Dear Members of the Geoffrey Ogram Memorial Research Grant Review Committee,

I am pleased to submit this Letter of Intent to apply for funding for our project titled "Development of a miRNA-Based Multiplex Lateral Flow Device for Early-Stage Lung Cancer Detection" This project is a collaboration between Dr. Fei Geng (School of Biomedical Engineering, McMaster University), Dr. Rosalyn Juergens (Department of Oncology, McMaster University and Juravinski Cancer Centre), and Dr. Monsur Ali (Biointerfaces Institute, McMaster University).

Lung cancer remains the leading cause of cancer-related mortality worldwide, with most cases diagnosed at advanced stages where treatment options are limited, and survival rates are drastically reduced. Early detection is crucial for improving these outcomes, but current screening methods primarily target high-risk populations, leaving many individuals without access to early detection tools.

Therefore, our proposed research focuses on the following two aspects:

- Research into Early Detection Methodologies: Our project aims to advance the field of early-stage lung cancer detection by developing a multiplex lateral flow device capable of detecting a panel of microRNAs (miRs-126, 145, 210, and 205-5p) associated with earlystage lung cancer. This blood-based, non-invasive assay will offer a cost-effective, rapid, and accessible method for lung cancer detection, particularly for individuals outside current screening criteria.
- 2. Research into the Etiology of Lung Cancer in Different Populations and Demographics: Specifically, this project will target light ex-smokers and non-smokers, who are currently underserved by existing screening programs. By focusing on this population, we aim to fill the gap in early detection methodologies and investigate the differences in lung cancer etiology across diverse groups.

Our proof-of-concept studies have demonstrated a broad dynamic range, high specificity, and dose-dependent amplification of synthetic miRNA samples. Additionally, we observed a strong linear correlation between miRNA concentration and readout signal, further confirming the technical feasibility of this system. These promising results underscore the potential of our system to implement early lung cancer screening on a broader scale, expanding access to early detection for a wider population. By validating this platform, we aim to create an accessible and scalable point-of-care tool for early-stage lung cancer detection. Future iterations of the device will include additional miRNAs to enhance diagnostic accuracy, further reducing the likelihood of false positives and negatives.

With over 20 years of experience in lung cancer biomarker discovery, my team, in collaboration with Dr. Juergens and Dr. Ali, is well-equipped to carry out this transformative project. We believe this work aligns closely with the mission of the Geoffrey Ogram Memorial Research Grant, and we are excited about the potential impact this device will have on the future of lung cancer screening.

Thank you for considering our application.

Sincerely,

Dr. Fei Geng, Dr. Dr. Rosalyn Juergens, and Dr. Monsur Ali

# Development of a miRNA-Based Multiplex Lateral Flow Device for Early-Stage Lung Cancer Detection

## INTRODUCTION

Lung cancer remains the leading cause of cancer death worldwide, with the majority of cases diagnosed in an advanced stage. Thus, lung cancer early detection is the frontrunner strategies to reduce this burden. Currently, radiological evaluation is the main screening methods for lung cancer diagnosis and treatment planning<sup>1,2</sup>. However, there are a large number of patients who do not meet the current screening criteria (e.g. the population that are younger than 50-year-old and/or have no smoking history). This gap underscores the urgent need for a cost-effective, sustainable, and accessible alternative screening method that can expand early-stage lung cancer detection to a broader and more diverse demographic.

Our previous research has demonstrated the correlation of lung cancer detection to prognosis<sup>3,4</sup>. MicroRNAs (miRNAs) involved in regulating gene expression, have been identified as highly sensitive and specific biomarkers for early-stage lung cancer detection<sup>5,6</sup>. Recently a plasma miRNA signature (miRs-126, 145, 210, and 205-5p) with the best prediction has been developed, producing 91.5% sensitivity and 96.2% specificity for lung cancer detection<sup>5–9</sup>. Consequently, the plasma miRNA signature holds promise as a blood-based assay for early-stage lung cancer screening<sup>1,7,9</sup>. To translate the miRNA signatures into a practical screening tool, lateral flow devices offer a simple, rapid, and cost-effective platform for point-of-care diagnostics, making them ideal for population-wide screening<sup>10,11</sup>. These devices are designed to detect plasma miRNAs, providing quick results without the need for specialized laboratory equipment<sup>10,11</sup>.

