumoral space of the cryoablated tumors had higher levels of pro-inflammatory cytokines, including IL-1β, IL-2, IL-6, IL-12β, TNF-α, and IFN-γ after cryoablation compared to controls (17). These findings were highly suggestive that the “abscopal-like” effect of cryoablation of lung cancer was immunological in nature, and mediated both by cellular and cytokine effector mechanisms.

Enhancement of Anti-Tumor Immune Response with Cryoablation Combined with Immunotherapy in Lewis Lung Cancer Murine Model

Immunocompetent mice (C57BL/6J strain) were implanted with Lewis Lung carcinoma tumors in their intra-foot pads. In addition to treatment with cryoablation, bone marrow derived immature dendritic cells (DC) were isolated, cultured ex-vivo, and then co-administered with an immune adjuvant, CpG-ODN, by intratumoral injection one hour after cryoablation and again seven days after cryoablation. Additional treatment arms for comparison included treatment with and without cryoablation followed by intratumoral PBS, intratumoral DC or CpG-ODN, or intratumoral DC cells and CpG-ODN. The tumor draining lymph nodes were analyzed seven days after the second treatment (14 days after

cryoablation). In these lymph nodes, there were significant increases in $CD4^+$ T cells and $CD8^+$ T cells, and a significant decrease in T regulatory cells in the combined cryoablation and treatment with intratumoral DC and CpG-ODN group compared to all other arms. (T regulatory cells act to maintain tolerance to self-antigens and to downregulate immune responses. A decrease in the number of T regulatory cells can increase tumor immunity.(30)) Additionally, there were significantly increased $CD4^+$ T cells and $CD8^+$ T cell expression of IFN-gamma and TNF-alpha levels (involved in T_H1 mediated immune responses), but no increase in $CD4^+$ T cells or $CD8^+$ T cell expression of Il-4 (involved in T_H2 mediated immune responses)(18). Overall, combined cryoablation with intratumoral DC and CpG-ODN led to slower tumor growth, less lung cancer metastasis, and improved overall survival compared to other treatment groups (18).

Cryotherapy Combined with Anti-CTLA-4 Therapy for Enhanced Anti-Tumor Immune Response in Prostate Carcinoma Murine Model

In immunocompetent mice (C57BL/6J strain), cryoablation of a primary prostate tumor (Tramp C2 prostate cancer model) combined with anti-CTLA-4 therapy led to slower tumor growth of a secondary prostate tumor or complete rejection of prostate cancer cells upon after inoculation at a distant site compared to treatment with cryoablation alone, anti-CTLA-4 therapy alone, and placebo(31). After 50 days, tumor-free survival was 44% when treated with combination therapy compared to 0% in all other groups.

Additionally, secondary prostate tumors were infiltrated with higher levels of $CD4^+$ and $CD8^+$ cells and had a higher T effector cell to T regulatory cell ratio when treated with combined therapy compared to cryoablation alone, anti-CTLA-4 therapy alone, and placebo(31). The total number of $CD8^+$ T cells per mg of tumor increased 10-fold compared to cryoablation alone and placebo, and two-fold over anti-CTLA-4 therapy alone. The total number of $CD4^+$Foxp3- T cells per mg of tumor increased 8-fold compared to cryoablation alone and placebo, and 4-fold over anti-CTLA-4 therapy alone (31). In contrast to the significant anti-tumor immune response to cryoablation alone observed in the prior study using the Lewis Lung Cancer Murine Model, in this study, treatment of primary prostate tumor with cryoablation alone did not demonstrate an effect on secondary tumor growth or secondary tumor T cell infiltration. Despite this finding, the above significant synergistic immune and survival effects of cryoablation with anti-CTLA-4 therapy were observed.

2.2.2 Clinical Data to Date

Improved Outcomes When Targeted Therapy is Combined with Cryoablation for the Treatment of Advanced Non-Small Cell Lung Cancer

In a small, randomized controlled trial, 36 female patients who were non-smokers received either percutaneous cryoablation prior to gefitinib (epidermal growth factor receptor [EGFR] transcription kinase inhibitor) or gefitinib alone for treatment of stage IIIb and stage IV, EGFR positive, non-small cell lung cancer. Treatment with cryoablation prior to initiation of gefitinib was associated with significantly more partial regression (55.6% versus 27.8%), increased progression free survival (8.4 months versus 5.2 months), and increased 1-year survival rate (66.7% versus 33.3) (19). These studies support the use of combination therapies with cryoablation to improve clinical outcomes for treatments of advanced non-small cell lung cancer.

Enhanced Anti-Tumor Response and Slower Tumor Growth with Cryoablation Combined with Immunotherapy for the Treatment of Advanced Non-Small Cell Lung Cancer

In an experimental clinical trial, 60 patients with stage III and stage IV non-small cell lung cancer were randomized to receive percutaneous cryoablation alone or percutaneous cryoablation followed by

allogeneic natural killer (NK) cell immunotherapy. Percutaneous cryoablation of lung, liver, and bone tumors due to non-small cell lung cancer were performed. In the 30 patients who were treated with combination therapy, allogeneic donor peripheral blood was collected for NK cell culture and isolation. NK cell immunotherapy was administered 3-5 days after percutaneous cryoablation. Peripheral blood was collected 9 days after cryoablation. Compared to peripheral blood post-cryoablation alone, peripheral blood post-combined therapy was remarkable for significant increases in number of T cells, $CD8^+$ T cells, and specific cytokines involved in T_H1 mediated immune responses (Il-2 and IFN-gamma) (20). Maximal tumor diameters at 1 month post-treatment were significantly smaller in both groups compared to pre-treatment diameters. At 3 months post-treatment, maximal tumor diameter was significantly smaller in the combined group compared to the cryoablation alone group. Effective clinical response rates included complete responders (arterial enhancement imaging of tumor disappeared) and partial responders (total reduction in tumor diameter of more than 30%). The response rate 3 months post-treatment was 63.3% in the combined group compared to 43.3% in the cryoablation alone group ($p< 0.01$) (20).

Improved Outcomes When Chemotherapy and/or Immunotherapy Is Combined with Cryoablation for the Treatment of Advanced Non-Small Cell Lung Cancer

In a single center retrospective review of 161 patients treated for stage IV non-small cell lung cancer, there was an association between combination treatment regimens involving percutaneous cryoablation and improved survival compared to treatment regimens without percutaneous cryoablation(21). Eighty-six patients' treatment regimens included percutaneous cryoablation compared to 75 patients whose treatment regimens did not include percutaneous cryoablation. Treatment regimens also included platinum-based chemotherapy, dendritic cells co-cultured with cytokine-induced killer cells (DC-CIK), or both. Median overall survival was significantly longer at 20 months in patients whose treatment included cryoablation versus 10 months in patients treated without cryoablation. In 33 patients receiving all three treatments, median overall survival was 27 months. In comparison, median overall survival in 32 patients treated with cryoablation and chemotherapy and 21 patients treated with cryoablation and immunotherapy were 18 months and 17 months, respectively. In 44 patients who received chemotherapy alone and 31 patients who received chemotherapy with immunotherapy, median overall survival was 8.5 months and 12 months, respectively(21).

