Lung Ambition Awards - Letter of Intent

Project Title: Comparative Analysis of Bronchoalveolar Lavage Fluid and Blood ctDNA for Early Lung Cancer Detection Using a Novel Capture-Based Sequencing Panel – Pilot Study

Principal Investigator (Applicant): Emilian Olteanu, MD, Pathology Fellow, BC Cancer

Co-Principal Investigators (Mentors): Dr. Renelle Myers, MD, FRCPC, Vancouver General Hospital & BC Cancer; Dr. Diana N. Ionescu, MD, FRCP(C), FCAP, BC Cancer

Institution: BC Cancer

1. The Challenge: Early Lung Nodule Diagnosis is Failing Patients.

Indeterminate lung nodules from screening demand better diagnostics. Invasive biopsies are problematic; current blood liquid biopsies lack sensitivity. This project addresses this critical gap directly.

2. Our Innovation: Proximal Liquid Biopsy with Advanced Genomics.

We propose a paradigm shift: bronchoalveolar lavage (BAL) fluid, a tumor-proximal source, for highly sensitive ctDNA liquid biopsy. We will rigorously compare BAL vs. blood ctDNA using a **Novel CAD 80 Sequencing Panel**, engineered for superior performance in early lung cancer detection.

3. Key Novelty for Enhanced Impact:

- **BAL Fluid Focus:** Exploiting a more sensitive biofluid beyond blood.
- **CAD 80 Panel:** Optimized for degraded DNA, UMI-enhanced, guideline-aligned.
- **Direct Comparison:** Definitive BAL vs. blood ctDNA performance data.

4. Transformative Potential: Faster Diagnosis, Better Outcomes, Reduced Burden.

If successful, this research promises:

- **Rapid, Accurate Diagnosis:** Improving care pathways for indeterminate nodules.
- **Earlier Treatment, Improved Survival:** Detecting early-stage cancer for curative intervention.
- **Minimally Invasive Solution:** Reducing patient burden and healthcare costs.

5. Feasibility and Environment:

BC Cancer provides ideal infrastructure, with expert mentorship ensuring successful project completion and clinical translation.

6. Budget: ~$50,000 Direct Costs (Personnel, Patient Costs, NGS, Conference). No indirect costs.

7. No Other Funding Submitted.

Proposed Research: Comparative Analysis of Bronchoalveolar Lavage Fluid and Blood ctDNA for Early Lung Cancer Detection Using a Novel Capture-Based Sequencing Panel – Pilot Study

Background:

Early detection of lung cancer through screening programs is vital for improving patient survival. However, diagnosing small, peripheral lung nodules detected by screening remains a challenge, as bronchoscopic and CT-guided biopsies are often difficult to perform or inconclusive in these lesions. Liquid biopsies, analyzing circulating tumor DNA (ctDNA), offer a promising non-invasive alternative. While blood-based ctDNA assays are being developed, evidence suggests that ctDNA derived from bronchoalveolar lavage (BAL) fluid, obtained proximally to the tumor site, exhibits superior sensitivity for early-stage lung cancer detection. BAL of the pulmonary segment containing a nodule is readily achievable, even when the lesion is not accessible via biopsy forceps, making it an attractive source for early lung cancer biomarkers.

Novel Methodology: Capture-Based Sequencing Panel for Comparative ctDNA Analysis

To rigorously compare the diagnostic performance of ctDNA from BAL fluid and blood, we will employ a Novel Capture-Based Sequencing Panel. This advanced panel offers significant advantages for our proposed research:

Comprehensive Genomic Coverage: This panel is strategically designed to target 79 genes of highest relevance in lung cancer, based on current 2024 clinical guidelines and the most up-to-date genomic understanding of the disease.

Enhanced Sensitivity and Performance: Utilizing a capture-based enrichment strategy, the panel provides superior performance, particularly with degraded DNA samples often encountered in liquid biopsies and FFPE tissues. The capture approach is also optimized for sensitive fusion detection.

Unified Oligo Design for Multi-Analyte Analysis: Crucially, the panel utilizes the same oligo design for DNA, RNA, and ctDNA analysis, allowing for consistent and comparable data across different sample types and analytes. The panel is compatible with both IDPE and IRPE workflows.

UMI-Based Library Preparation for ctDNA: For optimal ctDNA analysis, we will utilize the Illumina cell-free DNA with Enrichment kit, a UMI (Unique Molecular Identifier)-based library preparation kit. This approach minimizes PCR duplicates and enhances the accuracy of low-frequency variant detection in ctDNA.

