September 25, 2024

Lung Cancer Canada

RE: Letter of Intent – Geoffrey Ogram Memorial Research Grant – PI: Dr. Natasha Leighl

Dear Award Selection Committee,

I wish to submit this research project entitled “Assessment of Novel Liquid Biopsy in ctDNA Lung Detect” for the Geoffrey Ogram Memorial Research Grant. This study will utilise a new technology from Foresight Diagnostics called PhasED-Seq, which is able to identify multiple single nucleotide variants within individual DNA fragments. This results in a higher sensitivity ctDNA assay compared to currently available personalized assays. Through our ongoing study, circulating tumour (ct)DNA Lung Detect (NCT05254782), we have banked tissue and blood samples from early-stage non-small cell lung cancer (NSCLC) patients who have undergone curative-intent surgery. The ctDNA Lung Detect study aims to establish the rate of pre-operative and post-operative ctDNA detection in patients with T1-T4 (T3,T4 multifocal only) node negative NSCLC and the association of ctDNA detection with relapse free survival. To date, we have recruited over 150 patients to this study and ctDNA is detected in 1 in 4 patients before surgery. More sensitive assays are needed to increase the value of this promising tool in clinical practice. Herein, we propose to assess the sensitivity of the novel PhasED-Seq assay in our banked samples from the ctDNA Detect trial. We will focus on samples from patients who have had cancer recurrence but did not have ctDNA detected in our study.

If we can demonstrate the superiority of this novel assay, we plan to incorporate it into our study, enabling more accurate prediction of who needs intensified curative therapy after surgery and who is cured with surgery alone. We will demonstrate the value of intensified therapy for patients through our ctDNA Lung RCT trial (NCT04966663), a randomized study of adjuvant chemo-immunotherapy versus standard of care observation following surgery for patients with stage I NSCLC and plasma ctDNA positivity.

This funding will enable us to improve the current state of the art through validation of novel technology.

Yours truly,

Natasha B. Leighl, MD, MMSc (Clin Epi), FRCPC FASCO

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2. Summary of Proposed Research

Background

Even when lung cancer is diagnosed and treated at its earliest stage, stage I, it remains a challenge to definitively cure. Currently, 20-30% of patients with non-small cell lung cancer (NSCLC) present with stage I disease. Standard treatment includes surgical resection or ablative radiotherapy if surgery is not feasible[1]. Patients with resected node positive disease or larger tumours are offered perioperative chemotherapy with immunotherapy or targeted therapy, while those with smaller node negative tumours undergo surveillance post surgical resection[2,3]. Despite curative surgery, ~20% of patients with resected Stage I NSCLC develop recurrence and die within 5 years[4]. In patients that have undergone curative surgery, the detection of circulating tumour (ct)DNA in plasma post-operatively, termed "minimal residual disease" (MRD), is associated with earlier cancer recurrence. Detection of MRD in plasma between 2-16 weeks after surgery is associated with a ~5-fold higher risk of death and ~15-fold higher risk of recurrence[5,6]. Studies have shown that ~50% of patients treated with surgery that later have cancer recurrence have detectable ctDNA levels in plasma ~5.5 months (median) earlier than evidence of relapse on imaging[7,8,9]. This indicates that patients with early stage lung cancer at risk of recurrence may be identified using ctDNA assays earlier than current detection techniques such as imaging. In particular, tumor-informed or bespoke assays are now commercially available and have a projected 95% lower limit of detection (LOD_{95}) of 1 in 10^{-5}. The establishment of clinical utility of these assays in clinical practice may provide an opportunity to intensify curative treatment for high risk individuals with early stage lung cancer, to improve the chance of cure.

The ctDNA Lung Detect study (NCT05254782, Appendix Figure 1), is a multicentre prospective study at three top tier thoracic surgery centres in the Greater Toronto Area (Princess Margaret Cancer Centre-University Health Network (UHN), Michael Garron Hospital, Unity Health). The primary objective is to establish the rate of perioperative ctDNA detection in patients with T1-T4 (T<4cm, T3, T4 multifocal only) node negative NSCLC and its association with relapse-free survival (RFS). Patients with detectable ctDNA are offered participation in ctDNA Lung RCT (NCT04966663), a randomized trial investigating the benefit of adjuvant chemo-immunotherapy versus standard of care observation in patients with resected stage I NSCLC and perioperative ctDNA detection. Demonstrating the clinical utility of ctDNA testing will lead to a sustainable increase in our ability to personalise treatment, determine prognosis and potentially help cure more patients with early stage lung cancers. We have an established programme evaluating ctDNA to improve our ability to detect and intensify therapy in those at risk for recurrent disease, and avoid toxicity in those cured by surgery alone.