Pre- and Post- Percutaneous Cryoablation Peripheral Blood Biomarkers

In a small clinical trial of five patients treated with percutaneous cryoablation for various malignancies, there were no significant changes in pre- cryoablation and post- cryoablation peripheral blood T cell subsets ($CD4^+$, $CD8^+$, and regulatory T cells)(32).

Similarly, in a small clinical trial of 30 patients with advanced non-small cell lung cancer treated with percutaneous cryoablation alone, post-cryoablation peripheral blood total T cells and $CD8^+$ T cells both only marginally increased by 1.1-fold compared to pre-cryoablation peripheral blood (20). However, there were significant increases in specific cytokines involved in T_H1 mediated immune responses levels in post-cryoablation peripheral blood compared to pre-cryoablation peripheral blood: Il-2 and IFN-gamma levels increased by 1.9-fold and 2.3-fold, respectively (20).

2.2.3 BCI Dose Rationale

BCI of peripheral lung tumors has not been performed previously. To maximize safety, the initial freeze time is shorter than standard freeze times for cryobiopsy of peripheral lesions (15). Our dose escalation will initiate patients at a safe freeze time for which there is extensive clinical experience with related procedures. If there is no complication from this, in the next cohort of patients we will gradually escalate dose to those higher than are routinely performed in bronchoscopic cryobiopsy.

Freeze times will be escalated up to 30 seconds based on tolerability. In comparison, freeze times of up to 20 minutes have been safely administered during percutaneous cryoablation of peripheral lung tumors (7, 10). Although an optimal freeze time duration is unknown, longer freeze times may be needed in order to induce anti-tumor immune responses. Based on tolerability, freeze times will be increased as described in the following table:

Dose Level	Freeze Time (per site)	Thaw Time
Level 1	1 second	Will last until the probe separates from target tissue*
Level 2	2 seconds	Will last until the probe separates from target tissue*
Level 3	3 seconds	Will last until the probe separates from target tissue*
Level 4	5 seconds	Will last until the probe separates from target tissue*
Level 5	10 seconds	Will last until the probe separates from target tissue*
Level 6	20 seconds	Will last until the probe separates from target tissue*
Level 7	30 seconds	Will last until the probe separates from target tissue*

* Thaw times at each dose level will be captured for each site.

2.2.4 Rationale for Performing BCI

Although anti-PD-1 monoclonal antibody therapy has emerged as first-line treatment in select patients with advanced non-small cell lung cancer who have tumor PDL-1 expression of over 50% (22), only around 20% of patients respond to immune checkpoint inhibitors in the majority of clinical trials (23). Combination therapy of cryoablation and anti-PD-1 therapy has the potential to enhance anti-tumor immune responses and improve clinical efficacy compared to treatment with anti-PD-1 therapy alone. In theory, BCI could stimulate anti-tumor specific T cell activation and proliferation, and PD-1 inhibition would prevent these tumor specific T cells from developing T cell anergy or immune tolerance by blocking PD-1/PD-L1 interactions.

Currently, percutaneous cryoablation is an accepted technique for establishing local control of peripheral lung tumors which are not amenable to surgical resection. There are, however, potential benefits to performing BCI instead of percutaneous cryoablation of peripheral lung tumors. Major innovations of BCI over percutaneous cryoablation of NSCLC include a lower theoretical rate of pneumothorax; significantly shorter treatment time; the ability to perform BCI during the same procedure as diagnostic bronchoscopy, and to concomitantly obtain tissue samples from primary tumor, draining lymph nodes, and periphery of lung. Additionally, unlike percutaneous cryoablation, BCI may allow for a greater capacity for safe, repeated procedures for future combination trials with ICI.

We hope that this trial will demonstrate that BCI is feasible and safe, and that BCI induces significant anti-tumor immune responses. These results would support future investigations of the

efficacy of BCI combined with systemic and/or local delivery of anti-PD-1 therapy for the treatment of select patients with advanced non-small cell lung cancer.

2.3 Potential Risks & Benefits

2.3.1 Known Potential Risks

Potential Risks of Bronchoscopic Cryo-Immunotherapy (BCI)

The radial endobronchial ultrasound probe, which will be used to identify lung tumors in BCI, could potentially cause airway trauma, bleeding, or pneumothorax in rare cases. When lung tumors undergo cryoablation in BCI, potential risks include bleeding, hemoptysis, and pneumothorax. Because BCI does not involve taking any biopsies or removing any lung tissue, the risk of bleeding, hemoptysis, and pneumothorax directly related to this intervention are anticipated to be low. After applying freezing temperatures, rewarming will occur to ensure that the cryoprobe does not remain frozen to the target lung tumor. This will avoid accidentally removing or performing a biopsy on the target tumor, which could cause bleeding, hemoptysis, or pneumothorax. However, if a large pneumothorax occurs and is not properly treated, or if severe bleeding occurs, which is not properly controlled, then these potential complications could theoretically cause severe hypoxemia and be life-threatening. If a pneumothorax occurs, treatment with oxygen and possibly tube thoracostomy, depending on the size of the pneumothorax, will be provided. If significant bleeding or hemoptysis occurs, treatment to stop the bleeding may include applying iced saline, topical epinephrine, or placement of an endobronchial blocker. There may be additional risks of BCI that are currently unforeseeable. We will take all due precautions to determine if a potential participant is at increased risk for any adverse events and to minimize any potential complications in advance of undergoing BCI.

BCI will involve exposure to radiation from fluoroscopy to guide the placement of the probe. The risks of receiving very small doses of radiation are thought to be low. These risks are not actually known. Pregnant women cannot be exposed to radiation. Women of child-bearing age must have a negative pregnancy test before they can have an X-ray and undergo appropriate lead shielding to minimize exposure to reproductive organs.

Participation in this study will lengthen the overall time of bronchoscopy and exposure to general anesthesia by approximately 15-25 minutes. In rare cases, risks associated with general anesthesia include arrhythmias, heart attack, stroke, and death. All due precautions will be taken to determine if you a potential participant is at increased risk for any of these complications. General anesthesia will be provided by an anesthesiologist throughout the procedure.

Potential Risks of Bronchoalveolar Lavage (BAL)

BAL is a low risk procedure, and sometimes is included as a standard of care component in routine bronchoscopy for lung cancer. BAL will be performed for research purposes in this study. Potential rare risks of bronchoalveolar lavage (BAL) are bronchospasm, airway trauma, bleeding, fever, infection, pneumothorax, and hypoxemia.

Potential Risks of Peripheral Blood Draw

Potential risks of blood draws are localized bruising, redness or swelling at the venipuncture site. This is normally self-limited, requiring no treatment.

Other Potential Risks

Participating in clinical research involving the access to, use, and sharing of health information involves a risk of loss of confidentiality. However, privacy procedures in place and good clinical practice guidelines are followed for the study to minimize risks associated with research procedures and participation. We will do our best to minimize this risk by labeling information and samples with a unique code and securely keeping identifiable information separates from data and samples. Enrollment in this study will have no impact on the standard of care portion of the bronchoscopy. Participation will also not delay you from receiving standard of care lung cancer treatment.