Efficient and Cost-Effective Sequencing Platforms: The panel is designed for use on both MiSeq i100 (for FFPE solid tumor analysis) and NextSeq 1000/2000 platforms (for both solid and ctDNA analysis). For this ctDNA comparison study, we will utilize the NextSeq 2000 platform, enabling cost-effective sequencing with an estimated cost of $550 per ctDNA sample from extracted sample to report, including library preparation, reagents, sequencing consumables, analysis, and Connected Insights.

Hypothesis:

We hypothesize that ctDNA analysis in BAL fluid, utilizing our novel capture-based sequencing panel, will demonstrate superior sensitivity for early lung cancer detection compared to ctDNA analysis in blood using the same panel. This will provide enhanced diagnostic accuracy for differentiating benign from malignant pulmonary nodules.

Objectives:

Primary Objective: To directly compare the diagnostic performance (sensitivity, specificity, and overall accuracy) of ctDNA analysis in BAL fluid versus blood for detecting early-stage lung cancer using our novel capture-based sequencing panel in an incidental pulmonary nodule cohort.

Exploratory Objective: To determine the correlation between ctDNA from BAL fluid and blood using the novel sequencing panel, with key clinical parameters including tumor burden, stage, and patient prognosis.

Methods:

Patient Enrollment: We will recruit 10 patients undergoing bronchoscopy for suspected early-stage lung cancer, focusing on individuals with pulmonary nodules <3 cm. A cohort of 10 control patients with benign nodules, nodules that have demonstrated radiographic stability for >than 2 years, will also be enrolled, as controls.

Sample Collection:

BAL Fluid: 20 mLs of BAL from the segment containing the nodule will be collected during clinically indicated bronchoscopy procedures.

Blood: Peripheral blood samples will be collected at the time of bronchoscopy.

ctDNA Analysis:

ctDNA Extraction and Sequencing: ctDNA will be extracted from both BAL fluid and plasma samples using optimized methods. Libraries will be prepared using the Illumina cell-free DNA with Enrichment kit and sequenced using our novel capture-based sequencing panel on the NextSeq 2000 platform.

Data Analysis:

Statistical methods will be employed to compare the diagnostic performance of ctDNA in BAL fluid versus blood. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value will be calculated for each biofluid.

Correlations between biomarker profiles and clinical parameters will be evaluated using appropriate statistical analyses and potentially incorporating machine learning approaches to identify predictive models.

Expected Outcomes:

We anticipate that ctDNA analysis in BAL fluid, when analyzed using our novel capture-based sequencing panel, will demonstrate significantly higher sensitivity for early lung cancer detection compared to ctDNA analysis in blood using the same advanced panel. Finally, we anticipate identifying correlations between biomarker profiles and tumor characteristics, potentially providing insights into patient prognosis.

Novelty:

- **Enhanced Sensitivity for Degraded DNA:** Our novel Capture-Based Sequencing Panel is specifically designed to perform optimally with degraded DNA, a common characteristic of cfDNA samples. This optimization for cfDNA inherent properties represents a key methodological advancement.
- **UMI-Based Library Preparation:** This study will employ UMI (Unique Molecular Identifiers) in ctDNA library preparation. This cutting-edge UMI approach significantly improves the accuracy of low-frequency variant detection in ctDNA by effectively minimizing PCR duplicates, a feature not utilized in previous studies like Nair et al.
- **Direct, Head-to-Head Comparison of Clinically Relevant Biofluids:** The core novelty lies in the rigorous, direct comparative analysis of BAL fluid and blood ctDNA. Blood is the clinically standard liquid biopsy matrix, making this direct comparison highly relevant for translational potential and clinical adoption.
- **Advanced, Clinically Focused Sequencing Panel (CAD 80 Panel):** We will utilize a next-generation capture-based sequencing panel (CAD 80) meticulously designed with features optimized for degraded DNA, UMI-based quantification, comprehensive, guideline-aligned gene coverage, and cost-effective sequencing platforms. This advanced panel represents a significant improvement in technology applied to this clinical question.
- **Enhanced Translational Emphasis:** This project is strongly focused on establishing the optimal biofluid for clinical application in early lung cancer detection. By highlighting the potential for a cost-effective and highly sensitive approach, this research is designed with clear translational aims for improved early detection and patient outcomes.

