Letter of intent

Montreal, Canada,

September 28, 2025

Dear Members of the Lung Cancer Canada Research Committee,

I am writing to submit my Letter of Intent for the 2025 *Give A Breath Research Award* for the research project entitled: **Liquid biopsy as a clinical tool to discriminate immune-related toxicity and lung infection from true cancer progression on first-line immunotherapy for non-small cell lung cancer**. As a clinician-scientist in thoracic oncology at the Centre hospitalier de l'Université de Montréal (CHUM), my research program is focused on precision medicine for patients with advanced non-small cell lung cancer (NSCLC). I am applying together with **Catalina Moreno, MSc Candidate**, a graduate student in my laboratory, as co-applicant.

Our proposal addresses one of the most urgent challenges in the care of patients treated with immunotherapy: accurately distinguishing true cancer progression from infection or immune-related pneumonitis when new lung opacities appear. *For many patients with stage III–IV NSCLC, immunotherapy is the only treatment that offers the possibility of long-term survival*. However, CT imaging cannot reliably separate progression from infection or toxicity, and current diagnostic tools provide limited information. *Misclassification can lead to premature discontinuation of effective first-line immunotherapy or unnecessary exposure to toxic or ineffective treatments.*

Our project introduces a *BAL–plasma cfDNA classifier* that integrates tumor-derived DNA, microbial signatures, and host-response signals through whole-genome sequencing. By applying this dual-specimen approach, we aim to:

1. Improve diagnostic accuracy beyond radiology alone, where management decisions critically affect patient prognosis.
2. Enable tailored interventions in advanced NSCLC – immunosuppression for pneumonitis, targeted antimicrobials for infection, or locoregional versus systemic therapy for progression.
3. Allow patients to safely continue immunotherapy when it remains effective, maintaining access to the only therapy with long-term survival potential, and avoiding premature transition to second-line chemotherapy with limited benefit.

This pilot study will generate feasibility and proof-of-concept data that could be scaled to larger national cohorts. By focusing squarely on patients living with advanced lung cancer *beyond first-line therapy*, the proposal aligns directly with the goals of the *Give A Breath Research Award*: to reduce the burden of lung cancer and optimize patient care after the first-line therapy setting.

Thanking you for your consideration,

Antoine Desilets, MD, MSc
Hematologist-Oncologist, CHUM
Principal Investigator, Cancer Axis, CRCHUM
Co-Director, Precision Oncology Program
Assistant Professor, University of Montreal

Catalina Moreno, BSc, MSc Candidate
Department of Cellular Biology and Pathology
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Research program summary

Context: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death in Canada. At diagnosis, 75% of patients present with stage III or IV disease[1], with poor survival often driven by thoracic-predominant progression (lung, pleura, nodes, lymphangitic spread), sometimes with limited extra-thoracic disease[2]. The introduction of immunotherapy (IO) has revolutionized cancer care, offering the hope of long-term benefit in a subset of patients[3-5].

For patients receiving IO, serial computed tomography (CT) scans are routinely performed to monitor tumor burden. However, these scans frequently reveal new lung opacities that are difficult to interpret. Radiological changes in the lung and lymph nodes may correspond to **cancer progression**, **immune-related toxicity** such as pneumonitis[6], or **pulmonary infection**[7,8]. Pneumonitis is the most frequent immune-related adverse event (irAE)[9], with presentations ranging from incidental radiological findings to life-threatening respiratory failure. Yet radiological patterns such as ground-glass opacities or sarcoid-like reactions[10] are nonspecific, and no validated biomarkers exist to reliably guide clinical decision-making. The differential diagnosis is further complicated by the high risk of pulmonary infections in NSCLC, driven by immunosuppression[11,12], post-obstructive pneumonia[7], and prior therapies[13], with reactive lymphadenopathy[14] further obscuring interpretation.