2.3.2 Known Potential Benefits

Although bronchoscopic cryo-immunotherapy may cause tissue necrosis of a portion of the target peripheral lung tumor and has the potential to induce anti-tumor immune responses, no potential benefits are anticipated to be conferred to participants in this study. Information derived from this study may benefit patients with lung cancer in the future.

3 Objectives and Purpose

3.1 Primary Objective: Demonstrate Feasibility and Determine the MTD

1) Demonstrate feasibility of BCI

We hypothesize that BCI will be feasible, defined as successful performance in at least 80% of patients in whom BCI is attempted. Successful performance of BCI will require identification of the target peripheral lung tumor by radial endobronchial ultrasound within 20 minutes followed by completed cryoablation of target tumor.

2) Determine the safety and MTD of BCI

We hypothesize that BCI will prove safe at each planned dose level. Using a standard 3+3 design, the trial will determine the maximally tolerated dose (MTD) by sequentially assessing increasing freeze times during the procedure. Dose-limiting toxicity (DLT) is defined as the occurrence of any of the following: grade 2 or 3 bleeding, pneumothorax necessitating tube thoracostomy, or any NCI CTCAE grade 4 or 5 adverse events, which may be possibly, probably, or definitely related to BCI (33). The monitoring period for dose limiting toxicity will be for seven days post-BCI.

3.2 Secondary Objectives: Assess BCI Induced Anti-Tumor Immune Responses

1) Pre- and Post-BCI Peripheral Blood Analysis

We hypothesize that BCI will induce activation and proliferation of peripheral blood T cells. Based on previous work (26, 27) (see section 2.1.IV for full details), we hypothesize that some combination of the following markers will be displayed on peripheral blood $CD8^+$ T cells after BCI: HLA-DR and CD38 (surface markers of $CD8^+$ T cell activation), Ki-67 (a cell cycle marker of proliferation), Bcl-2 (an anti-apoptotic protein downregulated on activated $CD8^+$ T cells), and PD-1. We also hypothesize that there will be significant changes in peripheral blood lymphocyte gene expression after BCI, which will be assessed by the PanCancer OncoImmune™ profile panel, a 770-plex gene expression panel covering innate and adaptive immune responses (NanoString® Technology, Seattle, WA).

2) Investigate BCI Association with PD-1 Phenotype of Effector T cells in NSCLC

We hypothesize that BCI will induce PD-1 phenotype in circulating effector T cells on single-cell RNA sequence analysis and that CD8+ T cell responses to BCI will be greater among subjects with high PD1 expressing T cells in BAL effluent.

4 Study Design and Endpoints

4.1 Description of Study Design

This is an open-label, phase I pilot study to primarily evaluate the feasibility and safety of bronchoscopic cryo-immunotherapy (BCI) of peripheral lung tumors in patients with non-small cell lung cancer or suspected non-small cel lung cancer who are undergoing a standard-of-care bronchoscopy for palliative and/or diagnostic purposes. Dose escalation will proceed using a standard 3+3 design.

4.2 Study Endpoints

4.2.1 Primary Study Endpoints: Feasibility and Safety

1) Each patient will be classified as having successfully undergone BCI or not. Feasibility of BCI is defined as successfully performing BCI in at least 80% of patients in whom BCI is attempted. Successful performance of BCI will require identification of the target peripheral lung tumor by radial endobronchial ultrasound within 20 minutes followed by completed cryoablation of target tumor. The target tumor will have freeze-thaw cycles applied every one centimeter of the largest diameter. Additional BCI features will be described as follows:
    a) Ability of radial endobronchial ultrasound to identify peripheral lung tumor
    b) Length of time to perform BCI
    c) Length of time of fluoroscopy exposure during BCI

2) Dose-limiting toxicities (DLT) will be used to guide the dose-escalation procedure and determine the maximum tolerated dose. A DLT is defined as any of the following occurring up to 7 days post-BCI:

    - Grade 2 or 3 bleeding, based on the following scale (34):
        a) Grade 0: absence of bleeding
        b) Grade 1: bleeding requiring suctioning to clear
        c) Grade 2: bleeding requiring an endoscopic procedure (iced saline, 1:20,000 topical epinephrine, or bronchial occlusion)
        d) Grade 3: severe bleeding (>100cc total volume). Severe bleeding will also be defined as bleeding which cannot be controlled endoscopically, requires ICU admission or interventional radiology/ surgical intervention, or results in respiratory or hemodynamic instability (34)
    - Pneumothorax necessitating tube thoracostomy; recorded features of pneumothorax to include:
        a) size
        b) associated symptomatology
        c) respiratory impairment
        d) need for chest tube placement
    - Any NCI CTCAE (33) grade 3, 4, or 5 adverse events, which may be possibly, probably, or definitely related to BCI

4.2.2 Secondary Study Endpoints: Immune Analysis

1) Assess BCI Induced Anti-Tumor Immune Responses in Peripheral Blood

Research Samples: Peripheral blood will be collected by the investigatory team prior to BCI on the day of procedure, and at approximately 7 days and 14 days after BCI (± 3 days). Specimens may be processed in the William N. Rom Environmental Lung Disease Laboratory, the Segal Translational Lung Biology Laboratory, or in the Chattopadhyay Laboratory on the campus of the New York University Langone Medical Center/Bellevue Hospital Center.

Flow cytometry: Peripheral blood samples will undergo Ficoll-Paque separation. The buffy coat containing peripheral blood mononuclear cells (PBMCs) will be isolated, and then washed with phosphate buffered saline. Isolated PBMC will undergo staining with antibodies against the markers described in Table 1 (Section 2.1: Background Information and Relevant Literature – Subsection VI Biomarkers to Assess for BCI Induced Anti-Tumor Immune Response) in Dr. Pratip Chattopadhyay's laboratory in the Perlmutter Cancer Center at NYU School of Medicine.

Data will be acquired on a BD Symphony A5 flow cytometer, and analyzed using FlowJo software and various R-based bioinformatics algorithms developed previously by Dr. Chattopadhyay and colleagues. The 30-parameter flow cytometry panels we plan to use (Table 1) will query multiple markers of activated cells (including a broad range of cytokines), and multiple markers associated with immune checkpoints (along with markers of T-cell differentiation). To ensure that our results do not reflect global T-cell activation or exhaustion, in follow-up experiments, we will substitute a cocktail of peptide MHC Class I multimers ("tetramers") against influenza and EBV responses into panel 1 (the cocktail will cover various epitopes and HLA-types) and stimulate a parallel aliquot of cells from panel 2 with a CEF (CMV/EBV/influenza) cocktail of peptides or the same tetramer cocktail used for Panel 1.

mRNA analysis: We will also be assessing a variety of other immune biomarkers from peripheral blood utilizing the NanoString™ platform, including cytokine gene expression, that may allow for the establishment of a variety of peripheral blood biomarkers to correlate with the effects of BCI in advanced NSCLC. Once sorted, the CD8+ and CD4+CD25- cells (non-Treg cells) will be processed for RNA extraction and NanoString™ mRNA quantitation. RNA extraction will be performed using the MARIS technique to insure RNA quality after sorting (35). We are particularly interested in determining gene expression of proteins involved in T cell activation and immune regulation, such as CTLA-4, PD-1, OX-40, TIM-3, IL-10, TGF-ß, IL-2, MHC-II and TNF-α. The transcriptome data will be processed in collaboration with the Biostatistics Division led by Dr. Andrea Troxel at the NYU School of Medicine to calculate the relative expression of the key genes of interest previously noted. The NanoString™ platform offers the convenience of straightforward high-throughput mRNA quantitation using RNA barcodes to probe and count mRNA transcripts of interest (28). Our preliminary analysis will be conducted with the pre-designed OncoImmune™ Profile panel, which measures 770 gene targets most relevant in tumor immunobiology.

