

## Specific aims

Despite recent progress, survival after lung transplantation is approximately six years which is significantly worse than that of other organ transplants (1). Acute rejection and chronic lung allograft dysfunction are frequent causes of morbidity and mortality for patients following lung transplantation (2, 3). Rejection can be clinically silent and thus diagnosis relies on a combination of clinical parameters, imaging, and lung biopsy specimens obtained by bronchoscopy. The International Society of Heart and Lung Transplantation (ISHLT) recommends that at least five pieces of “well-aerated” alveolated lung parenchyma with 100 alveoli per high power field are required for assessment of acute rejection via transbronchial biopsy (4). These statements are based on consensus agreement with limited evidence to back up this recommendation. There remains a critical gap in our understanding of the role bronchoscopy for the diagnosis of rejection, specifically in the size and number of specimens needed for assessment.

Transbronchial cryobiopsy has been proposed as a technique to increase diagnostic yield of flexible bronchoscopy by increasing specimen size and decreasing crush artifact (5). Our team has significant clinical experience with transbronchial cryobiopsy biopsy, and have published our early experience in lung transplant patients using a conventional cryoprobe (6). Based on these early experiences we recognized limitations of this conventional technique and lack of generalizability given that the conventional technique mandated that the bronchoscope had to be removed en bloc with the specimen exposing the patient to increased risk. As a result, we developed a smaller 1.1 mm sheath cryoprobe (ERBECRYO 2 Cryosurgical Unit; Tuebingen, Germany) to perform transbronchial cryobiopsy without having to remove the bronchoscope from the airway to improve safety. Specimens obtained with this probe are larger than transbronchial forceps biopsy with less crush artifact (figure 1; 7). This device received FDA 510(k) approval in 2020 (7) and was recently shown to be safe and effective in humans in the FROSTBITE-1 study showing safety data equivalent to transbronchial forceps (9).

At this time, there is little data evaluating the adequacy of transbronchial biopsy specimens obtained with cryobiopsy in the lung transplant population, and no data to support an optimal number of biopsy specimens needed to achieve adequate sampling. In theory, fewer specimens may be required if a cryoprobe is used as the samples obtained are larger and contain minimal to no crush artifact (5, unpublished data).

Since both transbronchial biopsy using either forceps or cryoprobe are associated with infrequent but significant risks (including severe bleeding and pneumothorax), defining the optimal number of specimens to establish specimen adequacy of either technique would be valuable. This proposal will leverage the resources and framework of a currently funded randomized controlled trial (FROSTBITE-2) evaluating the diagnostic yield of forceps biopsy versus a 1.1 mm sheath cryoprobe. This proposal is designed within the context of the FROSTBITE-2 parent study with the following aims:

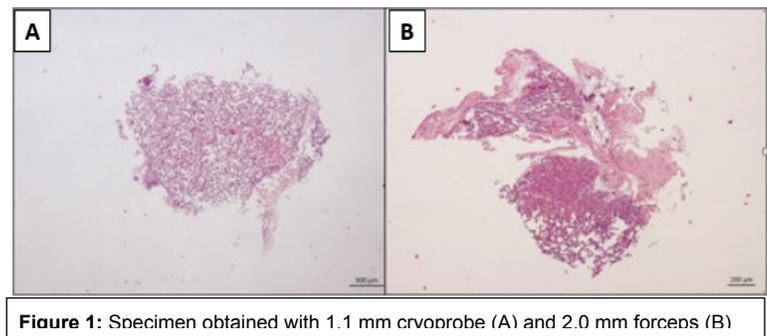


Figure 1: Specimen obtained with 1.1 mm cryoprobe (A) and 2.0 mm forceps (B)

**Aim 1.** To determine the optimal number of biopsy specimens needed to assess for lung transplant rejection using a novel 1.1 mm transbronchial cryoprobe

*Hypothesis.* A fewer number of specimens will be needed using a novel 1.1 mm transbronchial cryoprobe versus 2.0 mm forceps to establish an “adequate” sample.

**Aim 2.** To determine the optimal number of biopsy specimens needed to assess for lung transplant rejection using conventional 2.0 mm transbronchial forceps

*Hypothesis.* Fewer than 10 specimens will be needed using a 2.0 mm forceps in order to obtain an “adequate” sample for transplant rejection. This number may redefine the current ISHLT recommendation.

