Letter of Intent

Lung cancer is one of the most aggressive malignancies, not only in Canada but worldwide (Zhou, et al., Cancer Epidemiology, 2024.). As a thoracic surgeon, I have performed numerous lung cancer surgeries for the last 15 years. Through these experiences, I have observed and strongly believe that early detection and early resection are crucial to overcoming lung cancer. As such, I have conducted several fundamental- and clinical-studies on lung cancer, particularly focusing on early-stage small lung cancers and optimal resection methods (Miura et al., EJCTS, 2024; Miura et al., ICVTS, 2023, etc). These experiences have fueled my enthusiasm to further develop new technologies for early detection and early resection.

I am currently affiliated with the Latner Thoracic Research Laboratories in Toronto, where my primary research theme is the development of a novel lung cancer detection method using an activatable fluorescent contrast agent targeting active cathepsins. This reagent binds specifically to active cathepsins, which are frequently overexpressed in malignant tumors. Upon binding, the quencher is released, enabling exposure of the ICG signal and thus the subsequent excitability and emission of its fluorescent signal. This agent has characteristics of being non-invasive and a high specificity. It has been able to advance to a Phase III clinical trial is being prepared for thoracic surgery.

While this is very promising, several important clinical questions remain. First, which tumor types are detectable with this reagent, including ground-glass nodules and metastatic tumors; and second, whether tumors can be detected via a transbronchial approach. This study aims to address these critical questions. In other words, if the usefulness of this reagent in bronchoscopy can be confirmed, it may enable the early detection of early-stage lung cancers, including small peripheral lung cancers, as well as metastatic lung tumors. Such early detection would, in turn, allow earlier treatment and could ultimately lead to improved patient outcomes.

Specifically, we can say that we align very well/strongly for the research into early detection of lung cancer methodologies, but also that this is based on clinically available samples that we can then align with the contrast agents of today bit also help inform on drugs or contrast agents of tomorrow.

Research Summary

1. Introduction

Lung cancer is one of the most aggressive malignancies, not only in Canada but worldwide (Zhou, et al., Cancer Epidemiology, 2024). Early diagnosis and treatment, particularly complete surgical resection, are essential for achieving a cure. However, intraoperative detection of small lung cancers remains a major challenge for thoracic surgeons, as some tumors are not palpable. This issue is a critical topic in thoracic surgery. Recently, a novel fluorescent dye reagent targeting active cathepsins has been developed. After peripheral vein injection, this reagent binds to active cathepsins, frequently overexpressed in malignant tumors. Upon binding, the quencher is released, enabling excitation, emission, and ultimately detection of the ICG signal. Because of its non-invasive use and high specificity, a Phase III clinical trial is ongoing in thoracic surgery (Kennedy, et al., Clinical Cancer Research, 2022; Bou-Samra, et al., Ann Thorac Surg, 2024). Thus, this cathepsin-targeted reagent represents a promising approach for detecting small peripheral lung cancers. However, key clinical questions remain: first, which tumor types are detectable with this reagent, including ground-glass nodules and metastatic tumors, and second, whether tumors can be detected via a transbronchial approach. This study aims to address these questions.

2. Objectives

- To determine which cancer types exhibit strong fluorescence, reflecting high cathepsin expression, by immunohistochemistry (IHC) of human specimens.
- To evaluate whether pulmonary tumors and lymph node metastases can be detected transbronchially and transpulmonarily using an ultrathin bronchoscope and an active cathepsin-targeted reagent (*in vivo* and *ex vivo*).

3. Methods and expected results

We will collaborate with OK Fiber Technology Co., Ltd. (Kyoto, Japan) that has developed an ultrathin bronchoscope (<1mm in diameter) equipped with a laser capable of detecting ICG wavelengths. This ultrathin fiber allows transbronchial observation of the peripheral lung, and we have already confirmed that it enables visualization down to the alveolar structures. Using this scope and the active cathepsin-targeted reagent, we plan to conduct the following experiments (Figure 1).

