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September 30th, 2024
Lung Cancer Canada
133 Richmond St. W., Suite 208
Toronto, ON M5H 2L3

Re: Geoffrey Memorial Research Grant (GOMRG)

Dear Review Committee,

Please accept our application for the Geoffrey Memorial Research Grant (GOMRG). The intent of this application is to support our research into the surgery-induced systemic accumulation of extracellular vesicles in the blood stream which we have identified are associated with early cancer recurrence after lung cancer surgery. This has the potential to provide an insight into early detection with affordable blood-based biomarker assay to screen patients for early-stage lung cancer and also to perform surveillance on patients after lung cancer surgery. The ultimate aim is to reduce the burden of lung cancer and optimize patient care.

Our team combines the expertise of lung cancer clinical specialists and scientists including myself, a thoracic and foregut surgeon, and co-PIs Dr. Shantanu Banerji, a medical oncologist at Cancer Care Manitoba, and Senior Scientist at the CancerCare Manitoba Research Institute and Dr. Ayesha Saleem, a Principal Investigator at the Children's Hospital Research Institute of Manitoba and expert in molecular and cellular physiology, specializing in mitochondrial metabolism and extracellular vesicle (EV) biology. In a prospective study, the translational team will use serial samples from lung cancer patients immediately before and after surgery to measure novel markers of early cancer recurrence**. In this proposal we will focus on the potential of EV-DNA as a tumor-agnostic perioperative biomarker for early recurrence in lung cancer patients who receive lung resection.**

Our published work shows that lung surgery induces inflammatory changes in both the local airway and the systemic environment [1]. The current proposal we present promising preliminary data supporting a link between early recurrence and an increase in the release of EVs in the blood stream immediately following lung surgery. The budget within this proposal will support EV analysis of an additional 25 patients in the study and serve as the foundation for ongoing research investigating the role of tumour microenvironment, surgical trauma and exosomes play in long-term lung cancer survival.

Sincerely,

Dr. Biniam Kidane, MD, MSc, FRCSC*
Thoracic & Foregut Surgeon, Section of Thoracic Surgery
Associate Professor of Surgery, College of Medicine, University of Manitoba
* Denotes Medical Corporation
Enclosure

[1] Kormish J, Ghuman T, Liu RY, Srinathan SK, Tan L, Graham K, Enns S, Buduhan G, Halayko AJ, Pascoe CD, Kidane B. Temporal and Spatial Patterns of Inflammation and Tissue Injury in Patients with Postoperative Respiratory Failure after Lung Resection Surgery: A Nested Case-Control Study. Int J Mol Sci. 2023 Jun 13;24(12):10051.

TITLE: Extracellular Vesicles as Biomarkers of Early Detection of Lung Cancer Recurrence Post Non-Small Cell Lung Cancer (NSCLC) Surgery.

BACKGROUND: Preamble: Inflammation contributes to lung cancer initiation. Commonly used curative procedures like surgery and radiation can cause lung injury and potentially further increase inflammation. The degree of biological inflammation caused by these interventions and its impact on disease dissemination and patient outcomes has not been quantified. Currently, there is great need for liquid biopsies to allow for non-invasive and early detection of new lung cancer as well as lung cancer recurrence are expensive and require knowledge of the specific tumour mutations in that cancer (i.e. tumour-**INFORMED**). Our preliminary work suggests that less expensive biomarkers can potentially be used that do not require knowledge specific tumour mutations (i.e. tumour-**AGNOSTIC**). Surgery Treatment of Early-Stage Lung Cancer: Surgery has been the cornerstone of cure for patients with NSCLC. The vast majority of early-stage lung cancers are operable by minimally invasive techniques resulting in a short hospitalisation and a risk profile that has continued to improve over the years [1,2]. Recent data indicate that pulmonary resection is associated with a 1.3% 30-day mortality risk and a major morbidity risk of 7.9% [3]. The advantages of surgery for early-stage lung cancer are numerous: definitive diagnosis without risk of sampling error, low local recurrence rates, excellent regional control, and definitive nodal staging with opportunities for adjuvant systemic therapy for node-positive disease [4]. Emerging Biomarkers of Lung Cancer Dissemination: A major contributor to the poor outcomes associated with NSCLC is the rapid progression to metastatic/incurable disease. At the patient level, this represents disease spread to distant organs like the bone, brain, liver, contralateral lung, and adrenal glands via lymphatic and hematogenous spread. The exact molecular mechanisms that drive the transition from local to metastatic disease include many of the known Hallmarks of Cancer [5]. Blood-based surrogate biomarkers have been identified that closely correlate with disease burden and patient prognosis. These markers often measured in venous circulation include circulating tumour DNA (ctDNA) and extracellular vesicles (EVs), especially exosomes. The latter, EVs, represent discrete 'packages' containing genetic material (including somatic mutations), protein, and lipids that mirror the composition of the cells from which they arose [6]. EVs differ from each other based on size, site of origin and cargo content, with three main subtypes: exosomes, microvesicles and apoptotic bodies. Tumour-derived EVs promote remodelling of the extracellular matrix which increases tumour cell invasion and motility thereby enhancing metastasis [7]. *However, the direct effects of acute injury causing inflammation on NSCLC-associated EV generation has not been evaluated.*