References:

Nair VS et al. Genomic Profiling of Bronchoalveolar Lavage Fluid in Lung Cancer. Cancer Res. 2022;82(16):2838-2847. doi: 10.1158/0008-5472.CAN-22-0554.

Zhang H et al. Bronchoalveolar lavage fluid assessment facilitates precision medicine for lung cancer. Cancer Biol Med. 2024;21(3):230-251. doi: 10.20892/j.issn.2095-3941.2023.0381.

Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, Bruno DS, Chang JY, Chirieac LR, D'Amico TA, DeCamp M. Non–small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2022 May 1;20(5):497-530.

Guibert N, Hu Y, Feeney N, Kuang Y, Plagnol V, Jones G, Howarth K, Beeler JF, Paweletz CP, Oxnard GR. Amplicon-based next-generation sequencing of plasma cell-free DNA for detection of driver and resistance mutations in advanced non-small cell lung cancer. Annals of Oncology. 2018 Apr 1;29(4):1049-55.

Impact Statement:

This pilot study has the potential to exert a significant influence on the field of early lung cancer detection. By rigorously comparing the diagnostic performance of ctDNA from bronchoalveolar lavage fluid and blood using a novel, highly sensitive capture-based sequencing panel, this research directly addresses a critical unmet need for accurate early detection methods. If our hypothesis is confirmed – demonstrating superior sensitivity of BAL fluid ctDNA – it will provide strong evidence to shift the paradigm in liquid biopsy approaches for early lung cancer. This advancement promotes major progress in lung cancer research by validating a more proximal and informative biofluid source. In the short-to-medium term, successful completion of this pilot study will have tangible outcomes.

Optimized Patient Care: A more sensitive and specific non-invasive diagnostic test, particularly for small nodules where current methods are lacking, will improve clinical decision-making, potentially reducing the need for more invasive, resource heavy biopsy techniques such as robotic bronchoscopy, accelerating diagnosis and care.

Improved Treatment: Earlier and more accurate detection of lung cancer, especially in early stages, directly translates to improved treatment outcomes and increased survival rates as patients can be directed to curative therapies sooner.

Reduced Burden: BAL-based liquid biopsy approach, if proven superior, can be more accessible to patients and reduce the burden associated with current diagnostic uncertainties and invasive procedures. Ultimately, by advancing early detection methodologies, this research aims to contribute to reduced lung cancer mortality and improved patient quality of life within the Canadian Lung Cancer community.

Public Summary:

Lung cancer is often diagnosed at late stages when treatment is less effective. Finding lung cancer early, when surgically resectable greatly improves survival. Lung cancer screening with low dose CT scans has demonstrated a 25% mortality reduction. CT scans are highly sensitive for small nodules, however, the vast majority of the nodules found are not a cancer. Biopsing these very small nodules to determine if they are cancerous is very challenging. Liquid biopsies, which are blood tests that look for cancer signals like tumor DNA, are a promising tool to help detect cancer in those hard to reach nodules, but lack sensitivity. This research project focuses on improving liquid biopsies for early lung cancer detection, specifically in fluids from the lung. We will compare fluid from the lungs, obtained during a routine procedure called bronchoscopy (bronchoalveolar lavage fluid or BAL), to plasma (blood) for the same circulating tumor components/DNA. We believe that the BAL will be more sensitive as it's closer to the tumor. Our tests use a new very sensitive DNA sequencing to look for cancer DNA. If the BAL proves to be better than blood for early detection, this could lead to a new, more accurate test for lung cancer for very small screen detected lung cancers. This will reduce repeat procedures and improve time to diagnosis. Earlier diagnosis means earlier treatment, which could ultimately save lives and improve the health of Canadians affected by lung cancer. This research aims to make lung cancer diagnosis easier, faster, and less invasive for patients.

Budget Justification:

Personnel: The budget includes $11,000 for a Part-time Research Assistant (0.2 FTE) for one year. This individual will be responsible for patient recruitment and consenting, organization of sample collection (BAL fluid and blood), processing of BAL fluid and blood samples, and data management. This role requires a bachelor's degree in a biological science or related field and experience in clinical research coordination and sample handling. The 0.2 FTE commitment is appropriate for the estimated patient enrolment and sample processing workload of this pilot study. At (0.2 FTE) x $50,000, total ask is $11,000.