(i) Immunohistochemistry (IHC) using human surgical specimens

The cathepsin-targeted reagent binds to cathepsins B, L, S, and X (Suurs, et al.,EJNMMI Res, 2020.). We will perform IHC on human clinical samples (primary tumors and lymph node metastases). Using the HALO image analysis software

(HALO®, Indica Labs, Albuquerque, NM, USA), we will evaluate which malignant tumors are more detectable with this reagent. The H-score calculated by the HALO system will be adopted as an objective evaluation method. We have already conducted IHC using a few human lung adenocarcinoma specimens and optimized the IHC conditions. (Figure 2)

(ii) Mice experiments (*in vivo*)

We will use subcutaneous tumor models generated from human lung cancer cell lines (A549 and H460) to confirm whether ICG signals can be detected after reagent injection using the ultrathin bronchoscope. Although optimal timing and concentration after injection have been reported in previous studies, we will verify reproducibility and system characterization.

(iii) Rabbit experiment (*in vivo*)

We have established a rabbit lung cancer model using VX2, derived from rabbit sarcoma. In this model, we will inject the reagent and confirm ICG signals via a transbronchial approach using the ultrathin bronchoscope under general anesthesia.

(iv) Pig experiment (*in vivo*)

After creating subcutaneous tumors in mice and injecting the reagent, we will harvest these tumors at the optimal time point and transplant them into the lungs, bronchial wall, and trachea of pigs. We will then attempt to detect ICG signals via a transbronchial approach using the ultrathin bronchoscope under general anesthesia.

(v) Human declined lung experiment (*ex vivo*)

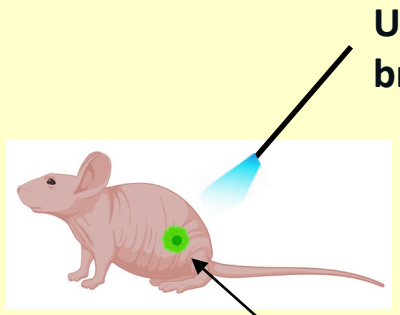
Our institution is one of the world's largest lung transplant centers. As such we are able to access human lungs that were declined for transplantation. After creating subcutaneous tumors in mice and injecting the reagent, we will harvest these tumors and transplant them into declined lungs and the tracheobronchial wall. We plan to insert the ultrathin bronchoscope via the trachea to confirm whether ICG signals can be observed through human bronchial walls and lungs.

4. Impact

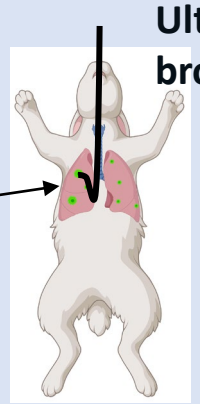
The IHC results are expected to clarify which tumor types are most detectable with this reagent, providing direct value to thoracic surgeons in both surgery and bronchoscopy. If applicable via bronchoscopy as well as intraoperatively, this optical approach could greatly improve early diagnosis and treatment of lung cancer. Moreover, in the era of sublobar resection, bronchoscopic detection of stump recurrence would allow timely reintervention, directly improving patient outcomes. In addition, assessing lymph node metastasis with this technique would further enhance its clinical utility by guiding treatment decisions and optimizing surgical strategies.

Figure 1: Study plans of in vivo and ex vivo experiments.