2) Investigate BCI Association with PD1 phenotype of Effector T cells in NSCLC

Research Samples: In addition to peripheral blood specimens as described previously, BAL fluid will be collected from regions of the primary tumor and of the distal portion of the same lung.

Using a subset of samples from enrolled subjects enrolled with sufficient BAL specimens, we will perform single-cell RNA sequencing on sorted CD3+ T cells from BAL and PBMCs obtained at the time of BCI. This approach will be repeated in PBMC after 7 and 14 days post BCI. The percentage of PD-1 expressing T lymphocytes will be determined in $CD4^+$ and $CD8^+$ cells from both BAL and PBMC compartments. $PD1^+$ cell percentage and transcriptome will be compared between responder categories. RNA sequencing and subsequent data analysis will be performed in the Genomic Technology and Bioinformatics Cores of NYU School of Medicine.

4.2.3 Sub-Set Analysis and Exploratory Endpoints

1) Investigation of changes in peripheral blood $CD8^+$ T cells displaying a combination of $Ki67^+$,PD-1+, HLA-DR^+, $CD38^+$, and Bcl-2^{low} after BCI at each dose level. This sub-set analysis will investigate if there is an optimal freeze time duration for inducing anti-tumor immune responses.

2) Investigation of changes in peripheral blood $CD8^+$ T cells displaying a combination of $Ki67^+$,PD-1+, HLA-DR^+, $CD38^+$, and Bcl-2^{low} after BCI at each dose level. This sub-set analysis will investigate if the combination of cryoablation and current active cancer treatment will lead to enhanced increases in percent of peripheral blood $CD8^+$ T cell expression of PD-1 and Ki67 and/or $CD8^+$ T cells displaying an activated effector-like phenotype.

5 Study Enrollment and Withdrawal

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Biopsy-proven, advanced, inoperable non-small cell lung cancer (stages IIIA/B/C and IVA/B based on the 8th edition TNM staging guidelines) or suspected inoperable non-small cell lung cancer (stage as defined above) who may undergo on-site pathology review of tissue specimen obtained at time of diagnostic and staging bronchoscopy.
    - If on-site pathology review is not feasible or is unable to confirm the diagnosis of non-small cell lung cancer, patients with high-risk features for the diagnosis of non-small cancer including past or current cigarette smoking, history of COPD, spiculated lesion with bronchus sign on chest CT, presence of mediastinal/hilar adenopathy or evidence of distant metastatic disease on chest CT scan or PET CT scan may be included.
- Pre-procedure chest CT scan with the presence of an airway path leading directly to the peripheral lung tumor (also known as a “bronchus sign”), and the peripheral lung tumor will need to be visualized by radial endobronchial ultrasound at time of bronchoscopy
- Undergoing bronchoscopy for diagnostic and/or palliative purpose unrelated to this study
- Age of 22 years or older
- Ability to provide informed consent
- Concomitant chemotherapy, immunotherapy, and/or radiation therapy are allowed
- ECOG performance status of ≤2

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Pregnancy (negative pregnancy test required for women of child-bearing age)
- Currently on a platelet inhibitor (such as clopidogrel) other than aspirin or NSAIDS, or on a blood thinner (such as heparin, enoxaparin, or a novel oral anticoagulant), which is unable to be held for planned bronchoscopy
- INR ≥ 1.5 (after correction and only if checked pre-operatively based on clinical indication)
- Platelets ≤ 100,000 (after correction)
- Absence of tissue diagnosis of non-small cell lung cancer either prior to procedure or during on-site pathology review at time of bronchoscopy in patients without the above specified high-risk features

- Absolute contraindication to bronchoscopy

5.3 Vulnerable Subjects

Vulnerable subjects will not be included in this study.

5.4 Inclusion of Men and Women

Men and women of any races and ethnic groups are eligible for this trial.

5.5 Strategies for Recruitment and Retention

Participants will be recruited from outpatient interventional pulmonary clinic visits and from the inpatient setting at NYU Langone Medical Center. Potential participants will be identified based on referrals to the interventional pulmonary team for a diagnostic and/or therapeutic bronchoscopy. Patients who have been identified as being eligible to participate in this study will be approached in the inpatient setting, in the Ambulatory Surgery/Endoscopy unit or during interventional pulmonary clinic visits.

We expect to recruit women and minorities. The number of women and minorities recruited will be dependent on the demographics of the lung cancer population at NYULMC. Our recruitment plan will not include the use of any NYULMC media services or recruitment material.

5.5.1 Use of DataCore/Epic Information for Recruitment Purposes

This study will utilize EPIC to identify subjects. Any recruitment information sent by email will utilize Send Safe email.

Once potential subjects have been identified, the study team will notify the treating physician (TP) that they have patients eligible to participate as follows:

- Provide TP with names of potential subjects, and discuss directly with TP whether or not enrollment of particular patients would be appropriate
- Only if TP agrees that particular patient would be appropriate for this study, potential subjects will be directly contacted during office clinic visit, on the Ambulatory Sugery/Endoscopy unit, or inpatient hospital stay

Once contact is made, approved recruitment language will be used to communicate the reason they are being contacted and subjects will be asked if they are interested in participating in this specific study. Should the potential subjects agree, the study team will provide the subjects with information regarding the next steps for participation.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858.

5.6 Duration of Study Participation

The total duration of study participation is 14 days. BCI will be performed at time of standard of care bronchoscopy on day 0. Peripheral blood will be collected by the investigatory team prior to BCI on the day of procedure, which will be drawn off the intravenous catheter inserted for the standard of care bronchoscopy to avoid a separate blood draw whenever possible. The investigatory team will also collect blood samples at approximately 7 days and 14 days after BCI (± 3 days). During each blood

draw, the volume of blood collected will be approximately 2 teaspoons (10 ml). This will occur at routine standard of care follow up clinic visits whenever possible. Alternatively, the investigatory team will collect blood samples at approximately 7 days and 14 days after BCI at the Perlmutter Cancer Center.

Total Number of Participants and Sites

- Total number of participants: 15-24
- Sites: New York University Langone Medical Center

5.7 Participant Withdrawal or Termination

5.7.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

5.7.2 Handling of Participant Withdrawals or Termination

If participant withdraws after treatment with BCI, the participant will be contacted via phone to monitor for any adverse events during the seven day period post-BCI. If participant withdraws after post-BCI blood draws have been performed, then their samples will be discarded.

If participant misses follow up appointment for blood draws on day 7 and 14 post-BCI, the participant will be contacted via phone. If participant does not respond to three phone calls, the participant will be considered lost to follow up.