As of April 2024, 231 patients have been screened and 154 had bespoke ctDNA panels successfully constructed. Approximately 1 in 5 patients with clinical stage I NSCLC (22.7%) had ctDNA detected pre-operatively using the tumour-informed, bespoke Inivata Residual Disease and Recurrence (RaDaR) assay[10]. However, the rate of detection in patients with pathologic stage I NSCLC was significantly lower, and no patient with completely resected stage I NSCLC had detection of ctDNA at the post-operative landmark timepoint (3-6 weeks post resection). Detection of ctDNA pre-operatively was associated with a significant increase in the risk of tumour recurrence including in patients with pathologic stage I NSCLC. At a median follow up of 13.9 months, 10 patients have experienced cancer recurrence[10]. Further study data remain embargoed at this time until December 2024.

RaDaR uses whole exome sequencing of resected tumour to generate a custom panel of ~48 tumour-specific variants (median) tested against plasma samples to determine if ctDNA is detectable with a LOD_{95} of 0.0011% variant allele frequency (10^{-5})[6]. Though detection of ctDNA has a high positive predictive value for recurrence, false negative results remain a challenge, i.e. patients who go on to relapse despite a negative ctDNA result, especially in adenocarcinoma cases which are the most common type of NSCLC. A new approach has been developed known as the Phased variant Enrichment Sequencing (PhasED-Seq) assay (Diehn laboratory, Stanford University, USA)[11]. This assay tracks multiple phased variants instead of single nucleotide variants within individual DNA fragments. Initial analyses suggest this approach has >90-fold better LOD_{95} than currently available assays,[12] particularly in adenocarcinoma cases.

In our proposed study, we aim to determine if the multiple phased variant approach with PhasED-Seq is more sensitive and specific than currently available state-of-the art tumour informed or bespoke assays in patients with stage I NSCLC (RaDaR). This comparison will be the first of its kind in the stage I NSCLC population.

Aim: To characterize the ability of the PhasED-Seq assay to detect ctDNA in longitudinal plasma samples in a subset of patients in the ctDNA Lung Detect trial.

Objectives:

1. To determine the rate of pre-operative, 3 to 6 weeks post-operative and 12-month post-operative ctDNA detection in patients with T1-T4 (T3,T4 multifocal only) node-negative NSCLC using a multi-phased variant approach (PhasED-Seq);
2. To compare the detection rate, sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of whole genome sequencing multiple phased variant approach PhasED-Seq to whole exome sequencing single variant approach RaDaR.

Methods

We will analyse banked samples from 50 patients that participated in the ctDNA Lung Detect study using the PhasED-Seq assay (Diehn laboratory, Stanford University, USA; letter of commitment attached).

Study Population: The key eligibility criteria for ctDNA Lung Detect included patients with:

1. T1-T4 (T3,T4 multifocal) N0M0 NSCLC who were planned for complete surgical resection;
2. Tumour size was required to be at least 1 cm (unidimensional) and had at least some solid component on imaging;
3. Prior radiation, surgery or chemotherapy for the current diagnosis of NSCLC was not permitted.
4. Patients with banked samples at all timepoints (pre-op, 3-6 weeks post-op, 1-year post-op) plus banked tissue available will be included into 1 of 4 cohorts (Appendix Table 1).

PhasED-Seq methods: Samples will be sent to the Diehn laboratory or commercialized Foresight Diagnostics laboratory in the USA. Patient-specific phase variants will be identified following whole genome sequencing of tumour and peripheral blood mononuclear cells (for germline DNA)[11]. Subsequently, patient specific polyvariants will be identified by designing custom hybrid capture oligonucleotide pools allowing ctDNA to be assessed. PhasED-Seq decreases background error rates and increases sensitivity by requiring the concordant detection of two or more distinct somatic mutations within a single DNA molecule. PhasED-Seq v1.0 will be used for analysis. Following analysis of samples with PhasED-Seq, ctDNA call for each sample in molecules per mL and eVAF (variant allele frequency) will be compared with RaDaR results. Patients who had cancer recurrence by imaging with detected ctDNA in plasma at any timepoint will be termed “true positive” (TP) cases. Those who recurred without ctDNA detected will be termed “false negative” (FN) cases. Those without ctDNA detected and without recurrence will be termed “true negative” (TN) cases and those that had detectable ctDNA but no clinical evidence of recurrence will be “false positive” (FP) cases (Appendix Table 1). Samples from the 10 patients who experienced cancer recurrence will be included in this study and we will randomly select 40 cases of patients without recurrence using a computer-generated random selection algorithm. From this, detection rates, PPV, NPV, SN, and SP will be calculated. The external Diehn lab will be blinded to the disease status of each patient.