Consequently, management decisions differ dramatically depending on the underlying cause:

- **Cancer progression** required second-line treatment, either radiation for locoregional recurrence or chemotherapy for metastatic disease, though benefits are limited.
- **Immune-related pneumonitis** requires urgent initiation of immunosuppressive therapy to reverse the effects of IO and prevent pneumonitis-related mortality – though this carries the risk of accelerating cancer progression and infection.
- **Lung infection** requires pathogen-targeted antimicrobial therapy, as unnecessary antibiotics disrupt the microbiome and have consistently been linked to worse survival in patients on IO.

CT findings are nonspecific, and no discriminant biomarkers are currently available. Misclassification may lead to inappropriate treatment decisions, withholding effective IO from responding patients, exposing others to harmful interventions, and ultimately worsening prognosis.

Current standard and limitations: Bronchoscopy with bronchoalveolar lavage (BAL) is often used to investigate new opacities. However, current analyses are mainly restricted to excluding bacterial infection and provide no insight into tumor progression or immune-mediated toxicity. Clinicians are often forced to make high-stakes decisions with incomplete information.

Whole-genome sequencing (WGS) of cell-free DNA (cfDNA) offers a novel opportunity to resolve this diagnostic dilemma by providing multidimensional insights in a single assay:

- **Cancer progression**: direct detection of circulating tumor DNA (ctDNA) using tumor–normal WGS, enabling (1) quantification of tumor fraction (TF; i.e. ctDNA/cfDNA ratio) and (2) identification of genomic alterations associated with IO resistance.
- **Immune-related pneumonitis**: profiling of fragmentomics, end motifs, and cell-of-origin methylation patterns, enabling characterization of inflammation and tissue damage associated with immune-related pneumonitis.
- **Lung infection**: species-level pathogen detection by shotgun metagenomic WGS as demonstrated by collaborators[15], enabling rapid identification of typical and atypical organisms (e.g., mycobacteria) and guiding tailored therapy beyond limits of slow culture-based methods.

Applying WGS-based cfDNA profiling to BAL fluid, alongside plasma, could fundamentally change how IO-treated NSCLC patients with new lung opacities are evaluated.

Hypothesis: We hypothesize that a WGS-based cfDNA classifier using BAL and plasma can accurately distinguish progression, pneumonitis, and infection in IO-treated NSCLC, offering greater specificity than radiology and guiding clinical decision-making.

Research program summary

Aim 1: **Identify cfDNA signatures in BAL and plasma that discriminate between cancer progression, immune-related pneumonitis, and infection in a discovery cohort (n=10)**

Sub-Aim 1.1: Define composite cfDNA signatures integrating ctDNA, microbial DNA, and immune activation signals, linked to adjudicated outcomes and orthogonal biomarkers.

- *Objective*: To retrospectively evaluate the diagnostic performance of a WGS-based cfDNA classifier in patients with radiological lung opacities during IO, complemented by orthogonal cytokine panels (IL-6 and CXCL9/10) immune cell profiling, and standard BAL microbiology.

Sub-Aim 1.2: Assess the relative contribution of BAL and plasma ctDNA in patients retrospectively confirmed to have progression.

- *Objective*: To determine the diagnostic value of BAL versus plasma ctDNA in identifying cancer progression among IO-treated NSCLC patients, and to discriminate between lung-predominant progression (potentially benefiting from radiation therapy with continuation of IO) versus systemic progression requiring transition to second-line systemic therapy

Aim 2: **Validate the diagnostic performance of the WGS-based cfDNA classifier in an independent patient cohort (n=10) and determine whether it improves diagnostic accuracy over radiology alone.**

- *Objective*: To demonstrate that cfDNA-based classification more reliably distinguishes progression, pneumonitis, and infection than radiological interpretation.

Methods: This pilot study will enroll patients with unresectable stage III–IV NSCLC receiving IO who develop new lung opacities and undergo bronchoscopy. BAL fluid and paired plasma will be collected during routine procedures. cfDNA will be extracted and analyzed with tumor-normal WGS to generate tumor, microbial, and immune signatures, which will be integrated into a classifier tested against adjudicated clinical outcomes. Interpretation will follow a predefined framework:

1. **Progression**: BAL-dominant ctDNA signal (BAL TF > plasma TF) supports intrathoracic progression corresponding to lung-limited radiological changes.
2. **Pathogen detection**: strong pathogen reads in BAL cfDNA with low or absent TF indicate infection, providing species-level resolution for targeted antimicrobial therapy.
3. **Immune-related pneumonitis**: inflammatory/epithelial cfDNA in BAL ± plasma, with low TF and no pathogen reads, supported by immune signatures (cell-of-origin methylation showing immune lineage cfDNA and fragmentomic shifts with shorter fragments and altered end-motifs).