## OBJECTIVES

The proposed research directly addresses two primary objectives: advancing **research into early detection methodologies** for lung cancer (Fig. 1) and investigating **the etiology of lung cancer across different populations and demographics**, particularly light ex-smokers and non-smokers who are typically underserved by current screening protocols.

**Short-Term Goals (Phases 1 and 2):** Develop a multiplex lateral flow device for the simultaneous quantitative detection of a panel of four miRNAs (miRs-126, 145, 210, and 205-5p), which are highly sensitive and specific for the early detection of lung cancer.

**Long-Term Goals (Phase 3):** Lay the groundwork for future iterations of the device, building upon the successful development of the initial miRNA panel to incorporate additional miRNAs, which will further enhance the detection accuracy and reliability of the device in a clinical setting.

The goal of this project is to develop a miRNA lateral flow device capable of detecting these four miRNAs, providing a low-cost, rapid, and accessible screening tool. **This proposal is exclusively focused on Phase 1** (the development and validation of miRNA detection module) and **Phase 2** (the development of miRNA Multiplex Lateral Flow Device). The proposed budget will fully support these short-term goals. Clinical validation and performance assessment of miRNA multiplex lateral flow device (**Phase 3**) will be addressed in subsequent research efforts.

# FEASBILITY STATEMENT

The project is led by Dr. Fei Geng (20+ years in lung cancer biomarker discovery), Dr. Rosalyn Juergens (global leader in lung cancer therapeutics), and Dr. Monsur Ali (25+ years in biosensors and lateral flow systems). With access to state-of-the-art facilities at McMaster University and prior success in miRNA detection assays, the team is well-equipped to develop a functional multiplex lateral flow device for early-stage lung cancer screening. The combined clinical and research experience ensures that the biomarker panel selected for this project is both clinically relevant and optimized for early-stage detection.

## **RESEARCH DESIGN AND METHODS**

To address the above-mentioned objectives, the proposed research is outlined in the following: Phase 1 The Development and Validation of Detection Module for the miRNA Lung Cancer Panel (Dec 2024 - May 2025): In this phase, we will develop and validate the detection module for the miRNA lung cancer panel, focusing on the quantitative detection of miRs-126, 145, 210, and 205-5p (Fig. 2A). The validation process will begin with fluorescence signaling using synthetic miRNAs (Fig. 2Bi), followed by spiked miRNA samples in human serum to closely simulate clinical conditions. Our primary objective is to achieve high sensitivity and specificity for each miRNA, while minimizing cross-reactivity with other miRNAs. This will involve iterative optimization of key parameters, including reagent concentrations, amplification efficiency, and probe specificity. Simultaneously, we will optimize assay conditions such as reagent concentrations. reaction temperature, and incubation times to guarantee reproducible and accurate results (Fig. 2). The proof-of-concept experiment (Fig. 3) demonstrated a clear dose-dependent amplification of miR-145, with ideal sensitivity, dynamic range ( $10^{-7} \mu M$  to  $10^{-4} \mu M$ ) and linearity (R<sup>2</sup> = 0.9547). These data indicate the platform's strong potential for sensitive and accurate detection of miRNAs. making it suitable for further development as part of the proposed lateral flow-based diagnostic tool for early-stage lung cancer.

**Phase 2: The Development of miRNA Multiplex Lateral Flow Device for Lung Cancer Early Detection (May 2025 - Nov 2025)**: In this phase, we will integrate the validated detection module from Phase 1 with conjugated gold nanoparticles to develop a functional lateral flow device for the early detection of lung cancer. The device will be optimized to detect the selected miRNA panel (miRs-126, 145, 210, and 205-5p) through a highly sensitive and specific assay. Building on previous research<sup>10,11</sup>, we will incorporate both test and control lines using specific oligo probes designed to capture the miRNA targets effectively (Fig. 2Bii). Immobilizing miRNA-specific capture probes and control probes on the lateral flow devices will be a key focus, as this is essential for enhancing both the sensitivity and specificity of the assay. Ensuring compatibility between reagents and materials will be critical to prevent cross-reactivity, interference, or degradation, which could impact assay reliability. Comprehensive performance evaluations will follow, assessing the sensitivity and specificity of the device by testing a range of miRNA concentrations. The assay's performance will also be tested against a panel of related miRNAs to verify specificity. Additionally, we will evaluate the device's performance in terms of dilutional linearity, spike and recovery, and its overall reliability by comparing it to the gold standard qPCR system.