5.8 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance with protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the IRB.

6 Procedural Intervention

6.1 Description of BCI

6.1.1 Administration of BCI

This will be an open label, phase I pilot study without a control group. As described in Section 2.2, the Flexible ERBECRYO™ Probe of 1.9mm outer diameter (ERBE, Inc., Tubingen, Germany) will be advanced through a bronchoscope to reach the target peripheral tumor, alternatively the ERBECRYO™2 single-use flexible 1.1mm outer diameter cryoprobe will be used if robotic bronchoscopy is being performed . The flexible cryoprobe, which reaches a minimum reported temperature of -89^{0}C, will be applied to the target tumor. Freeze time will be administered based on a dose escalation protocol, as described below. The freeze phase will be followed by a passive thaw phase, which will occur until the flexible cryoprobe has separated from the target tissue. The total number of freeze-thaw cycles will be performed (depending on tumor size) from distal to proximal within the target tumor. For each one centimeter of the longest diameter, one freeze-thaw cycle will be performed. The flexible cryoprobe will only be inserted into one airway to facilitate the cryoablation. Providers will be instructed to remain greater than 2 cm away from critical structures. The objective will not be to completely engulf the peripheral tumor in an ice ball, but to cryoablate a portion of the peripheral tumor.

We will evaluate for formation of an “ice ball” with fluoroscopy as well as via bronchoscopic visualization (when feasible) as we perform BCI. We will note the presence or absence of discernible ice ball formation for each patient at each dose level after completion of the BCI procedure.

6.1.2 Dose Escalation Protocol

A standard 3+3 dose escalation strategy will be used. The table below shows the doses that will be provided. Three participants will be initially enrolled at Level 1. If none of the first three participants at a given dose experiences dose-limiting toxicity (DLT), the dose will be escalated. If two or more of the first three participants experience DLT, the prior dose will be deemed the maximum tolerated dose (MTD) and the study will be terminated. If one of the first three participants experiences DLT, an additional three participants will be enrolled at the same dose. If among these six, only one experiences DLT, the dose will be escalated. If two or more of six experience DLT, the prior dose will be deemed the MTD and the study will be terminated. If the MTD is reached and only three participants have been treated at that dose, an additional three will be treated to ensure six subjects are treated at the MTD. DLT are defined in Section 4.1.1 (Primary Study Endpoints: Feasibility and Safety).

Dose Level	Freeze Time (per site)	Thaw Time
Level 1	1 second	Will last until the probe separates from target tissue*
Level 2	2 seconds	Will last until the probe separates from target tissue*
Level 3	3 seconds	Will last until the probe separates from target tissue*
Level 4	5 seconds	Will last until the probe separates from target tissue*
Level 5	10 seconds	Will last until the probe separates from target tissue*
Level 6	20 seconds	Will last until the probe separates from target tissue*

Level 7	30 seconds	Will last until the probe separates from target tissue*

6.1.3 Procedures for Training of Clinicians on Procedural Intervention

The NYU Interventional Pulmonary service has extensive experience using the flexible cryoprobe for debulking of central airway tumors. BCI will be performed by a board-certified, NYU Interventional Pulmonologist.

Only patients with the presence of a bronchus or airway path leading directly to the peripheral lung tumor will be eligible to undergo BCI. The peripheral tumor will also need to be identified by radial EBUS prior to performing BCI. If the peripheral tumor is unable to be identified by radial ultrasound within 20 minutes, then the patient will not be a candidate to undergo BCI.

6.1.4 Assessment of Compliance with Study Procedural Intervention

Not applicable

7 Study Procedures and Schedule

7.1 Study Procedures/Evaluations

7.1.1 Study Specific Procedures

- Bronchoalveolar lavage from regions of the primary tumor and from the distal portion of the same lung on day 0
- BCI on day 0
- Blood draws on day 0 (prior to BCI) and on days 7 and 14 (± 3 days) (post-BCI)

7.1.2 Standard of Care Study Procedures

Pre-BCI screening:

- Medical history (including cancer and cancer treatment history, co-morbidities, functional status, and social history)
- Medication and treatment history (specifically if patient is on a platelet inhibitor other than aspirin or anticoagulant), and if patient has previously or is currently receiving chemotherapy, radiation therapy, or immunotherapy.
- Physical examination (vital and full physical exam will be performed by clinical team prior to determining patient's fitness for undergoing bronchoscopy
- Chest CT assessment to evaluate for the presence of a bronchus or airway path leading directly to the peripheral lung tumor
- Screening laboratory evaluation as described in Section 7.2
- Pre-BCI blood collection for immune analysis

During bronchoscopy:

- Bronchoalveolar lavage (BAL) will be performed in two locations, which are the regions of the primary tumor and of the distal portion of the same lung. BAL is a standard of care component of routine bronchoscopy for lung cancer.

- If there is no pre-existing biopsy-proven non-small cell lung cancer diagnosis, on-site pathology review of a tissue biopsy obtained during bronchoscopy may be performed, if technically feasible or if the patient does not have high risk features for possible non-small cell lung cancer (see above).
- After the standard-of-care portion of the bronchoscopy is complete, the peripheral tumor will need to be identified by radial EBUS. If the peripheral tumor cannot be identified within 20 minutes, then BCI will not be performed.
- BCI will be performed in eligible candidates

Post-BCI:

- Monitor for any adverse events from day 0 to day 7 Post-BCI blood collection for immune analysis on day 7 ± 3
- Post-BCI blood collection for immune analysis on day 14 ± 3
- Patients who undergo BCI and are found not to have non-small cell lung cancer will be monitored for adverse events from day 0 to day 7 and be followed up at a standard of care pose-bronchoscopy visit within 7 ± 3 days. These patients will not have post-BCI blood collected.

7.2 Laboratory Procedures/Evaluations

7.2.1 Clinical Laboratory Evaluations

Screening Laboratory Evaluation:

- **Hematology:** hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count.
- **Biochemistry:** basic metabolic panel
- **Coagulation Panel:** INR, PTT (only if deemed necessary by the proceduralist as part of standard of care pre-bronchoscopy clinical assessment)
- **Pregnancy test:** In pre-menopausal women (women who have had a menstrual period within the past twelve months), a pregnancy test is to be done within 24 hours prior to study intervention and results must be available prior to administration of study procedure.

Study Laboratory Evaluation:

- **Pre-BCI blood collection:** Two lavender top blood tubes (approximately 10cc) will be collected for analysis of peripheral blood mononuclear cells and serum
- **Bronchoalveolar lavage:** Collected from regions of the primary tumor and of the distal portion of the same lung
- **Post-BCI day 7 blood collection:** Two lavender top blood tubes (approximately 10cc) will be collected for analysis of peripheral blood mononuclear cells and serum
- **Post-BCI day 14 blood collection:** Two lavender top blood tubes (approximately 10cc) will be collected for analysis of peripheral blood mononuclear cells and serum

For further details, pre- and post-BCI peripheral blood immune analysis is described in detail in Section 4.1.2 (Secondary Study Endpoints: Immune Analysis).