## Background

This grant is an analysis of a population included in a multicenter randomized controlled trial (FROSTBITE-2). This trial will compare transbronchial biopsy with a novel 1.1 mm sheath cryoprobe versus conventional forceps for evaluation of a variety of lung diseases. Several patient cohorts will be included, specifically those with diffuse lung diseases, discrete pulmonary lesions, and lung transplantation. In the lung transplant population, a planned 260 bronchoscopies will be

included (130 forceps and 130 cryobiopsy). Primary outcomes of FROSTBITE 2 include adequacy and diagnostic yield of biopsies obtained by transbronchial cryobiopsy compared to forceps biopsy.

Further, the novel 1.1 mm sheath cryoprobe being used in this study was developed by our lab (7). The FROSTBITE-1 trial demonstrated safety in a multicenter, prospective, single-arm study which was just completed and data will be presented at ERS 2021. In FROSTBITE-1, 21 patients were lung transplant recipients. No severe complications were related to biopsy with the transbronchial cryoprobe in this cohort (9).

### **Hypothesis**

The current ISHLT recommendation (at least 5 pieces of alveolated lung parenchyma) is not the optimal number of transbronchial forceps biopsies to assess for lung transplant rejection with regard to specimen adequacy and patient safety. Further, the number of specimens required for assessment of lung transplant rejection using a novel 1.1 mm flexible transbronchial cryoprobe will be less than that required for transbronchial forceps. An optimal number of biopsies will be defined based on objective parameters using data from a prospective, randomized controlled trial (FROSTBITE-2).

### **Innovation**

ISHLT criteria for determination of specimen adequacy in the lung transplant population are based on conventional transbronchial forceps and are largely consensus opinion. A novel 1.1 mm flexible cryoprobe has the potential to be a safer and more efficient tool for transbronchial biopsy and has not been used to define sample adequacy in a lung transplant population. Additionally, an optimal number of transbronchial biopsies to obtain both an adequate sample and maximize patient safety with either technique has significant clinical utility.

### **Preliminary Data**

Our group performed a retrospective review of all patients who underwent lung transplantation and had bronchoscopy with transbronchial forceps biopsy over a period of four years (2011-2016) at the Johns Hopkins Hospital. Adequacy (defined by the ISHLT as at least 5 alveolated samples) and diagnostic yield were recorded. A sample was considered diagnostic if the histopathologic report was consistent with the clinical suspicion for bronchoscopy (eg, rejection or infection). This revealed a diagnostic yield of 13.2%, and 32.8% rate of technically inadequate samples (unpublished date). These data suggest there is much room for improvement in technique and instrumentation which may aid in the diagnosis of acute rejection.

In addition, we performed a pilot study evaluating transbronchial cryoprobe biopsy in lung transplant patients using a 1.8 mm cryoprobe (ERBE; Tuebingen, Germany) (6). Twenty-one bronchoscopies were performed in 17 patients after lung transplantation. Specimen area and percent open alveoli were significantly greater using the transbronchial cryoprobe for biopsy compared with conventional forceps ( $P < 0.05$ ) with minimal crush artifact in the cry probe arm. No clinically significant procedural complications occurred and all patients were discharged the day of the procedure.

We then developed a novel smaller sheathed cryoprobe (1.1 mm) to allow better generalizability and safety of the procedure. To study the safety profile of the 1.1 mm sheath cryoprobe, a multicenter prospective single-arm study enrolling 50 patients was just completed (FROSTBITE-1). The primary outcome of this study was the composite rates of bleeding, pneumothorax, and 30-day respiratory failure following transbronchial cryobiopsy. Of the 50 cases, 21/50 (42%) of the patients enrolled were lung transplant recipients. There were no significant complications, demonstrating a similar safety profile to forceps biopsy in this cohort. Based on this data, we have organized a multi-center randomized controlled trial (FROSTBITE-2) using the 1.1 mm sheath cryoprobe in a variety of lung diseases.

### **Approach**

The primary data utilized in this grant will be generated from the FROSTBITE-2 randomized clinical trial which is scheduled to recruit in July 2021. This sub-study will capitalize on the resources of FROSTBITE-2, with a specific clinical question related to the lung transplant population which is distinct from the aims of the parent study (diagnostic yield in a broader patient cohort). Patients are eligible to enroll in FROSTBITE-2 if they are undergoing clinically indicated or surveillance bronchoscopy following lung transplantation at one of the recruiting centers (Johns Hopkins University, Duke University, Vanderbilt University, and the University of Pittsburgh). At the time of the procedure, patients will be randomized in a 1:1 fashion to transbronchial biopsy via (a) standard 2.0 mm forceps or (b) 1.1 mm sheath cryoprobe. Patients will undergo flexible bronchoscopy in the standard fashion with 6 biopsies obtained using the cryoprobe or 10 to 12 biopsies using standard forceps. Given the difference in technique and equipment, bronchoscopists are unblinded to the biopsy technique. The reviewing pathologist and the patient will be blinded to biopsy method.