Clinical outcomes will be adjudicated by a multidisciplinary committee to ensure accurate classification of progression, pneumonitis, and infection. Diagnostic performance will be benchmarked against radiology alone, with sensitivity, specificity, and area under the curve (AUC) as key metrics. This pilot will proceed through a discovery cohort followed by a small validation set.

Impact: This study will establish a **BAL-plasma cfDNA classifier** that redefines pneumonitis from a "diagnosis of exclusion" into a molecularly defined entity supported by host-response signals.

The approach is designed to:

- **Improve diagnostic accuracy** when CT findings are nonspecific, as management decisions directly affect prognosis, *particularly if effective first-line IO is prematurely discontinued.*
- **Enable tailored precision therapy** with urgent immunosuppression for pneumonitis, pathogen-directed antimicrobials for infection (avoiding unnecessary antibiotics impairing IO benefit), and appropriate locoregional vs systemic treatment for intrathoracic versus disseminated progression.
- **Provide a scalable framework** for integrating tumor, pathogen, and immune signals into real-time clinical decision-making.

If validated, this dual-specimen assay could be adopted as a point-of-care adjunct to bronchoscopy, reducing diagnostic uncertainty, expediting treatment, and improving outcomes for patients with advanced NSCLC following initiation of first-line IO.

Public non-scientific summary

Lung cancer is the leading cause of cancer-related death in Canada, and most patients are diagnosed at an advanced stage. In recent years, immunotherapy has transformed treatment for many people with advanced non-small cell lung cancer (NSCLC), offering the chance of long-term survival. However, for patients receiving immunotherapy, routine CT scans often reveal new spots or "opacities" in the lungs. These changes are difficult to interpret: they may represent true cancer progression, a lung infection, or inflammation caused by immunotherapy (known as immune-related pneumonitis). Each possibility requires a completely different treatment, and making the wrong call can seriously harm patients. **For many, first-line immunotherapy is the only treatment with long-term survival potential: stopping it unnecessarily can take away their best chance of living longer.**

If cancer is progressing, patients need to switch to another line of therapy. If the cause is pneumonitis, urgent immunosuppression is required to reverse the effects of immunotherapy and prevent severe, sometimes fatal, lung damage. If infection is the cause, patients need targeted antibiotics – but unnecessary or broad antibiotic use can disrupt the gut microbiome which reduces the effectiveness of immunotherapy. Unfortunately, CT scans cannot reliably distinguish among these scenarios.

Today, bronchoscopy with bronchoalveolar lavage (BAL) is often performed when CT scans show unclear findings. In this procedure, doctors wash a small part of the lung and analyze the fluid. While BAL can sometimes identify infections, it does not determine whether the cancer is progressing or whether immunotherapy has triggered inflammation. Physicians are often left making high-stakes decisions with incomplete information.

Our project introduces a new approach: using whole-genome sequencing (WGS) to analyze cell-free DNA (cfDNA) from BAL fluid and blood plasma. cfDNA consists of tiny fragments of genetic material released when cells die. By studying cfDNA, we can extract multiple layers of information from a single test:

- **Cancer progression**: tumor-derived cfDNA fragments reveal the presence of cancer and genetic changes linked to immunotherapy resistance.
- **Infection**: cfDNA from bacteria, viruses, or fungi can be identified at the species level, enabling precise antimicrobial treatment.
- **Pneumonitis**: cfDNA fragmentation and methylation patterns can reveal inflammation and immune activation specific to immunotherapy-related toxicity.