7.2.2 Other Assays or Procedures

None

7.2.3 Specimen Preparation, Handling, and Storage

Peripheral blood will be collected by the investigatory team prior to BCI on the day of procedure, which will be drawn off the intravenous catheter inserted for the standard of care bronchoscopy to avoid a separate blood draw whenever possible. This will occur in pre-op area, bronchoscopy suite, or operating room. Bronchoalveolar fluid will be collected during the bronchoscopy. The investigatory team will also collect blood samples at approximately 7 days and 14 days after BCI. This will occur at routine standard of care follow up clinic visits whenever possible. Alternatively, the investigatory team will collect blood samples at approximately 7 days and 14 days after BCI at the Perlmutter Cancer Center.

The investigators will store bronchoalveolar lavage fluid and blood samples on ice, and transport samples to the William N. Rom Environmental Lung Disease Laboratory at NYU/Bellevue Medical Center or the Segal Translational Lung Biology Laboratory at NYU Medical Center. The investigators will then process specimens. After isolated peripheral mononuclear cells undergo FACS, if additional processing is not to occur immediately, samples will be stored in a -80C freezer in the locked William N. Rom Environmental Lung Disease Laboratory at NYU/Bellevue Medical Center or the Segal Translational Lung Biology Laboratory at NYU Medical Center.

7.2.4 Specimen Shipment

Not applicable

7.3 Study Schedule

7.3.1 Screening

Screening Visit (Day -28 to 0)

- Review medical history, medications, and chest CT scan.
- Determine eligibility based on inclusion/exclusion criteria.
- Obtain informed consent of potential participant verified by signature on written informed consent form.
- Collect screening labs (if not already collected by clinical team): basic metabolic panel, complete blood count, and coagulation panel (if deemed necessary by the proceduralist).
- Schedule study visits for participants for day 7 and day 14 post-BCI (will attempt to coincide with scheduled office visit if possible).

7.3.2 Enrollment

Enrollment (Day -28 to Day 0)

- Obtain informed consent of potential participant (if not collected above) verified by signature on study informed consent form.
- Verify inclusion/exclusion criteria.
- Obtain urine pregnancy test (if applicable).
- Confirm demographic information, medical history, medication history, alcohol and tobacco use history.
- Record vital signs, results of examinations, other assessments.
- Collect pre-BCI peripheral blood sample
- Perform bronchoalveolar lavage during standard-of-care bronchoscopy
- Perform BCI

7.3.3 Study Visits

7.3.3.1 Visit 1: Day 7 (± 3) post-BCI

- Vital signs and physical exam
- Record adverse events as reported by participant or observed by investigator
- Collect blood for immune analysis

7.3.3.2 Visit 2: Day 14 (± 3) post-BCI

- Vital signs and physical exam
- Record adverse events as reported by participant or observed by investigator
- Collect blood for immune analysis

7.3.4 Data Collection

Data on patients' pathologic diagnosis, cancer surveillance imaging results, demographics, smoking history, cancer history, and medical history will be collected by researchers. Tumor sizes on any available cancer surveillance imaging, cancer treatment history, and mortality data will also be collected for up to one-year post-bronchoscopic cryo-immunotherapy by researchers.

7.3.5 Withdrawal/Early Termination Visit

If the subject withdraws or if early termination occurs, the participant will not be asked to provide further blood specimens.

7.3.6 Unscheduled Visit

Unscheduled visits will not occur. In the event of an adverse event requiring medical attention, the participant will be directed to seek immediate attention from their clinical team or to the emergency department.

7.4 Concomitant Medications, Treatments, and Procedures

All concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications.

In treatment naïve patients undergoing a diagnostic bronchoscopy, enrollment in this study will not lead to a delay in appropriate, standard of care treatment. Involvement in this study is not anticipated to preclude enrollment in any other ongoing clinical trial. In patients undergoing a diagnostic or therapeutic bronchoscopy who are already receiving treatment with chemotherapy, radiation, or immunotherapy, combination therapy with BCI could theoretically affect anti-tumor immune responses.

7.5 Justification for Sensitive Procedures

Not applicable

7.5.1 Precautionary Medications, Treatments, and Procedures

Not applicable

7.6 Prohibited Medications, Treatments, and Procedures

Patients currently on a platelet inhibitor (such as clopidogrel) other than aspirin or NSAIDs, or on a blood thinner (such as heparin, enoxaparin, or a novel oral anticoagulant) may be at increased risk for bleeding during their standard of care bronchoscopy or due to BCI. These medications will need to be held prior to planned bronchoscopy at the discretion of the proceduralist. If prohibited medications are not discontinued, then the patient will not be a candidate for BCI.

7.7 Prophylactic Medications, Treatments, and Procedures

None

7.8 Rescue Medications, Treatments, and Procedures

In the event of dose-limiting toxicity, rescue medication, treatments, and procedures may be necessary.

- Grade 2 bleeding may be treated with iced saline, topical epinephrine, or placement of an endobronchial blocker
- Grade 3 bleeding may require bronchial artery embolization or surgical intervention
- Pneumothorax may require chest tube placement based on size and other clinical features

7.9 Participant Access to Study Agent at Study Closure

BCI will only be performed once at time of standard of care bronchoscopy.

8 Assessment of Safety

8.1 Specification of Safety Parameters

Dose-limiting toxicities (DLT) will be used to guide the dose-escalation procedure and determine the maximum tolerated dose. A DLT is defined as any of the following occurring up to 7 days post-BCI:

- Grade 2 or 3 bleeding, based on the following scale (34):
  a) Grade 0: absence of bleeding
  b) Grade 1: bleeding requiring suctioning to clear
  c) Grade 2: bleeding requiring an endoscopic procedure (iced saline, 1:20,000 topical epinephrine, or bronchial occlusion)
  d) Grade 3: severe bleeding (>100cc total volume). Severe bleeding will also be defined as bleeding which cannot be controlled endoscopically, requires ICU admission or interventional radiology/ surgical intervention, or results in respiratory or hemodynamic instability (34)
- pneumothorax necessitating tube thoracostomy; recorded features of pneumothorax to include:
  a) size
  b) associated symptomatology
  c) respiratory impairment
  d) need for chest tube placement
- any NCI CTCAE (33) Grade 3, 4, or 5 adverse events, which may be possibly, probably, or definitely related to BCI

8.1.1 Definition of Adverse Events (AE)

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event
Adverse events are classified as serious or non-serious. A *serious adverse event* is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as *non-serious adverse events*.

8.1.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 Relationship to BCI

The clinician's assessment of an AE's relationship to BCI is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with cryoablation, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.
- **Not Related** – There is not a reasonable possibility that BCI caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established.

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after BCI, is unlikely to be attributed to concurrent disease or other drugs or chemicals.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after BCI). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to BCI makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of BCI and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.2.3 Expectedness

Dr. Jun-Chieh Tsay (Assistant Professor, Division of Pulmonary, Critical Care, and Sleep Medicine) will be the study's designated medical monitor who will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.2.4 Time Period and Frequency for Event Assessment

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

8.3 Reporting Procedures

8.3.1 Adverse Event Reporting

Any adverse events or serious adverse events possibly, probably, or definitely related to BCI will be reported in real-time notification by the clinical investigators to the Data and Safety Monitoring Board and Data Coordinating Center. In accordance with 21 CFR 812.150(a)(1), the clinical investigators will also report to the IRB as soon as possible and within 10 working days.