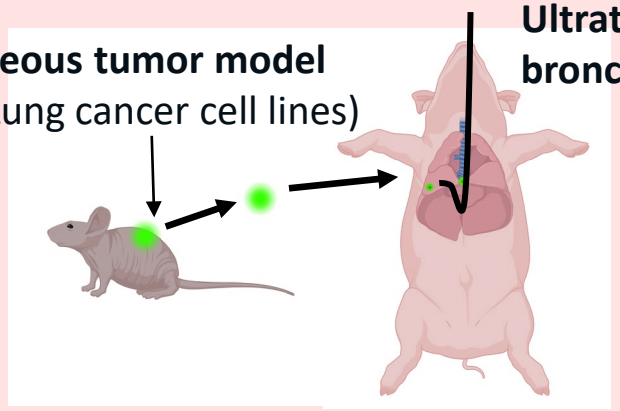
(1) Mouse model experiments



(2) Rabbit model experiments



(3) Pig model experiments



(4) Human declined lung model

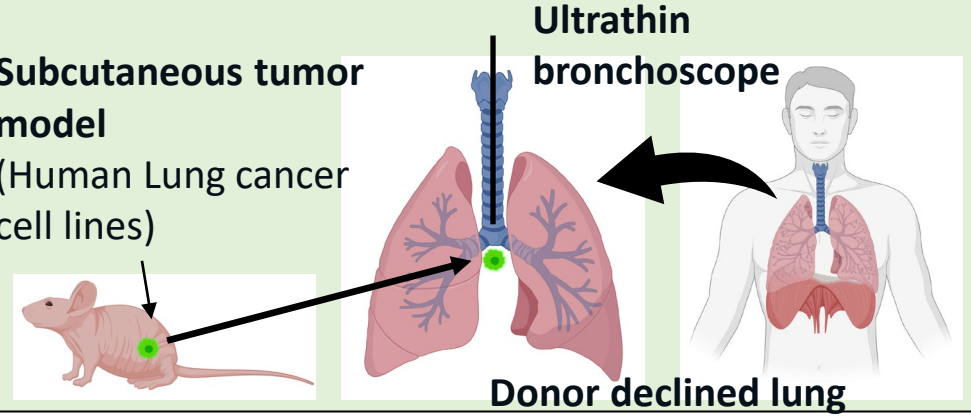
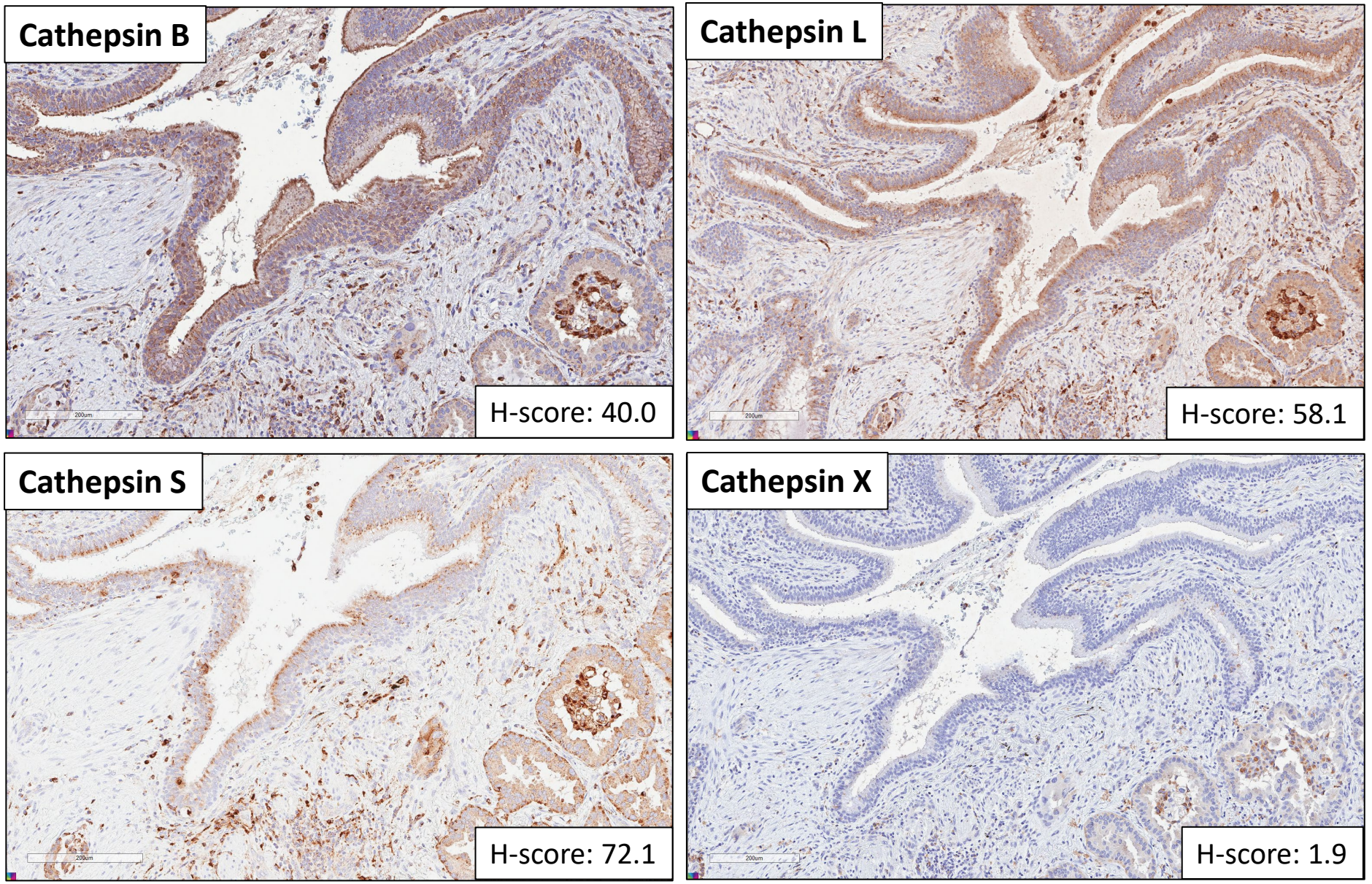