By integrating these signals, we aim to build a diagnostic classifier that more accurately determines whether new lung opacities are caused by cancer, infection, or pneumonitis. The test can also indicate whether the disease is confined to the chest (BAL-dominant signal), where patients may still benefit from radiation while continuing immunotherapy, or whether it is more widespread (plasma-dominant signal), supporting a switch to second-line chemotherapy for more diffuse cancer progression that is not yet visible on routine scans.

This project would deliver a point-of-care test that reduces diagnostic uncertainty, guides the right treatment faster, and ultimately improves both survival and quality of life for patients with advanced lung cancer. **By allowing doctors to safely continue immunotherapy when it is still effective, this test could help more patients maintain access to the only treatment with long-term survival potential, avoiding premature transition to second-line therapy with limited benefit**

Figures

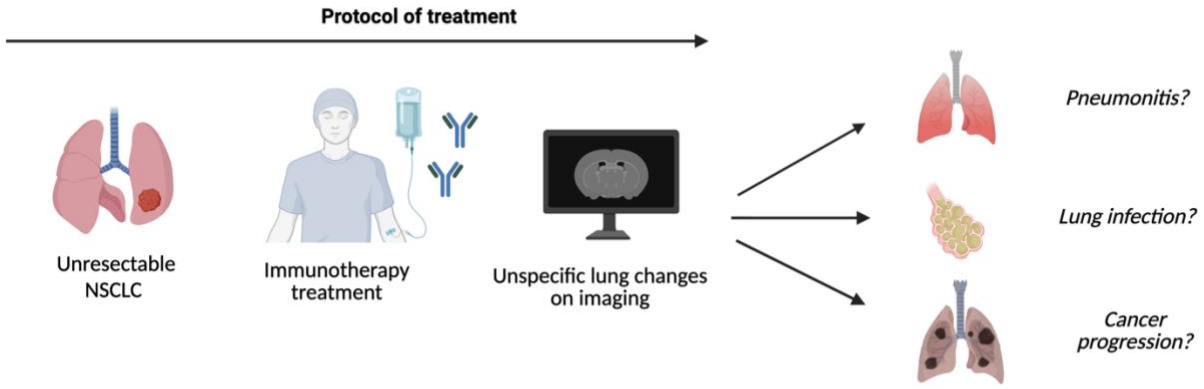

Figure 1: Limitation of radiological evaluation in discriminating immune-related events: General treatment protocol for unrespectable non-small cell lung cancer (NSCLC) patients involved immune checkpoint inhibitor. Following the immunotherapy, routine scan are performed to monitor disease progression. However, pulmonary opacities may be related to immune-related pneumonitis or lung infection, rather than cancer itself. These non-specific clinical findings can lead to a misdiagnosis and affect the course of treatment. Image created with BioRender (license: Antoine Desilets and Catalina Moreno)