**Phase 3: Clinical Validation and Performance Assessment Using Lung Cancer Patient Blood Samples (Starting in Nov 2025 and beyond)**: Following the successful validation in Phase 2, we will conduct a pilot study to evaluate the performance of the miRNA-based lateral flow device using blood samples from lung cancer patients and healthy individuals. The first step will involve developing and optimizing a robust sample extraction protocol to ensure the isolation of high-quality miRNA from patient blood samples. Next, we will assess the device's performance through a pilot screening that includes two groups: one consisting of clinically diagnosed lung cancer patients (n=10, aged 30-55, light ex-smokers or non-smokers) and the other of healthy individuals (n=10, aged 30-55, light ex-smokers or non-smokers). The results from the lateral flow device will be compared with qPCR, the conventional gold-standard assay, to validate accuracy and reliability. This phase will provide critical data on the feasibility, sensitivity, and effectiveness of the device for early lung cancer detection in real-world settings, helping to determine its potential for large-scale screening efforts across Canada.

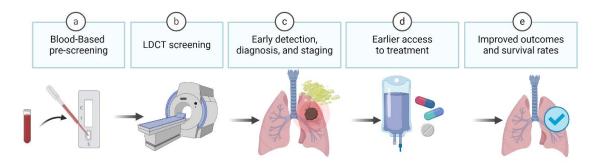
#### SUMMARY

This project aims to develop a rapid, affordable miRNA-based lateral flow device for early lung cancer detection, specifically addressing the urgent need for a cost-effective, sustainable, and accessible screening method. By targeting underserved populations such as light ex-smokers and non-smokers, this project seeks to fill critical gaps in current screening protocols and broaden early detection to a more diverse demographic.

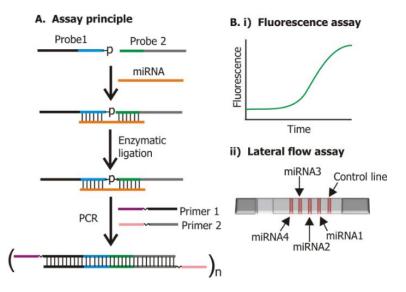
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# Appendix



**Figure 1.** The workflow for early-stage lung cancer detection. (a) Lung cancer pre-screening test is performed to identify high-risk individuals using miRNA-Based Multiplex Lateral Flow Device. (b) Positive pre-screening results lead to further examination using low-dose computed tomography (LDCT) for lung cancer screening. (c) LDCT facilitates early detection, diagnosis, and staging of lung cancer. (d) Early diagnosis allows timely access to treatment, including chemotherapy, targeted therapy, and other interventions. (e) The combination of early detection and treatment improves patient outcomes and increases survival rates.



**Figure 2**. Schematic of workflow in the development of miRNA detection module (left) and multiplex lateral flow device (right) for the early-stage lung cancer detection. **(A)** Assay principle: miRNA binds to complementary regions on Probe 1 and Probe 2, followed by enzymatic ligation to form a complete detection template. The ligated product is amplified via PCR using Primer 1 and Primer 2, generating multiple copies of the target sequence. **(B.i)** Fluorescence assay: The amplification is monitored through fluorescence, where an increase in fluorescence over time indicates the presence of miRNA. **(B.ii)** Lateral flow assay: The amplified products are visualized on a lateral flow strip, where miRNA targets (miRNA1, miRNA2, miRNA3, and miRNA4) are detected as distinct bands, with a control line ensuring proper detection performance.