Patient Costs: The Clinical Research Unit (CRU) fees for 20 blood draws at $69 per draw (including blood draw fee ($20), and processing fee ($40), and a 15% administrative fee). This is a standard cost for blood draws through the CRU at BC Cancer. At $69.00 per patient x 20 patients, total ask is $1380.

Lab Costs: The major cost component is $36,620 for Next-Generation Sequencing (NGS) analysis of ctDNA from both BAL fluid and blood samples for 20 patients (x 2 per patient = 40 samples). The cost is estimated at $1831 per patient, encompassing ctDNA extraction, library preparation using the Illumina cell-free DNA with Enrichment kit, sequencing using the novel capture-based sequencing panel on the NextSeq 2000 platform, bioinformatics analysis, and generation of clinical reports. This cost is based on quotes for similar services and is crucial for achieving the study objectives of comparing the diagnostic performance of ctDNA from the two biofluid sources using a comprehensive genomic panel. At $1831 per sample x 20 samples, total ask is $36,620.

Conference/Travel Support: $1,000 is budgeted to support conference travel and registration fees to present the findings of this study at a Canadian lung cancer conference. Dissemination of results at a national conference is a requirement of the Lung Ambition Awards, and this allocation ensures the study findings will be shared with the Canadian lung cancer community.

Other Funding: This project is not part of a larger initiative, and there are no other sources of funding dedicated to the specific aims outlined in this proposal. The requested funds are solely for this pilot study. Indirect costs and contingency costs are not included in this budget as per the grant guidelines to maximize funds available for direct research activities and stay within the funding limit.

Budget: Pilot Study - 20 Patients (10 Cases, 10 Controls) - ~ $50,000

Budget Category	Description	Estimated Cost
Personnel		
Part-time RA (0.2 FTE)	Recruitment, consenting, sample organization, blood/BAL processing	$11,000
Patient Costs		
Blood Draws (20 patients)	20 blood draws x $69/draw (including processing and 15% admin fee).	$1,380
Lab Costs		
NGS Sequencing (20 patients)	20 patients x $1831/patient (BAL & Blood ctDNA sequencing per patient) – includes all workflow.	$36,620
Other Costs		
Conference/Travel Support	Estimated for conference presentation of findings (limited to guideline max).	$1,000
Total Direct Costs		$50,000

Investigator Information and Curriculum Vitae:

Principal Investigator (Applicant): Emilian Olteanu, MD Pathology Fellow BC Cancer, Curriculum Vitae for Dr. Olteanu will be provided as a separate document.

Co-Principal Investigators (Mentors):

Dr. Renelle Myers, MD, FRCPC Interventional Respirologist, Vancouver General Hospital & BC Cancer, Clinical Associate Professor, Medicine, UBC Clinician Scientist, Department of Integrative Oncology, BC Cancer Research Institute, Curriculum Vitae for Dr. Myers will be provided as a separate document.

Dr. Diana N. Ionescu, MD, FRCP(C), FCAP Consultant Pathologist, BC Cancer, Medical Lead Anatomical Pathology, BC Cancer Agency, Clinical Professor of Pathology, University of British Columbia, Curriculum Vitae for Dr. Ionescu will be provided as a separate document.

February 7, 2025

Review Committee Cancer Research Society

Dear Review Committee,

It is with great pleasure that I am writing this letter in support of Dr. Diana Ionescu, Renelle Myers, and Dr. Emilian Olteanu for the Lung Cancer Canada's Lung Ambition Award application.

This project addresses an urgent need to find highly specific and sensitive methods for the detection of early lung cancer. The proposed study will improve our understanding on the utility of bronchoalveolar lavage fluid versus blood for ctDNA-based lung cancer detection.

As the head of the department of BC Cancer Pathology, I can confirm the team has the laboratory space, equipment and other resources to complete the proposed research. In addition, infrastructure support is available to ensure the future translation of the research findings.

I believe this is an outstanding research proposal that will address a priority area of unmet need in early detection of lung cancer. I support with enthusiasm the application of this project to the Lung Ambition Award Society.

Sincerely,

Gang Wang

Gang Wang, MD, PhD, FRCPC

Department Head of British Columbia Cancer Pathology, and lead principal pathologist of British Columbia Cancer Genito-Urinary BioBank.

Clinical Associate Professor, Pathology and Laboratory Medicine, Faculty of Medicine, University of British Columbia