Figure 2: Immunohistochemistry (Cathepsin B, L, S, and X) of human lung adenocarcinoma.



Impact Statement

Lung cancer remains the leading cause of cancer-related mortality worldwide, with poor survival rates largely attributable to late-stage diagnosis (Zhou, et al., Cancer Epidemiology, 2024.). Despite advances in surgical techniques, systemic therapies, and immunotherapy, the prognosis of lung cancer patients has not substantially improved over the past decades unless the disease is detected at an early stage (Schabath, et al., Cancer Epidemiol Biomarkers Prev, 2019). Therefore, innovative strategies for early detection represent one of the most powerful approaches to reducing lung cancer incidence, mortality, and the overall burden on patients and healthcare systems.

Lung tumors frequently overexpress active cathepsins, making them a promising biomarker for early detection. The proposed research leverages this biological insight by evaluating a novel fluorescent dye reagent that binds specifically to active cathepsins, enabling endoscopic visualization of tumors. This reagent, with a Phase III clinical trial scheduled to begin in thoracic surgery, offers high specificity and a non-invasive application. However, its full clinical potential remains unexplored. By systematically investigating its utility in the transbronchial setting and across diverse tumor types—including small peripheral lung cancers, ground-glass nodules, and metastatic tumors—this project aims to address critical clinical questions that will determine how cathepsin-targeted imaging can be integrated into patient care.

The **short-term impact** of this research will be the establishment of robust clinical evidence regarding the diagnostic capabilities of this reagent in endoscopic applications. Such evidence will provide clinicians with new tools to visualize and characterize lung tumors during bronchoscopy, thereby facilitating timely diagnosis and surgical planning. By enabling earlier intervention, the study has the potential to significantly improve resection rates for small, early-stage lung cancers, which are often difficult to detect with current modalities.

In the **medium-term**, the outcomes of this research are expected to accelerate the integration of molecularly targeted fluorescence imaging into routine clinical practice. If validated, this technology could become a standard adjunct to bronchoscopy, complementing existing imaging techniques such as CT and PET scans. The ability to detect tumors in patients who do not meet current screening criteria—such as those without a heavy smoking history—would represent a major advancement in inclusive lung cancer screening and diagnosis. Moreover, the potential to detect metastatic lesions expands its relevance beyond primary lung cancer, addressing a critical gap in oncologic care.