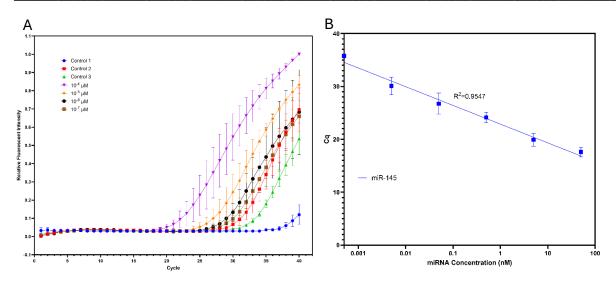


Figure 3. The specificity, dynamic range, sensitivity, and linearity of miRNA detection. (A) Amplification curves depict the relative fluorescence intensity of miR-145 at varying concentrations across 40 PCR cycles. The tested concentrations range from  $10^{-4} \mu M$  to  $10^{-7} \mu M$ , represented by the purple, orange, black, green, and brown curves, respectively. Control samples (Control 1, 2, and 3) are shown in blue, red, and violet curves. Each data point represents the mean fluorescence intensity  $\pm$  standard deviation (n = 3). (B) Standard curve for miR-145 detection shows the relationship between miR-145 concentration (nM) and the quantification cycle (Cq) over a concentration range of 0.001 nM to 100 nM ( $10^{-7} \mu$ M to  $10^{-4} \mu$ M). The linear regression fit (blue line) demonstrates a strong correlation (R<sup>2</sup> = 0.9547) between Cq values and miRNA concentrations, indicating robust detection sensitivity and linearity. Error bars represent the standard deviation (n = 3) for each concentration point. The results demonstrated a clear dosedependent amplification of miR-145, with higher concentrations reaching detectable fluorescence earlier in the cycle. The controls remain flat, indicating no amplification, thus validating the specificity of the assay. This data supports the sensitivity and dynamic range of miR-145 detection within the miRNA panel for the proposed lateral flow device for the early-stage lung cancer detection.

Our pioneering project to develop a blood-based lateral flow device for the simultaneous detection of a microRNA (miRNA) panel is set to exert a sustained and transformative influence on lung cancer research. By addressing the unmet need for early detection in populations currently excluded from conventional screening criteria—such as light ex-smokers and non-smokers—this project has the potential to significantly reduce lung cancer incidence and mortality while improving patient quality of life.

Lung cancer remains the leading cause of global cancer-related deaths, largely due to the challenges of early detection. Most cases are diagnosed at an advanced stage, where treatment options are limited and prognoses are poor. Our project offers a new path forward by introducing a non-invasive, cost-effective, and accessible screening tool that targets individuals who are underserved by existing screening programs. This device will expand the reach of early-stage lung cancer screening, providing timely intervention to broader populations, which is critical to reducing mortality.

This innovative approach also promotes a major advancement in lung cancer research by accelerating the translation of scientific findings into real-world applications. Led by a team of researcher, oncologist and engineer, including Dr. Fei Geng, Dr. Rosalyn Juergens, and Dr. Monsur Ali, this endeavor is poised to exert a sustained, powerful influence on lung cancer research and significantly reshape the landscape of patient outcomes. By leveraging a novel miRNA panel, we aim to bring laboratory discoveries into clinical settings, where they can deliver tangible benefits to patients. In the short to medium term, our lateral flow device has the potential to become a powerful tool in routine screening, significantly improving the chances of early diagnosis and, consequently, better treatment outcomes.

Additionally, this project will foster a deeper understanding of lung cancer etiology across different populations, particularly those not covered by current screening methods. By focusing on these underserved groups, we aim to advance research into the diverse biological pathways of lung cancer, ultimately leading to more tailored and effective screening strategies.

Our project's key innovation lies not only in its diagnostic capability but also in its ability to reshape early detection strategies. By enabling earlier and broader detection, we anticipate a reduction in late-stage diagnoses, which in turn will facilitate more effective and less aggressive treatment options. This will optimize patient care, reduce the healthcare burden, and improve long-term survival and quality of life.

In the short term, we will focus on refining and validating the lateral flow device through rigorous testing, ensuring that it meets the high standards required for reliability and clinical use. As the project progresses, the knowledge gained will propel major advancements in lung cancer research, positioning the device for widespread implementation. In the medium term, we envision this lateral flow device becoming an integral component of lung cancer screening protocols, driving a more inclusive and effective approach to early detection.

Ultimately, this project has the potential to revolutionize lung cancer screening. By providing a scalable and practical solution for early detection, we aim to reduce lung cancer incidence and mortality while enhancing patient well-being. Through the accelerated translation of scientific knowledge into optimized patient care, this project is poised to make a lasting impact on the global fight against lung cancer.

Lung cancer remains one of the deadliest cancers worldwide, largely because it is often diagnosed at advanced stages when treatment options are limited and survival rates are low. Our project, led by a distinguished team of researchers, aims to achieve **early-stage lung cancer detection** using a microRNA (miRNA)-based lateral flow device. This innovative approach will significantly broaden access to early-stage lung cancer screening, particularly for individuals who do not meet the current criteria for traditional screening methods, such as light ex-smokers, non-smokers, and younger individuals.