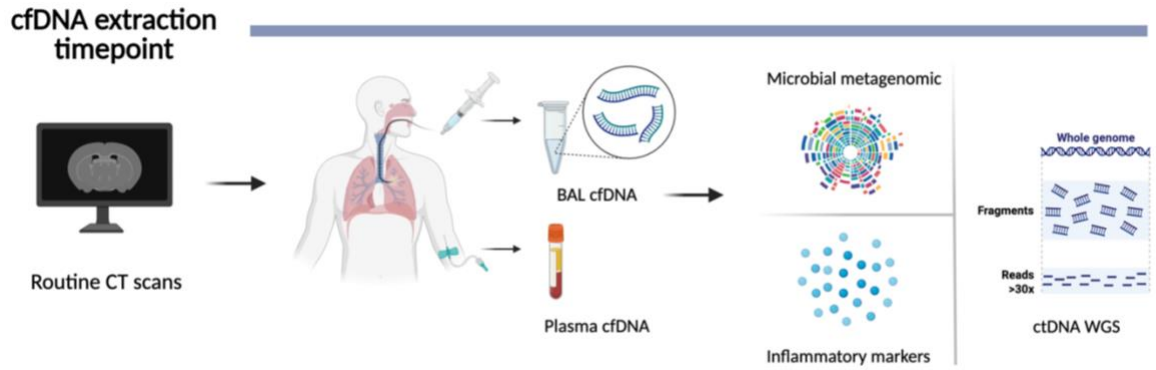

Figure 2: cfDNA extraction timepoint: Computed tomography (CT) scans allow the detection of lung opacities. Bronchioalveolar lavage (BAL) and plasma sampling will be performed on patients to extract circulating free DNA (cfDNA). Using whole-genome sequencing (WGS), cfDNA signatures will be analysed to discriminate cancer-related circulant tumor DNA (ctDNA) from microbial DNA and immune activation signals. Image created with BioRender (license: Antoine Desilets and Catalina Moreno)

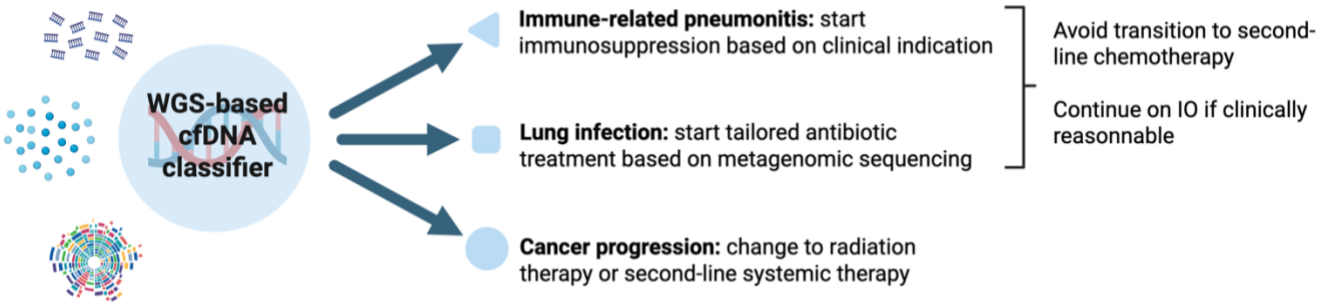

Figure 3: Determination of lung opacities with WGS classifier: The whole-genome sequencing (WGS)-based circulating free DNA (cfDNA) classifier will allow to discriminate between immune-related pneumonitis, lung infection and cancer progression, hence managing patients specifically based on their bronchoalveolar lavage (BAL) and plasma cfDNA genetic signatures. Image created with BioRender (license: Antoine Desilets and Catalina Moreno)

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Budget

LEGEND – in **green: pre-existing contributions** through research grants and institutional support to indirectly sustain the liquid biopsy program. **Total samples**: n= 20

PRE-EXISTING AND NON-OVERLAPPING GRANTS: Institutional support and research funds already in place indirectly supporting the liquid biopsy program		
	Value	*Description*
Institut du Cancer de Montréal - Rapatriement des Cerveaux	250 000 $	$250,000 start-up fund over 5 years for equipment, research staff (already hired), and platform services.
CRCHUM	225 000 $	$225,000 start-up fund over 3 years for equipment, research staff (already hired), and platform services.
CHUM Foundation & Samuel McLaughlin Fellowship	125 000 $	$125,000 fellowship over 3 years for a research fellowship in precision oncology and liquid biopsy at Memorial Sloan Kettering Cancer Center, New York.
Guy-Lafleur Precision Oncology Program	4 000 000 $	$4,000,000 co-directed by Anne-Marie Mes-Masson and Antoine Desilets to deploy a clinically accredited sequencing platform in molecular pathology at CRCHUM, with bioinformatics pipeline and sequencing report aligned with pan-Canadian standards in collaboration with the Terry Fox Foundation and Marathon of Hope Cancer Centres.
Clinician Scientist Award - Terry Fox Marathon of Hope Cancer Centers Network	450 000 $	3-year research grant on whole-genome sequencing liquid biopsy for minimal residual disease detection in early-stage NSCLC