PRELIMINARY WORK

We have shown that lung surgery induces inflammatory changes in both the local airway and systemic environments. We have established an *in vivo* human protocol for collecting serial local lung airway and blood-based samples in patients undergoing lung surgery for malignant and benign indications **(Figure 1, Table 1)**. Patient-derived samples have been used to measure dynamic changes in biomarkers of local and systemic inflammation. Preliminary results (**Figure 2)**, highlight their ability to detect changes in inflammatory biomarkers, **comparing levels at the beginning of surgery to levels at the end of surgery**. Furthermore, heterogeneity exists in the inflammatory responses to surgery which will be important to characterise the inflammatory profile associated with cancer dissemination. In parallel, Dr. Saleem, a close collaborator, has established protocols for the purification and characterization of EVs, and routinely performs EV co-culture experiments using *ex vivo* models. In a pilot study of 16 human patients (**Table 2)**, we assessed the hypothesis that patients with early recurrence would have different EV population characteristics, differentially expressed proteins and different concentration of EV-DNA (**Figure 3**). Indeed, EV concentration and DNA content was observed to significantly increase in early recurrence; however, given the small sample size, we seek to validate these promising results in larger cohort of all patients having recurrence within <6 months post-surgery.

HYPOTHESES AND SPECIFIC AIMS: We hypothesize that commonly used local-modality therapies, such as lung surgery, influence cancer dissemination dependent on baseline and dynamic changes in systemic inflammation and emerging markers of cancer dissemination (e.g. EVs). Further, we posit that the causal link between these biomarkers and cancer recurrence will be evident through both *in vitro* experimentation (Aims 1 and 2) and in patient outcomes using clinical data (Aim 3).

Aim 1: *Assess the relative intra-patient dynamic changes in systemic markers of inflammation and dissemination compared to baseline in patients receiving surgery.* **1A)** Perform high-throughput screens to identify inflammatory markers that change most significantly post-surgery. **1B)** Measure dynamic changes in emerging markers of cancer dissemination (EVs). **1C)** Assess if there is a relative association between markers of inflammation and

dissemination. **Aim 2***: Asses the biological significance of biomarkers associated with inflammation and dissemination using in vitro cell line models.* **Aim 3**: *Identify the relationship between routinely available lab-based markers of global inflammation with early cancer recurrence and survival in a real-world setting.* **3A)** To assess the relationship between markers of inflammation and survival and recurrence-free survival. 3**B)** To assess the interaction between markers of inflammation and treatment (surgery) and their relationship with survival.

RESEARCH PLAN:

Aim 1: Using our existing strengths as a multidisciplinary research team, we will determine how surgery influences systemic markers of inflammation and emerging biomarkers of tumour dissemination (EVs). We will focus on patients with Stage I or II (node negative) biopsy-proven NSCLC referred to the CancerCare Manitoba (CCMB) Thoracic Disease Site Group for curative treatment. Patients will consent for peripheral blood collection, baseline demographics, and medical history. Blood will be drawn from each patient at 2 time points (**Figure 1)** and plasma will be collected from blood tubes for downstream analysis of inflammatory biomarkers and EVs. We will collect specimens from 25 patients (with equal male:female sex distribution) undergoing surgery in the first two years of the study. ***Aim 1A:*** Plasma samples will be characterized using the Mesoscale Discovery V-plex human biomarker 54-plex panel to determine the effect of the treatment (i.e. surgery) on the levels of protein markers [8]. This system is the gold standard for the multiplexed quantification of circulating inflammatory biomarkers and includes inflammatory biomarkers relevant to many chronic diseases, including cancer. Our research team has previous success using these panels to quantify circulating inflammatory markers following lung surgery [9]. ***Aim 1B:*** The size and yield of EVs is linked with EV cargo and sub-type, and subsequently function. Isolated EVs will be characterized biochemically following the Minimal Information for Studies of Extracellular Vesicles (MISEV) 2018 guidelines [10]. EVs will be isolated from plasma using size exclusion chromatography [11] to derive functionally intact EVs that can be used for downstream co-culture experiments. EV biophysical properties will be measured using Tunable Resistive Pulse Sensing technology (Exoid, Izon Sciences), in a single-blind fashion as shown in Figure 2 and described before [12]. Protein content will be determined using a Micro BCA kit and EVs subjected to gel electrophoresis to measure the expression of proteins associated with exosomes and microvesicles. Electron microscopy (Figure 2) imaging will be used to confirm integrity and purity of isolated EVs, and the presence of exosomes. Intra-patient changes will be analysed using a one-way ANOVA or paired Student's t-test. A two-way ANOVA will be used to compare samples at all time points. ***Aim 1C:*** Using methods described in sub aim 1A, we will explore associations between inflammatory biomarker abundance and presence of EVs. Inflammatory biomarkers that significantly change pre/post-surgery will be classified into categorical values as increased, decreased, or not changed in individual samples. EVs will be categorized as static variables based on size, yield, or stability, or dynamic variable. Fisher's exact test will be used to determine if there is a significant association between inflammatory marker and EV features or dynamics. Those markers that exhibit consistent association will be prioritized for functional validation in Aim 2.

Aim 2: To understand the role of inflammatory biomarkers in disease progression, we will use cell-based models to assess the ability of biomarkers identified in Aim 1 to promote migration and invasion. We will use a panel of lung cancer cell lines that harbour genetic mutations most common in NSCLC and are frequently used as models of NSCLC biology *in vitro*. This includes cell lines from ATCC - The Global Bioresource Center, A549 (*KRAS* mutation), H1770 (*TP53* mutation), and H1975 (*EGFR* mutation). We will employ scratch assays and transwell migration assays to assess changes in migration and invasion, respectively, in response to inflammatory biomarkers identified in Aim 1 [13,14]. Based on preliminary data, IL-7, IL-1β, and CRP will be investigated due to their high change in abundance post-surgery. Further evidence of invasiveness will be assessed by looking for changes in markers of epithelial-to-mesenchymal transition, including e-cadherin and vimentin. These will be characterized using immunofluorescence in fixed cell specimens. In parallel, we will also examine the impact of extracellular cargo contained in EVs using these same *in vitro* assays. **Aim 3:** 25 surgical NSCLC cases from Aim 1 will be assessed for 3-year cancer recurrence. Cases will be annotated with baseline demographics and status of significant biomarkers as determined in Aims 1A/B. Dr Kidane's ongoing study has prospectively collected data/biospecimens on >550 patients, including perioperative changes in inflammatory markers as well as cancer recurrence. Based on the first 186 patients assessed, approximately 33% (n=61) demonstrate recurrence/metastasis within 2 years of surgery. Thus, it is reasonable to expect 25 surgical patients in this Aim will result in sufficient recurrence events.

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Figure 1. Specimen collection, perioperative and patient follow-up workflow diagram.