Current screening methods, such as low-dose computed tomography (LDCT), are limited to highrisk groups (e.g., heavy smokers and older adults), leaving a large segment of the population without effective early detection tools. Our platform uses miRNAs—small molecules found in blood that can reflect disease states—as the basis for a new, non-invasive, and cost-effective approach to detecting lung cancer at its earliest stages. While miRNAs such as miRs-126, 145, 210, and 205-5p have already been shown in research to be associated with lung cancer, our goal is to develop a lateral flow assay that can efficiently and accurately detect these markers to facilitate earlier interventions.

This project directly supports two objectives: First, it advances **research into early detection methodologies** by creating a blood-based screening tool that is easy to use, rapid, and widely accessible. Second, it investigates the **etiology of lung cancer in diverse populations**, specifically focusing on light ex-smokers and non-smokers, who are often excluded from traditional screening criteria despite their potential risk of developing the disease.

Our project is structured into three phases. Initially, we will optimize the miRNA detection platform to ensure high sensitivity and specificity. Following this, the lateral flow assay components will be incorporated into an easy-to-use device similar to a home pregnancy test, offering a rapid, point-of-care solution. Finally, we will validate the performance of the device by conducting pilot tests using blood samples from lung cancer patients and healthy individuals to assess its clinical accuracy and feasibility.

The potential impact of this project is far-reaching. By developing this miRNA-based lateral flow platform, we are creating a practical solution that can be used in a variety of healthcare settings, including those with limited resources. It is not only designed to improve lung cancer detection among high-risk populations but also to extend early detection capabilities to those who do not meet traditional screening guidelines, such as younger individuals and non-smokers.

In the future, this platform could revolutionize lung cancer screening practices, leading to earlier diagnosis, more effective treatments, and improved survival rates. With strong proof-of-concept data, we plan to seek additional support, such as a CIHR research grant, to expand the testing of this platform across larger patient cohorts. Our ultimate vision is to make early lung cancer detection accessible to all, reducing lung cancer incidence and mortality while improving patient quality of life on a global scale.

# **BUDGET JUSTIFICATION**

Institution:		McMaster University	
		FROM:	TO:
DETAILED BUDGET		12/1/2024	11/30/2025
PERSONNEL		SALARY REQUESTED	
NAME	ROLE	Year 1	TOTALS
Dr. Fei Geng	PI	N/A	N/A
Dr. Rosalyn Juergens	Co-PI	N/A	N/A
Dr. Monsur Ali	Co-PI	N/A	N/A
Arjun Raha	Ph.D. Student	\$12,500	\$12,500
PERSONNEL TOTAL		\$12,500	\$12,500
SUPPLIES		•	
DNA-modified gold nanoparticles Normal human serum		\$700 \$500	\$700 \$500
Synthetic miRNAs		\$1,000	\$1,000
Primer and probe synthesis		\$800	\$800
Conjugation reagents		\$1,000 \$2,000	\$1,000 \$2,000
Lateral flow strip supplies (nitrocellulose Membrane, sample pad, backing card, etc.)		\$2,000	\$2,000
			\$6,000
FACILITY USAGE			
Lateral flow strip dispensing Test strip cutting and assembly		\$3,500	\$3,500
		\$1,500	\$1,500
			\$5,000
OTHER EXPENSES Computer purchase		\$1,500	<u>Ф1 БОО</u>
		ψ1,000	\$1,500 <b>\$1,500</b>
			φ1,500
TOTAL DIRECT COST	S		\$25,000
TOTAL COSTS			\$25,000

# PERSONNEL

The research team for the proposed study includes PI Dr. Fei Geng (Program Chair in Biotechnology at McMaster), Co-PI Dr. Rosalyn Juergens (Oncologist specializing in lung cancer), Co-PI Dr. Monsur Ali (Biomedical Engineer specializing in lateral flow-based system), and PhD student Mr. Arjun Raha.

Here's the information regarding the personnel required for the study:

## Principal Investigator (PI): Dr. Fei Geng

Education: Dr. Geng received his M.D. degree from Jining Medical University (China) and his Ph.D. in Biochemistry from McMaster University.

Current Position: Associate Professor in School of Biomedical Engineering and Program Chair in Biotechnology at McMaster University.