The **long-term impact** of this research lies in its contribution to a paradigm shift in lung

cancer management. By bridging the gap between molecular biology and endoscopic practice, this project will promote the translation of laboratory findings into tangible patient benefits. The anticipated reduction in delayed diagnoses, improvement in surgical outcomes, and subsequent decrease in lung cancer mortality will exert a sustained and powerful influence on the field. Beyond clinical care, the widespread adoption of this technology could reduce healthcare costs by minimizing late-stage treatments and improving resource allocation.

In summary, this project embodies the mission of the grant by focusing on innovative early detection strategies for lung cancer. By anchoring the approach in tumor biology—specifically cathepsin expression, which varies among patients—it will generate high-impact evidence to clarify which tumors are most suitable for cathepsin-targeted imaging. While several promising agents are now emerging, the strength of this project lies in showing how COF technology can provide the anatomical access and visualization needed to integrate these agents into clinical practice. Ultimately, the successful completion of this study will improve patient quality of life, extend survival, and reduce the societal burden of lung cancer.

Public, Non-scientific Summary

Lung cancer is the leading cause of cancer-related deaths in Canada and worldwide. One of the main reasons lung cancer is so deadly is that it is often discovered too late, when it has already spread or become too advanced to treat effectively. If lung cancer can be detected earlier, patients can receive treatment sooner, which offers a much greater chance of survival and a better quality of life.

Our research focuses on improving the early detection of lung cancer. We are studying a special fluorescent dye that attaches to certain enzymes, called cathepsins, which are more active in cancer cells than in normal cells. When the dye binds to these enzymes, it produces a light signal that doctors can see with a camera.

In surgery, this approach has already shown promise by helping surgeons see lung tumors more clearly and remove them more precisely. The next step is to study which kinds of tumors and cancer types can be detected most reliably with this method. After that, we will investigate whether the same signal can also be seen through the airways with a bronchoscope, not just during surgery.

If these questions can be answered, this method could provide doctors with a powerful new way to detect lung cancer earlier and more accurately. Finding cancer at an earlier and more treatable stage would allow patients to receive timely treatment, improve their chances of cure, and reduce the physical and emotional burden of late-stage therapies such as chemotherapy and radiation.

In the future, this technology may also help detect lung cancer in people who do not meet current screening criteria, such as those without a heavy smoking history, making early detection more accessible to a broader population. Our ultimate vision is that this research will bring us closer to a future where lung cancer is found early, treated promptly, and no longer the leading cause of cancer deaths.

Budget justification

A. Personal

Research Fellow (0.6 FTE) – Salary: $31,800 / Benefits: $8,000

Total: $39,800

The applicant is the sole research fellow responsible for executing the entire project, dedicating 60% of their research time (0.6 FTE). With an MD background and extensive expertise in lung cancer biology and animal models, the fellow will lead the study design, perform all experimental procedures, analyze data, and present findings.

B. Equipment and Facility Access

- Animal facility usage: $3,000
- Imaging cores (bioluminescence and CT): $3,000
- Analysis system of immunohistochemistry (HALO system): $700

Total: $6,700

These facilities are critical for orthotopic transplantation and non-invasive monitoring of tumor growth and therapeutic response in vivo.

C. Experimental Animals

Mice (immunodeficient): $3,000

Rabbit: $3,000

Pig: $5,000

Required for creating the subcutaneous tumor model (mice), lung cancer models (rabbit and pig) including purchase, housing, and care.

Total: $11,000

D. Materials and supplies

Medium: $500

Dishes: $500

Matrigel: $500

PBS: $300

Tips: $300

Centrifuge tubes: $300

Antibodies (Cathepsin B, L, S, and X): $2,400

Total: $4,800

These consumables support the full experimental pipeline: cell culture,

immunohistochemistry, and mechanistic studies.

E. Immunohistochemistry

Cathepsin immunohistochemistry (including antibody optimization), 100 samples x 4 antibodies: $6,500

All procedures will outsourced to BioBank.