In the event of any unexpected fatal or life-threatening suspected adverse reaction, the Data and Safety Monitoring Board and IRB will be notified as soon as possible and within 2 working days.

8.3.2 Unanticipated Problem Reporting

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB within 3 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB within 3 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures) within 3 days of the IR's receipt of the report of the problem from the investigator.

8.3.3 Reporting of Pregnancy

Pre-menopausal persons of child-bearing age will be screened with a pregnancy test prior to enrollment. Pregnant people will be excluded from enrollment in this study. However, if an enrolled participant becomes pregnant after BCI is performed, they will be eligible to remain in the study without any modifications to study procedures.

8.4 Study Halting Rules

If 2 or more participants experience DLT at any dose level, then the prior dose will be determined to be the MTD and the study will be terminated. Participants already enrolled in the study will be followed post procedure as per protocol. The study will be terminated in the event of death, massive hemoptysis, or acute respiratory distress syndrome (ARDS).

Additionally, the study will be suspended when three grade 3 AEs determined to be “probably related.”. The medical monitor will notify the investigators immediately when the third grade 3 event is reported and enrollment screens will stop accepting new study participants. The investigators will inform the DSMB members within 24 hours of this occurrence and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding or halting the study.

8.5 Safety Oversight:

8.5.1 Data Safety Monitoring Board (DSMB)

Safety oversight will be under the direction of a Data Safety Monitoring Board (DSMB) composed of Dr. John Hay (Associate Chief of Staff for research at the VA NY Harbor Healthcare System and Associate Professor in the Division of Pulmonary, Critical Care, and Sleep Medicine) and Dr. Jeffrey Weber (Deputy Director of the Perlmutter Cancer Center and Professor of Oncology).

The members of the DSMB have appropriate medical and research expertise, and are not involved in this study. The DSMB will meet at least semiannually to assess safety and efficacy data on each research subject undergoing BCI. The primary responsibilities of the DSMB will be to periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and make recommendations concerning the continuation, modification, or termination of the trial. Pre-defined stopping rules are described in Section 8.4: Study Halting Rules. The outcome of each DSMB review and decision made (eg, continuing the study, continuing with modifications, suspending the study, or terminating the study) will be submitted to the IRB.

Dr. Jun-Chieh Tsay will serve as a medical monitor who be responsible for overseeing participant safety. Dr. Tsay has extensive expertise in both bronchoscopy and translational research. He will 1) review all AEs (including DLT and unanticipated events) on a regular basis throughout the trial; 2) be available to advise the investigators on trial-related medical questions or problems, and 3) evaluate cumulative participant safety data and make recommendations regarding the safe continuation of the study. He will identify/evaluate AE/SAEs, determine seriousness, severity, causality, and monitor and follow subjects to resolution. He will report the events and will notify the IRB. In the event of DLT, the IRB will be notified as soon as possible and within three days.

9 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by an assigned medical monitor
- On-site monitoring will take place throughout the study, which will include a comprehensive review of all data and safety
- Details of clinical site monitoring are documented in a clinical monitoring plan. This describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10 Statistical Considerations

10.1 Statistical and Analytical Plans

All dose-limiting toxicities will be tabulated by type of DLT and dose level.

In addition to tabulating toxicities, we will record data on pre- and post-BCI peripheral blood biomarkers. For the continuous immunologic outcomes, we will calculate means by sampling time and perform exploratory graphical analyses, but with the small sample sizes and potentially irregular lengths of the series of observations it will not be possible to perform formal longitudinal analysis. We will apply non-parametric tests to evaluate changes in immunologic outcomes before and after BCI. Details are provided below.

10.2 Statistical Hypotheses

1) BCI will be feasible and safe, with a maximum tolerated dose to be determined in this trial.
2) Post-BCI peripheral blood will demonstrate an increase in the percent of $CD8^+$ T cells expressing a combination of $Ki67^+$,PD-1+, HLA-DR^+, $CD38^+$, and Bcl-2^{low} compared to pre-BCI peripheral blood $CD8^+$ T cells.
3) CD8+ T cell responses to BCI will be greater among subjects with high PD-1 expressing T cells in BAL effluent.

10.3 Analysis Datasets

All participants who were treated with BCI will be included.

10.4 Description of Statistical Methods

10.4.1 General Approach

- Continuous data will be summarized with means and standard deviations.
- Categorical data will be summarized with frequencies and percentages.
- *P* values will be considered statistically significant at the 0.05 level. Note that the study is designed to determine the MTD, and thus is not powered to detect statistically significant differences in any of the secondary outcomes.

10.4.2 Analysis of the Primary Endpoint: Feasibility

The proportion of patients in whom BCI is successfully completed will be estimated, along with its 95% exact confidence interval.

10.4.3 Analysis of the Primary Endpoint: Safety

Safety endpoints will be descriptive in nature, and any possible, probable, or definite adverse event will be counted only once for a given participant. Frequency and severity of all adverse events will be reported, both in the total study population and stratified by dose level.

Dose-limiting toxicities will be tabulated in the whole population and by dose level. Bleeding severity will be graded based on severity scale as described in Section 4.1.1 (Primary Study Endpoints: Feasibility and Safety). Pneumothoraces will be reported and described based on size, associated

symptomatology and respiratory impairment, and need for chest tube placement. Length of time to perform BCI and length of time of fluoroscopy exposure during BCI will be reported in minutes. Other adverse events will be recorded and categorized as per the NCI Common Terminology Criteria for Adverse Events (CTCAE) (33).

The maximum tolerated dose (MTD) will be determined based on the dose escalation rules described below and in Section 6.1.1. The MTD is the dose at which fewer than 1 of 3 or at most 1 of 6 participants on that dose experience a DLT.

10.4.4 Analysis of the Secondary Endpoint(s)

Wilcoxon signed-rank tests will be used to assess paired pre- and post-BCI peripheral blood measurements, and to assess paired post-BCI peripheral blood $CD8^+$ T cell responses and T cell PD-1 expression in BAL effluent.

10.4.5 Baseline Descriptive Statistics

All demographic and clinical characteristics of participants and tumor will be summarized and tabulated. Pre-BCI peripheral blood T cell levels and expression of surface and intracellular markers will be measured. Continuous data such as measured levels of T cell subsets will be presented with means and standard deviations. Categorical data such as presence of an activated effector-like phenotype will be presented with frequencies and percentages.

10.4.6 Planned Interim Analysis

10.4.6.1 Safety Review

Ongoing safety review will occur as part of the 3+3 dose escalation scheme, which requires evaluation of dose-limiting toxicities in order to escalate to the next dose or de-escalate to a lower dose.

10.4.6.2 Efficacy Review

Our primary focus will be on safety and feasibility. Formal interim analyses to review for efficacy will not be performed.