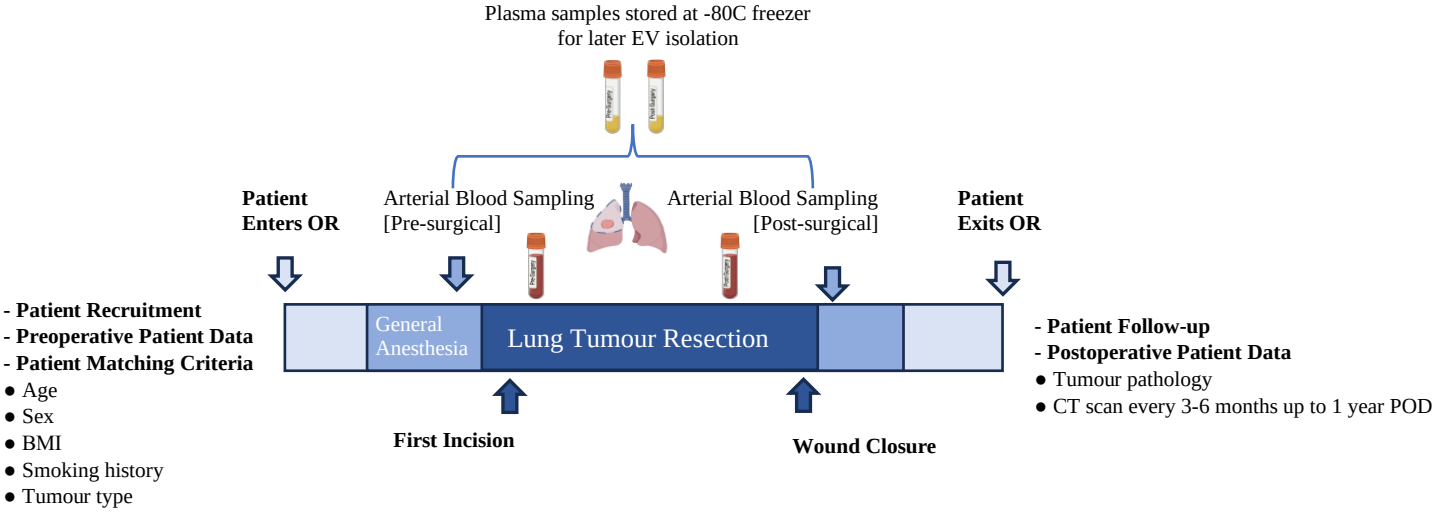

Table 1.

Part A: Proportion of Lung Surgery Patients with Primary Lung Tumours (n=375) % population (n)	
Primary Tumour No Metastasis	64.5 % (242)
Primary Tumour With Metastasis	17.1 % (64)
No Primary Lung Tumour	18.4 % (69)

Sex of Lung Surgery Patients (n=507)

56.8% (288) Female 43.2 % (219) Male

Part B: Age of Patients at Time of Procedure (n=506)		
Mean (STD)	66.4 (12.5)	
Median	68.8	
Range	18.2-88.6	
Age Distribution	# of patients	% of Total Patients
<46	34	6.7%
46 to 55	27	5.3%
56 to 65	124	24.5%
66 to 75	216	42.6%
76 to 85	102	20.1%
86 to 95	3	0.6%

Figure 1, Table 1 Part A, and Part B: EV analysis includes two perioperative time points. Our team has collected arterial plasma samples for over 500 lung surgery patients, including pre- and post-surgical samples collected in the operating room, (OR) immediately before and after lung surgery. Of these 500 lung surgery patients the majority (64.5%) have primary lung tumours. Distributions within patient matching criteria including sex, age, BMI and smoking history indicate that we have a sufficient patient base for effective control matching.

Figure 2:

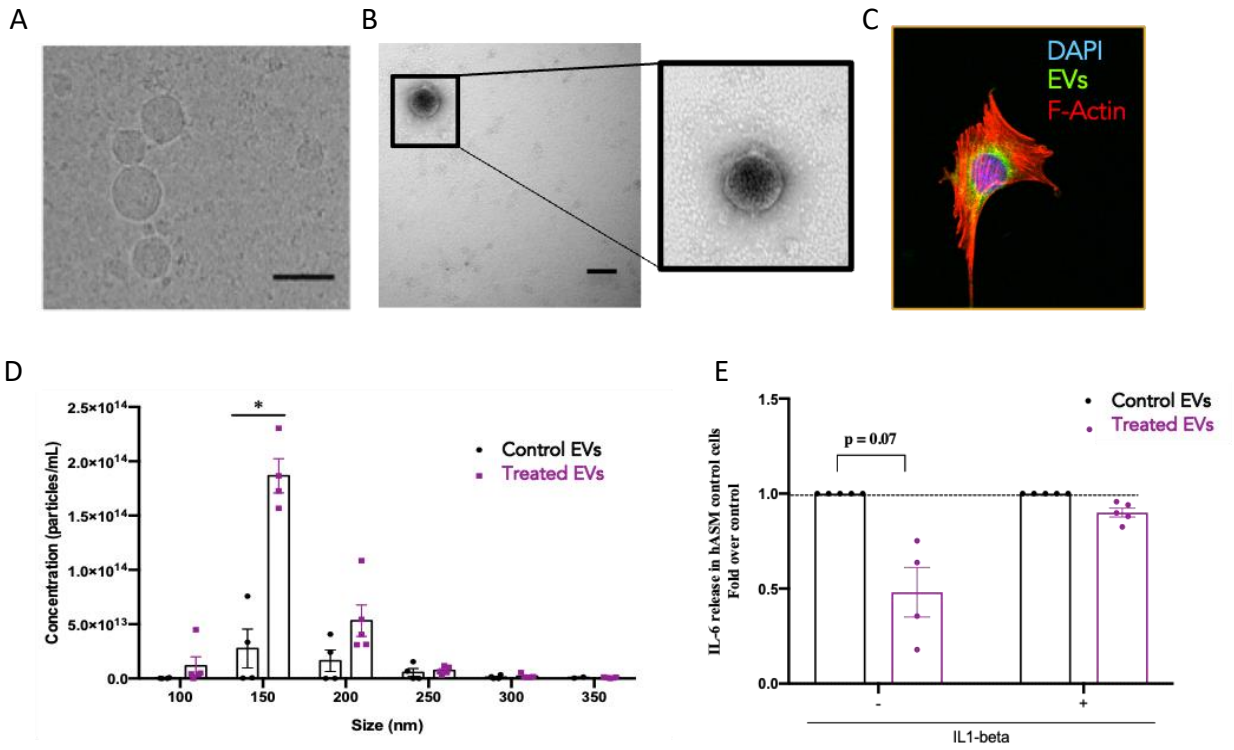

Figure 2: Extracellular vesicle (EV) biophysical characterization, cellular uptake and effect on cytokine release from cells. (A) Representative Cryo-EM (*left*, scale bar = 50µm) and **(B)** transmission electron microscopy image (*right*, scale bar = 100µm) clearly depicts the shape, purity, size and heterogeneity of exosomes. **(C).** EVs were labelled with a fluorescent dye (PKH67), and then incubated with recipient cells for 24hrs. Treated cells were stained with DAPI (nuclei) and rhodamine phalloidin (F-Actin) and imaged using confocal microscopy. The image shows distinct cellular uptake of EVs. **(D**) Representative biophysical characterization of EVs obtained using tunable resistive pulse sensing (TRPS) technology (Izon). **(E)** Cells incubated with EVs from the treatment group for 2 days showed a decrease in release of inflammatory cytokine IL-6 (p=0.07, N=4) demonstrating the capacity of EVs to modulate cellular function. Measurements included EV concentration (particles/ml), and size distribution. The heterogeneity and cellular uptake of extracellular vesicles is well documented. The ability of isolated exosomes to stimulate cytokine production in cell lines supports a model for exosome-mediated modification of the tumour microenvironment. Dr. Saleem, a co-PI on this proposal has the expertise and instrumentation to measure exosome properties and size distribution.

Table 2: Pilot Patient Population - Early Recurrence Detected at before 1-year post-operation, (total n=16).

Early Recurrence						Matched Control - No Recurrence (1 year)					
ID	**Age**	**Sex**	**BMI**	**Smoking History (Pack years)**	**Lung Cancer Pathology**	**ID**	**Age**	**Sex**	**BMI**	**Smoking History (PY)**	**Lung Cancer Pathology**
50	78	F	38.7	20	pT1 - ADENOCA	**93**	69	F	32.0	12	pT1- ADENOCA
51	76	M	31.44	45	pT2bN0-IADENOCA	**150**	70	M	34.72	40	pT2aN0(3)- ADENOCA
52	79	M	34.4	55	pT2a - ADENOCA	**79**	76	M	28.4	45	pT1bN0-IADENOCA
70	75	F	25.96	non-smoker	rpT4N0-IADENOCA	**62**	66	F	27.77	5.5	pT2aN2-ADENOCA
73	84	M	29.4	5.5	pT3N1-ISCC	**45**	76	F	20.3	8.3	pT3N0-SCC
95	76	F	27.73	27	pT4N1-MADENOCA	**276**	69	M	44	22.5	pT4N0-IMADENOCA
98	88	M	23.08	non-smoker	pT2a(4)N0-ADENOCA	**89**	59	M	26.17	non-smoker	pT1bN0
120	62	F	23.42	40	pT2bN0-SCC	**138**	78	F	32.26	100	rpT1bN0-SCC

Abbreviations: adenocarcinoma (ADENOCA), invasive adenocarcinoma (IADENOCA), squamous cell carcinoma (SCC), invasive squamous cell carcinoma (ISSC), mucinous adenocarcinoma (MADENOCA), invasive mucinous adenocarcinoma (IMADENOCA).

Figure 3.