Other Sources of Funding

Preliminary studies were supported in part by the William Coco Chair in Surgical Innovation for Lung Cancer. These supports were specifically allocated to the acquisition of non-consumable equipment that is already installed in our laboratory.

The current proposal requests funding solely for the execution of new experimental work, including animal facility usage, imaging core access and materials required for in vivo and in vitro studies. No part of this proposed project is supported by any other source.

Total Budget:

A. Personnel: $39,800

B. Equipment and Facility Access: $6,700

C. Experimental Animals: $11,000

D. Materials and Supplies: $4,800

E. Immunohistochemistry: $6,500

Grand Total: $66,800

Kazuhiro Yasufuku MD, PhD, FRCSC
Head, Division of Thoracic Surgery, University Health Network · FG Pearson – RJ Ginsberg Chair in Thoracic Surgery · William Coco Chair in Surgical Innovation for Lung Cancer · Senior Scientist, Toronto General Hospital Research Institute · Director of Endoscopy, University Health Network · Director, Interventional Thoracic Surgery Program · Professor and Chair, Division of Thoracic Surgery, University of Toronto

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September 24, 2025

To Geoffrey Ogram Memorial Research Grant, Lung Cancer Canada

I am pleased to provide this letter of institutional support for Dr. Kentaro Miura in connection with his application to the Geoffrey Ogram Memorial Research Grant from Lung Cancer Canada. Dr. Miura, a thoracic surgeon and current postdoctoral fellow, focuses his research on lung cancer detection. This study builds on a stepwise approach that begins with the collection of patient samples and biomarker analysis, with an initial focus on cathepsins, which are already being explored in clinical trials. Based on these findings, the project will integrate a fluorescent agent that specifically targets active cathepsins. Finally, by combining this agent with an ultrathin bronchoscope (COF system), the study aims to establish a practical method of fluorescence imaging for the early detection and treatment of small lung cancers.

I confirm that the University Health Network (UHN) provides a world-class research environment fully supportive of this project. Specifically, Dr. Miura has access to:

- A highly collaborative research community with strong expertise in pathology.
- Ultrathin bronchoscopy equipment (OK Fiber Technology Co., Ltd., Kyoto, Japan) and the active cathepsin-targeted reagent.
- UHN's advanced animal research facilities and preclinical imaging cores.
- Mentorship from leading thoracic oncology surgeons and researchers at Toronto General Hospital and Princess Margaret Cancer Centre.

Dr. Miura's project is strongly aligned with UHN's commitment to fostering translational research that directly impacts patient care. The insights generated through this work are expected to accelerate the development of next-generation strategies for the early detection and treatment of lung cancer and have the potential to improve patient outcomes by enabling earlier tumor identification.

Our division and institution are fully committed to supporting Dr. Miura's proposed research with both the necessary research resources and academic mentorship. We are confident that Dr. Miura will conduct this work with the highest scientific and ethical standards, and that the outcomes will contribute meaningfully to the mission of Lung Cancer Canada by advancing early detection research and improving patient care.

Please feel free to contact me if you have any questions or concerns.

Sincerely,

Kazuhiro Yasufuku MD, PhD, FRCSC
Head, Division of Thoracic Surgery, University Health Network
FG Pearson - RJ Ginsberg Chair in Thoracic Surgery
William Coco Chair in Surgical Innovation for Lung Cancer
Senior Scientist, Toronto General Hospital Research Institute
Director of Endoscopy, University Health Network
Director, Interventional Thoracic Surgery Program, University Health Network
Professor and Chair, Division of Thoracic Surgery, University of Toronto

Division of Thoracic Surgery · Toronto General Hospital
200 Elizabeth St, 9N-957, Toronto, ON M5G2C4, Canada
Admin: 416-340-4798 (P), 416-340-3478 (F)
Clinical:416-340-4290 (P), 416-340-3660 (F)
kazuhiro.yasufuku@uhn.ca DOTScanada.com

University of Toronto UHN