10.4.7 Additional Sub-Group Analyses

Sub-group analyses as described below will employ Wilcoxon signed-rank tests as described above for secondary analyses. In particular:

1) We will compare changes in pre- and post BCI peripheral blood $CD8^+$ T cells expressing a combination of $Ki67^+$,PD-1+, $HLA\text{-}DR^+$, $CD38^+$, and $Bcl\text{-}2^{low}$ at each dose level.

2) In the subset of participants receiving active treatment (radiation therapy, chemotherapy, and/or immunotherapy in the past 30 days) for non-small cell lung cancer, we will investigate if the combination of cryoablation and his/her current treatment will lead to enhanced increases in the percent of $CD8^+$ T cells expressing a combination of $Ki67^+$,PD-1+, $HLA\text{-}DR^+$, $CD38^+$, and $Bcl\text{-}2^{low}$ compared to participants treated with BCI alone.

10.4.8 Tabulation of Individual Response Data

Individual participant data will be listed by time points of peripheral blood collection day.

10.5 Sample Size

We will aim to enroll approximately 24 patients. The exact number will be determined based on our dose escalation protocol (as described in section 6.1.2).

The table below gives the operating characteristics of this design, presenting the likelihood of escalation when the true DLT rate takes various values. For example, if the true DLT rate is 60%, the probability of escalation is only 0.08. If the true DLT rate is 10%, the probability of escalation is 0.91.

True DLT Rate	**10%**	**20%**	**30%**	**40%**	**50%**	**60%**	**70%**	**80%**	**90%**
Probability of Escalation	0.91	0.71	0.49	0.31	0.17	0.08	0.03	<0.001	<0.001

The small sample size will not provide sufficient power to definitively address the secondary endpoint of measuring BCI induced anti-tumor immune responses. For example, 15 subjects provide approximately 80% power to detect a difference of about 0.75 standard deviations between pre- and post-BCI peripheral blood $CD8^+$ T cell expression of PD-1 and Ki67, conservatively assuming a within-subject correlation between assessments of 0.5. However, our results will be critical for informing the design of further studies aimed at estimating response and survival rates with BCI combined with anti-PD1-therapy for the treatment of advanced non-small cell lung cancer.

10.6 Measures to Minimize Bias

10.6.1 Enrollment/Randomization/Masking Procedures

Not applicable

10.6.2 Evaluation of Success of Blinding

Not applicable

10.6.3 Breaking the Study Blind/Participant Code

Not applicable

11 Source Documents and Access to Source Data

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents. If this is the case, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12 Quality Assurance and Quality Control

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13 Ethics/Protection of Human Subjects

13.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 Informed Consent Process

13.3.1 Consent Form Provided to Participants

Informed consent form describing BCI, pre-BCI bronchoalveolar lavage, and pre- and post-BCI blood draws in detail including risks, potential benefits, and alternatives are given to the participant. Written documentation of informed consent is required prior to undergoing BCI. A detailed informed consent form has been submitted with this protocol to the IRB.

13.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

13.4 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and

genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NYU Langone Medical Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NYU Langone Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Medical Center.

13.4.1 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Samples and data collected under this protocol may be used to study the immune response to BCI in non-small lung cancer. No genetic testing will be performed.
- Storage: Access to stored samples will be limited to the research investigator and his research assistants. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- Tracking: Data will be tracked using RedCap.
    - Disposition at the completion of the study: All stored samples will be discarded. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

13.5 Optional Future Use of Stored Specimens

Data collected for this study will be analyzed and stored at the William Rom Laboratory at NYU/Bellevue Medical Center. After the study is completed, the de-identified, archived data will be transmitted to and stored at the William Rom Laboratory Data Repository, under the supervision of Dr. Daniel Sterman, for use by other researchers including those outside of the study. Permission to transmit data to the William Rom Laboratory Data Repository will be included in the informed consent.

The only PHI maintained in any research records are the research subjects' medical record number and date of birth, and these are stored only on the secure NYU server. Only investigators with approved permission (those on the IRB protocol who have undergone CITI training), will have access to the data and banked samples, and appropriate steps to maintain confidentiality of the data will be taken, including coding data with an anonymous identifying number unique to each subject.

Samples will be labelled with coding data using permanent marker. Samples will be banked for up to five years.

With the participant's approval and as approved by the IRB, de-identified biological samples will be stored at the William Rom laboratory and/or the Segal Translational Lung Biology Laboratory with the same goal as the sharing of data with the William Rom Laboratory Data Repository. These samples could be used for research into the causes of lung cancer, its complications and other conditions for which individuals with lung cancer are at increased risk, and to improve treatment. Genetic testing will not be done on the samples to identify risks for other conditions. The William Rom Laboratory Data Repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant. The PI will maintain the link between the sample code and the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. When the study is completed, access to study data and/or samples will be provided through the William Rom Laboratory Data Repository.

14 Data Handling and Record Keeping

14.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into RedCap, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Subject's research records will be confidential. Subject's blood specimens will be banked for future research without personal identifiers. Any identifying information that could link a subject to his or her blood sample by name will be stored in a locked cabinet by the investigators separate from any medical records. Subjects will be identified by code. Subjects will not be personally identified in any publication.

14.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close-out or 5 years after final reporting/publication. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

14.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI/study staff to use continuous vigilance to identify and report deviations within 1 working day of identification of the protocol deviation, or within 1 working days of the scheduled protocol-required activity.

All protocol deviations must be addressed in study source documents, reported to the IRB.

Protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

14.4 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee’s responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations of a product subject to FDA regulation;

- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.
- NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

15 Study Finances

15.1 Funding Source

The principal investigator, Dr. Daniel Sterman, has received a NIH Exploratory/Developmental Research Grant (R21CA229714-01A1).

15.2 Costs to the Participant

Bronchoscopic cryo-immunotherapy and pre- and post-blood draws and analysis are being supported by external grant funding, which will cover study-related costs.

Please note that the standard of care portion of the bronchoscopy, including the cost of anesthesia, and pathological evaluation of clinically indicated specimens obtained at bronchoscopy will be billed to the participant's insurance, with co-payments for these services as per standard protocol. Additionally, the cost of any standard of care follow-up blood work or radiographic imaging will also be billed to the participant's insurance, with co-payments for these services as per standard protocol.

15.3 Participant Reimbursements or Payments

There is no payment for participation in this study.

16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the NYU School of Medicine has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

17 References

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18 Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

Informed Consent Form
ERBOKRYO® CA – Cryosugical Unit with Flexible ERBECRYO™ Probe FDA 510K Approval Form

19 Schedule of Events

Activity	**Enrollment** (Day -28 to 0)	**Pre-BCI Peripheral Blood Collection** (Day 0)	**BCI** (Day 0)	**Post-BCI Peripheral Blood Collection** (Day 7 ± 3)	**Post-BCI Peripheral Blood Collection** (Day 14 ± 3)
Study Team Procedures					
Consent	X				
Medical history	X				
			X	X	X
Physical exam	X		X	X	X
Bronchoalveolar lavage			X		
BCI			X		
Laboratory Assessments					
Complete blood count	X				
Chemistry panel	X				
Pregnancy test (if childbearing potential)		X			
Immunologic analysis		X		X	X
Imaging Assessments					
Review Chest CT scan	X				