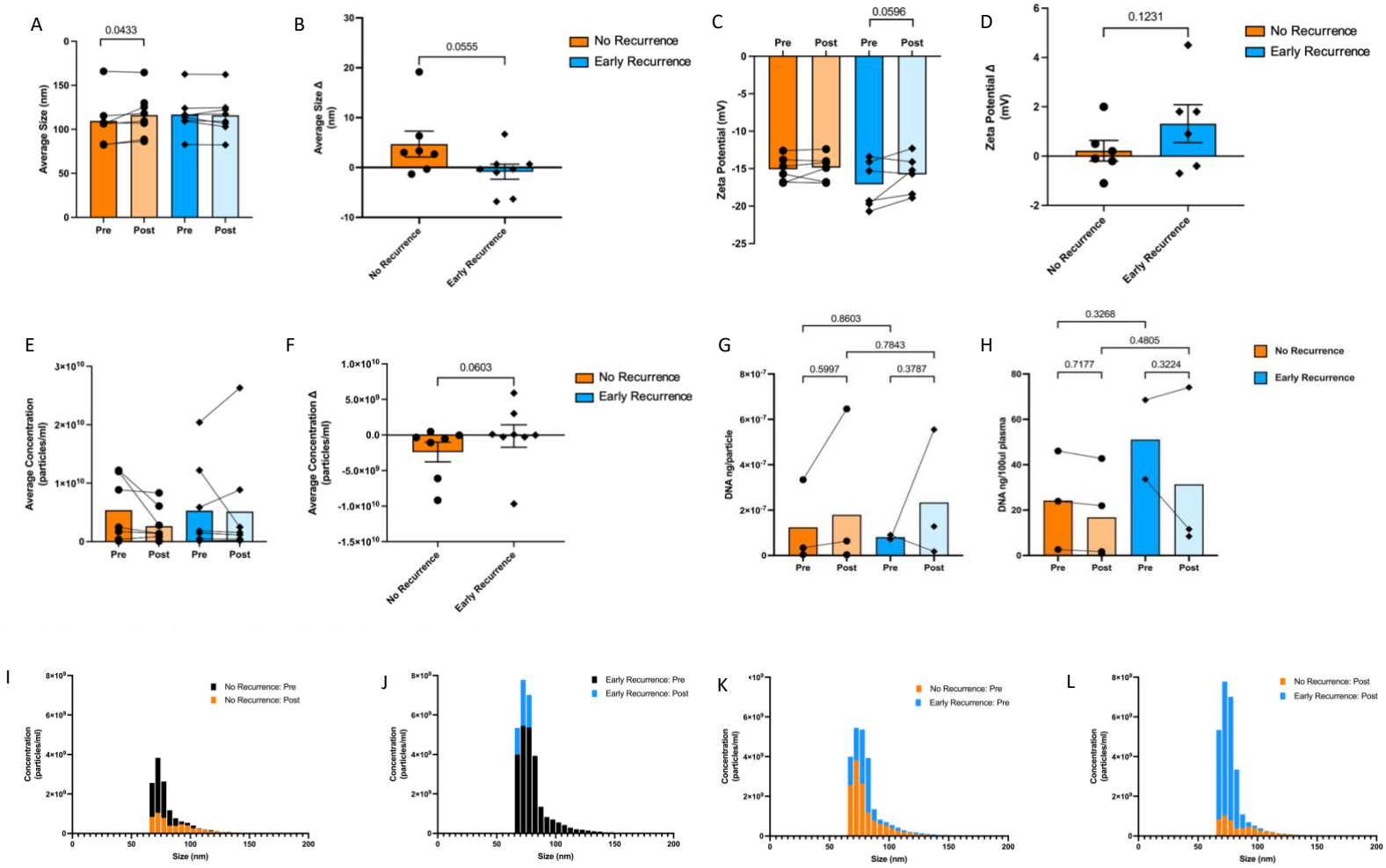

Figure 3: Biophysical characterization of EVs obtained from the sixteen patients outlined in Table 2. Arterial plasma samples collected immediately before (Pre) and following (Post) lung cancer. Our team has performed a pilot EV analysis on a subset of eight patients with early recurrence. These patients were matched to control patients that did not have early recurrence of the primary cancer but are well matched according to age, sex, BMI, smoking history and tumour characteristics. In this small patient population, there is a trend towards patients with early recurrence having higher concentrations of EV particles in the 80 nm size range in the post-surgical time point. **(A)** A main effect for interaction was noted and subsequent post-hoc analysis test demonstrated that small EVs (sEVs) were larger post-surgery by 6.1%, but only in those without recurrence (p=0.0433, n=7-8). **(B)** Mann-Whitney test of the difference between post- and pre-surgery sEV size found sEVs increased in size in no recurrence group post-surgery, with no change in size in patients with early recurrence (p=0.0555, n=7-8). **(C)** Two-way ANOVA of zeta potential (mV) showed no differences between groups or between conditions. Zeta potential increased by 7.8% post-surgery compared to pre-surgery in early recurrence patients (p=0.0596, n=6), which indicates reduced stability of particles. **(D)** Welch's T-test of the delta difference between post- and pre-surgery between the two groups indicated no statistically significant differences in response to surgery (p=0.1231, n=6). **(E)** No statistically significant results were observed between groups or between conditions, though data points clearly illustrate interpersonal variability in response to surgery between groups. **(F)** Mann-Whitney test on the difference between post- and pre-surgery sEV concentration (particles/ml) found sEV concentration decreased post-surgery in no recurrence group, compared to no change in patients with early recurrence (p=0.0603, n=7-8). **(G) (H)** EV concentration and DNA content was observed to significantly increase in early recurrence. Size distributions of small EVs (<200nm) in **(I)** pre- and post-surgery in patients without recurrence, and **(J)** pre- and post-surgery in patients with early recurrence; **(K)** pre-surgery in patients with early recurrence and those without recurrence; and **(L)** post-surgery in patients with early recurrence and those without recurrence.