GIVE A BREATH AWARD - RESEARCH GRANT APPLICATION			
CONSUMABLES AND PLATFORM SERVICES	*Unit cost**	*Value*	**Description (unit cost designation in parentheses)**
NovaSeq X Serie Plus Sequencer	1 400 000 $	- $	High-throughput whole-genome compatible sequencers for the liquid biopsy program (already covered by Durocher Fund).
CRCHUM Molecular Pathology Sequencing Platform	1 000 000 $	- $	Total costs for the upcoming tumor sequencing platform with ISO15189 clinical accreditation and research staff, excluding reagent costs (staff already covered by Guy Lafleur Program Fund).
Server Fees	0 $	- $	Secure post-sequencing genomic data hosting at CRCHUM (already covered by CRCHUM).
Streck® Tubes	20 $	400 $	Preservation tubes for plasma or urine liquid biopsy (unit cost per sample).
DNA Extraction	35 $	700 $	Reagents for nucleic acid (DNA) extraction from liquid biopsy samples (unit cost per sample).
Library Preparation	40 $	800 $	Reagents for next-generation sequencing library preparation (unit cost per sample).
Transport	30 $	600 $	Secure post-sequencing genomic data hosting at CRCHUM (already covered by CRCHUM).
Genetic Sequencing of Liquid Biopsies	350 $	7 000 $	Whole-genome sequencing (WGS) costs at Université de Montréal with Sainte-Justine Hospital, or eventually through the Guy Lafleur molecular pathology platform (unit cost per sample).
Genetic Sequencing of Solid Tumors and Normal Samples	775 $	15 500 $	
Total – Consumables and Platform Services:	1 250 $	**25 000 $**	

GRAND TOTAL	**25 000 $**

CRCHUM
CENTRE DE RECHERCHE
Centre hospitalier
de l'Université de Montréal

Université de Montréal

Montreal, September 25th 2025

Dr. Antoine Desilets (MD, MSc)
Centre de recherche du CHUM (CRCHUM)

OBJECT: Letter of institutional support for Dr. Desilets, MD, MSc – Lung Cancer Canada – Give a Breath Research Award

Dear Committee Members,

I hereby wish to express my strong support for Dr. Antoine Desilets' application for the Give a Breath Research Award.

Dr. Desilets' research program, which focuses on enhancing personalized medicine for non-small cell lung cancer patients through whole genome sequencing (WGS) and orthogonal biomarkers integration, aligns perfectly with the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) commitment to advancing innovative cancer treatments and precision medicine.

Dr. Desilets joined the CRCHUM as regular researcher in January 2025 and is an Assistant Professor of Medicine at the Université de Montréal. He is an emerging and promising researcher in hemato-oncology as demonstrated by his recent 2025 Clinician-Scientist Awards, funded by the Marathon of Hope Cancer Centres Network. At the CRCHUM, he is also part of the Cancer research theme which offers numerous opportunities for collaboration and expertise-sharing among highly experienced researchers. Dr. Desilets was appointed Co-Director of the Guy-Lafleur Precision Oncology Program at CRCHUM, working alongside Dr. Anne-Marie Mes-Masson, Scientific Director at the CRCHUM.

I confirm that the proposed research is feasible within our institution and that Dr. Desilets's team will have access to state-of-the-art facilities essential for the project, including high-capacity sequencers for WGS, common specialized equipment and the CRCHUM core facilities, along with technical support and collaboration with the CRCHUM Microbiome Center. Dr. Desilets' full research status at our institution ensures a minimum of 50% of protected time for research and remuneration for research time, access to a computer, storage space on the CRCHUM servers, benefit from identified spaces within our institution, usage of shared instruments and services of 19 platforms for which the services are subsidized up to 40%.

Our institution acknowledges and accepts the grant application guidelines and is fully committed to supporting Dr. Desilets throughout the award period. We will provide ongoing administrative assistance and will ensure the management of financial reporting. We are particularly excited about the potential impact this research program could have on the outcomes of Canadian patients with metastatic lung cancer with suspected progression on first-line immunotherapy.

Please accept, dear members of the committee, my warmest regards.

Director of Research and Innovation by interim - CHUM

Kathy-Thi Bao Khanh Lê

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