Each specimen obtained during FROSTBITE-2 (transbronchial forceps or cryobiopsy) will be assessed individually. Two pathologists with expertise in lung transplantation will sequentially review each transbronchial biopsy specimen and determine whether this is deemed “adequate” to assess for acute rejection based on pathologic criteria. The final pathologic diagnosis will be compared to the working diagnosis obtained after each sample is evaluated, and any changes in working diagnosis will be recorded. Due to concern for high inter-observer variability of histopathologic grading, slides will be read by at least two pathologists to provide a consensus. As objective measures, pathologists will also quantify (1) size of transbronchial biopsy, (2) number of alveoli, (3) presence or absence of bronchioles, (4) presence or absence of crush artifact, and (5) histopathologic evidence of rejection (graded via ISHLT criteria). The standards established for transbronchial forceps biopsy will be used as the “gold standard” for adequacy of sampling (2). The incidence of complications related to transbronchial biopsy (including bleeding, pneumothorax, and respiratory failure) will be tracked based on number of biopsies taken and type of biopsy instrument used.

*Sample size:* The sample size (n = 260 procedures) for the FROSTBITE-2 study was chosen based on a base rate of detection of 13.2% of acute rejection in lung transplant with transbronchial forceps based on retrospective review of data from this institution. With assumed clinically meaningful 15% improvement in detection of acute rejection with transbronchial cryobiopsy, a sample size of 130 biopsies in each arm was chosen to have an 80% power to detect a between group difference (alpha = 0.05). We are limited to the data structure of the FROSTBITE-2 study.

*Limitations:* A limitation of this study is the subjective nature of a pathologic definition of “adequacy.” We have included pathologists with expertise in lung transplantation, arranged for dual pathologist review, and defined objective criteria (eg, specimen size, quantification of alveoli) to mitigate these limitations. Pathologists will also be single blinded to final diagnosis as each specimen is interpreted to minimize bias. This variation may also be reduced by multicenter nature of the trial.

*Future applications:* Potential applications of this research include development of a new criteria (AABIP criteria) to define what constitutes an adequate sample for assessment of lung transplant rejection. This may include both the number of biopsy samples needed, area of alveolated lung parenchyma or other parameters identified in this study. These metrics will be obtained both for biopsies obtained by transbronchial forceps and the 1.1 mm cryoprobe. Based on these results, future studies may be planned to further define the role of transbronchial biopsy in assessment of lung transplant rejection more generally, including both surveillance and clinically indicated populations.

**Timeline**

This research question relies on data from the FROSTBITE 2 clinical trial, which will be open for enrollment in July 2021. Accrual of data is anticipated within 1 year. Given the lung transplant volumes at the centers participating and sequence of surveillance bronchoscopes at 1, 3, 6, 9, and 12 months in the first year we estimate the study recruitment will be fulfilled within 12 months. A preliminary analysis will be performed by 2022 and output of this analysis will be presented at the AABIP national meeting and international meetings and published in a peer-reviewed journal (see table 1).

<b>Table 1. TIMETABLE</b>	<b>2021</b>		<b>2022</b>	
<b>Quarter</b>	<b>3</b>	<b>4</b>	<b>1</b>	<b>2</b>
Patient Recruitment	■	■	■	■
Blinded Pathology Review		■	■	
Interim Progress Report		■		
Final Progress Report			■	■
Data analysis			■	■
Manuscript preparation			■	■
Development of future grant proposals			■	■
AABIP Annual Conference presentation				■ Aug. 2022

**Mentorship**

The primary goal for Dr. Andrew DeMaio is to acquire the knowledge and skills to become an independent investigator in the field of Interventional Pulmonology (IP). With increasing demands for IP services, there is a paucity of independently funded investigators. The training plan laid out in this proposal has been fashioned to ensure Andrew will receive training in aspects of translational science over the period of the award. His training will include formal classroom training at the Johns Hopkins University School of Public Health Science of Clinical Investigation program, laboratory based work and formal mentoring under Dr. Lonny Yarmus, who has a proven record of mentoring trainees who have gone on to launch independent research careers. The data gathered in this proposal will not only address critical gaps in our understanding in the role of bronchoscopy for assessment of transplant rejection, but will also support data for Andrew’s future National Institutes of Health (NIH) K award which he plans to submit in November 2022.

## References

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