Non-small cell lung cancer (NSCLC) is the most common form of lung cancer, accounting for 88% of incident cases in Canada[1]. Surgery has been the cornerstone of cure for patients with NSCLC. The vast majority of early-stage lung cancers are operable by minimally invasive techniques resulting in a short hospitalization and a risk profile that has continued to improve over the years[2]. The advantages of surgery for early-stage lung cancer are numerous: definitive diagnosis without risk of sampling error, low local recurrence rates, excellent regional control, and definitive nodal staging with opportunities for adjuvant systemic therapy for node-positive disease[3]. However, the invasive nature of the intervention, need for mechanical ventilation, and pre-existing patient comorbidities may prevent some from being appropriate surgical candidates.

A major contributor to the poor outcomes associated with NSCLC is the rapid progression to metastatic/incurable disease. At the patient level, this represents disease spread to distant organs like the bone, brain, liver, contralateral lung, and adrenal glands via lymphatic and hematogenous spread. The exact molecular mechanisms that drive the transition from local to metastatic disease include many of the known Hallmarks of Cancer[4]. Blood-based surrogate biomarkers have been identified that closely correlate with disease burden and patient prognosis. These markers often measured in venous circulation include extracellular vesicles (EVs), especially exosomes. EVs represent discrete 'packages' containing genetic material (including somatic mutations), protein, and lipids that mirror the composition of the cells from which they arose[5]. EVs differ from each other based on size, site of origin and cargo content, with three main subtypes: exosomes, microvesicles and apoptotic bodies. Tumour-derived EVs promote remodeling of the extracellular matrix which increases tumour cell invasion and motility thereby enhancing metastasis[6]. *The direct effects of acute injury causing inflammation on NSCLC-associated EV generation has not been evaluated.* ***Hence, our study will focus on dynamic intra-patient changes of circulating biomarkers of inflammation, tumour microenvironment and tissue trauma to understand how acute treatment-induced inflammation impacts metastases***.

The established collaboration between several of our team members using a cancer-agnostic model of surgery-associated inflammation has successfully detected changes in inflammatory biomarkers post-surgery[7]. Using a similar systematic approach to specimen collection in lung cancer patients, we expect to find novel inflammatory markers for further experimental validation. While the inflammatory cytokine, EV-DNA, and EV methodology in isolation is not novel, the combined analysis proposed by our team will provide unique insights into the mechanisms linking acute lung injury-induced inflammation and cancer recurrence. Our framework of parallel prospective and retrospective analyses positions our research team well to translate biological insights into appropriately powered prospective observational or randomized interventional clinical trials that will help determine which patients are best suited for specific curative local modality therapies, resulting on optimized patient care and improved treatment.

Our proposed research will advance our preliminary data suggesting that patients with early recurrence have higher concentrations of systemic EVs immediately following surgery. The long-term impact of our research will help select the ideal patients for lung resections and potentially develop strategies to identify and mitigate inflammation and tumour recurrence in patients with early-stage lung cancer. Currently, there is great need for liquid biopsies to allow for non-invasive and early detection of new lung cancer as well as lung cancer recurrence are expensive and require knowledge of the specific tumour mutations in that cancer (i.e. tumour-INFORMED). Our preliminary work suggests that less expensive biomarkers can potentially be used that do not require knowledge specific tumour mutations (i.e. tumour-AGNOSTIC). This has the potential to provide an insight into early detection with affordable blood-based biomarker assay to screen patients for early-stage lung cancer and also to perform surveillance on patients after lung cancer surgery. The ultimate aim is to reduce the burden of lung cancer and optimize patient care.