Experience: Dr. Geng has been working on biomarker discovery and detection of lung cancer since 2004. He is a member of the School of Biomedical Engineering and Biointerfaces Institute at McMaster University.

Role: Dr. Geng will oversee the entire study, providing guidance and direction and supervise the graduate student in the development of miRNA detection module (**Phase 1**). Responsibilities include project planning, technical management, securing funding, managing the budget, and ensuring adherence to ethical guidelines.

#### Co-PI (Oncologist): Dr. Rosalyn Juergens

Education: Dr. Juergens received her M.D. degree from Georgetown University and Ph.D. in Clinical Investigation from The Johns Hopkins Bloomberg School of Public Health.

Experience: Dr. Juergens is an oncologist specializing in lung cancer at Juravinski Cancer Centre. She has clinical expertise in lungl cancer and has held various leadership positions in cancer research and patient advocacy.

Role: Dr. Juergens will contribute to the study as a Co-PI, leveraging her expertise in oncology and contribute to the patient recruitment process and clinical analysis. Dr. Juergens will provide the clinical insight and guide the development of miRNA lateral flow device for lung cancer early detection (**Phases 1 and 2**)

#### Co-PI (Biomedical Engineer specializing in lateral flow-based system): Dr. Monsur Ali

Education: Dr. Ali received his PhD in Pharmaceutical Sciences in Kyushu University, Japan. Dr. Ali has been the Scientist at University of California and McMaster University.

Current Position: Researcher at the Biointerfaces Institute, McMaster University.

Experience: Dr. Ali is one of the most cited researchers in the field of biosensor and lateral flow system. He is recognized for his work in developing disposable paper-based point-of-care diagnostic devices.

Role: Dr. Ali will be responsible for miRNA lateral flow device development (**Phase 2**) and will provide the supervision of graduate student in the establishment of lateral flow device setup and system optimization.

### PhD student: Mr. Arjun Raha

Education: Mr. Arjun Raha received his bachelor's in biotechnology and master's degree in mechanical engineering at McMaster University and he is in his first year of PhD studies in School of Biomedical Engineering at McMaster University.

Role: The PhD student will be responsible for conducting experiments and analyzing data for **Phases 1 and 2**. They will work closely with the PI and Co-PIs to ensure the study is conducted rigorously and scientifically.

## OTHER SOURCES OF FUNDING

Lung Ambition Award (\$50,000) June 2024 to May 2025 Lung Ambition Award will be used to identify, characterize, and validate novel miRNA candidates for the panel focused on early detection of lung cancer. These activities are distinct and will not overlap with the research proposed for the Geoffrey Ogram Memorial Research Grant. The specific activities funded by Lung Ambition Award include:

- Leveraging existing datasets and computational tools to identify novel miRNA candidates that show promise for early lung cancer detection.
- Characterization of these identified miRNA candidates using cell lines and other biological models to assess their relevance and potential utility in early detection diagnostics.



Research Office for Administration, Development & Support Gilmour Hall, Room 305 1280 Main Street West Hamilton, ON, Canada, L8S 4L8 (905) 525-9140 https://roads.mcmaster.ca/

September 30, 2024

Dear Lung Cancer Canada:

McMaster enthusiastically supports the proposed research project by Dr. Fei Geng, associate professor at McMaster University. The project, titled "Development of a miRNA-Based Multiplex Lateral Flow Device for Early-Stage Lung Cancer Detection" and submitted for consideration within the Geoffrey Ogram Memorial Research Grant competition, is a compelling and innovative endeavor that aligns seamlessly with our institution's research objectives and goals.

Having thoroughly reviewed the details of the proposed research, we are confident in its feasibility within our institution. Our institution possesses the necessary infrastructure, resources, and expertise to facilitate the successful execution of this project. Furthermore, we acknowledge Dr. Fei Geng's expertise and dedication to their work. Their proven track record and commitment to excellence make us confident in their ability to carry out this research successfully. We anticipate that the outcomes of this research will not only enhance the academic reputation of our institution but also contribute meaningfully to the broader scientific community.

McMaster University intends to provide support for this project in the areas of grant fund administration, data management consultations, and institutional administrative support. We look forward to the positive impact Dr. Fei Geng's research will have on our institution and the broader academic community.

Sincerely, Sherrise Webb

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Director, Research Office for Administration, Development and Support