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Lung cancer is the leading cause of cancer mortality in Canada. The earlier cancer is treated, the better chance of survival. In no other cancer is this more important than lung cancer, where even early-stage disease has 30-50% five-year survival despite improvements in treatment and screening. Surgery and radiation remain the two best curative options for early lung cancer, but what if those treatments actually cause the cancer to spread in some patients? Our team combines the expertise of a lung cancer clinical specialist, thoracic surgeon and biological researcher, with the aim of identifying biomarkers during lung surgery that are predictive of early recurrence in lung cancer patients. Immune-mediated inflammation within the lung micro-environment is associated with lung cancer initiation and metastases. Our translational team will use serial plasma samples from treated cancer patients immediately before and after surgery to measure and correlate circulating extracellular vesicles (EVs) characteristics to patients that have early recurrence of lung cancer. Together, the multidisciplinary team will provide a new understanding of the effect treatment-related inflammation has on patient survival and possible ways to intervene in the future. Currently, there is great need for liquid biopsies to allow for non-invasive and early detection of new lung cancer as well as lung cancer recurrence are expensive and require knowledge of the specific tumour mutations in that cancer (i.e. tumour-INFORMED). Our preliminary work suggests that less expensive biomarkers can potentially be used that do not require knowledge specific tumour mutations (i.e. tumour-AGNOSTIC). This has the potential to provide an insight into early detection with affordable blood-based biomarker assay to screen patients for early stage lung cancer and also to perform surveillance on patients after lung cancer surgery. The ultimate aim is to reduce the burden of lung cancer and optimize patient care.

- We are requesting funds to complete EV analysis on 25 additional patients that have early cancer recurrence within one-year post-operation.
- Two sampling time points will be analyzed (i.e. pre-surgical and post-surgical plasma).
- In parallel, 25 matched controls will be identified and have the same sample series analyzed.
- A total of 100 plasma samples will be analyzed at a cost of $250 / sample time point, costing a total of CAD 25,000.
- Sample costs include extracellular vesicle isolation and characterization, including technical support and laboratory expendables.

Our team combines the expertise of lung cancer clinical specialists and scientists including:

1. Principal Investigator: **Dr. Biniam Kidane**, Thoracic and Foregut Surgeon, Section of Thoracic Surgery. Associate Professor of Surgery, College of Medicine, University of Manitoba.

2. Co-Principal Investigator: **Dr. Ayesha Saleem**, Principal Investigator at the Children's Hospital Research Institute of Manitoba and expert in molecular and cellular physiology, specializing in mitochondrial metabolism and extracellular vesicle (EV) biology. Associate Professor of Kinesiology, Faculty of Kinesiology and Recreation Management.

3. Co-Principal Investigator: **Dr. Shantanu Banerji**, Medical Oncologist at Cancer Care Manitoba, Senior Scientist at the CancerCare Manitoba Research Institute.

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February 27, 2024

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To whom it may concern,

RE: Letter of Support - Lung Ambition Awards– Dr. Biniam Kidane

The Department of Surgery and the University of Manitoba are pleased to provide a letter of support for Dr. Biniam Kidane's application to the 2024 Lung Ambition Awards.

Dr. Kidane's proposed research on identifying serum and liquid (i.e. sputum) biomarkers of early recurrence is an outgrowth of his established and successful translational research program. Over the last 6 years, Dr. Kidane has built a strong multi-disciplinary clinical and translational research program that includes a clinical arm at the Health Sciences Centre and a translational fundamental science arm in the Kidane Lab at the University of Manitoba. This includes close collaborations with fundamental scientists like Dr Shantanu Banerji and Dr. Ayesha Saleem, with whom he has co-developed this current work. Through his research program, Dr. Kidane has demonstrated a proven ability to execute research proposals to completion. He was awarded the Research Manitoba New Investigator Award in 2018 to establish a cohort study of lung cancer surgery patients with biospecimen and clinical data acquisition. He has been able to exceed his projected goals and has now accrued more than 550 patients with complete intra-operative and post-operative biospecimen and clinical data. This patient population will be the basis of this proposed research grant and thus it is clear that the proposed research will be feasible. Furthermore, Dr. Kidane and Dr. Saleem co-supervised a summer medical student who generated pilot data investigating the role of extracellular vesicles in the early detection lung cancer recurrence in a small subset of patients, which demonstrated feasibility of this proposed research and also demonstrated that there is already a mechanistic signal in the pilot study.

In summary, I confirm that this research is feasible in this institution.

Sincerely,

Edward W Buchel, MD, FACS
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Surgery Site Director- Health Sciences Centre
Program Director, University of Manitoba, Microvascular Reconstructive Fellowship