Statistics: ctDNA detection rates at the pre-operative, post-operative landmark and 12-month follow-up will be calculated, and their associated Clopper-Pearson exact 95% confidence interval (CI) will be provided. SN and SP will be calculated per definition, together with their associated 95% CI. PPV and NPV will be adjusted for the prevalence of cancer recurrence in this study population. ctDNA detection based on the whole genome sequencing multiple phased variant approach PhasED-Seq vs. those based on the RaDaR whole exome sequencing single variant approach will be cross-tabulated and compared using the McNemar’s test.

3. Impact Statement

This project holds great potential to demonstrate the pivotal role that liquid biopsy plays in early stage lung cancer patient outcomes. The standard of care for this patient population following surgery is observation, but recurrence remains a challenge for 20% of these patients. Trials which specifically target stage 1 NSCLC remain sparse, with our ongoing studies, ctDNA Lung Detect (NCT05254782) and ctDNA Lung RCT (NCT04966663), aiming to address this key population with unmet need. If we are able to predict who will recur before imaging in ctDNA Lung Detect, we will be able to offer them escalated potentially beneficial treatment with ctDNA Lung RCT. In our study, 10 patients had cancer recurrence (median time 13.9 months) and four of these tested negative for ctDNA[10]. This shows us that our assay is not sensitive enough. Novel assays have emerged that present new opportunities to advance the clinical utility of this technology. Notably, PhasED-Seq developed by the Stanford University group has demonstrated superiority in detecting ctDNA in early stage NSCLC suggesting feasibility of this application[12] using its unique multivariant approach compared to single variants. Our invaluable repository of banked patient samples from our larger established study ctDNA Lung Detect will assess whether this assay will outperform previous assays by detecting ctDNA in previously negative ctDNA samples (false negatives).

By demonstrating the ability of newer technologies to better select patients at risk and for treatment intensification, we believe this project will strengthen our potentially practice changing studies ctDNA Lung Detect and RCT. Specifically, improving our ability to identify patients that are not cured by surgery and that may benefit from intensified adjuvant therapy may reduce lung cancer mortality and improve morbidity and quality of life. In addition, those who are unlikely to benefit are spared treatment. Lastly, it is hoped early intensification will improve cure rates of early stage lung cancer, preventing the need for treatment at advanced stages, and improving patient outcomes. Analysis of these samples in the short term, allows amendments of the clinical studies in the medium term, which we hope will allow identification of high-risk individuals who can be treated before relapse. We hope this will improve cure, reduce morbidity, optimise patient care and patient treatment for long-term impact.

We will disseminate our findings in a high impact journal, at leading thoracic oncology conferences both nationally and internationally, as well as community-based events led by the Princess Margaret Cancer Foundation and events requested by Lung Cancer Canada. This analysis of patient samples has the potential to improve our ability to detect MRD and patients at risk of relapse. The validation and incorporation of this novel technology will help us demonstrate the benefit of intensified adjuvant therapy (through ctDNA Lung RCT) in those at risk of lung cancer relapse.

4. Public, non-scientific summary

Lung cancer causes the highest number of cancer deaths in Canada and globally. In Canada, we are able to detect lung cancers at an early stage and offer curative surgery. Despite standard of care, 1 in 3 patients' cancers return. Currently, we have no reliable way to predict whose cancer will return. Scientists have developed a blood test, known as a liquid biopsy, which detects small amounts of tumour genetic material (DNA) in the bloodstream, known as circulating tumour DNA (ctDNA). When ctDNA is detected in the blood, research suggests that these patients are more likely to have their cancers return than those who test negative.

We have an ongoing study to look at whether ctDNA can be detected in patients with early stage lung cancer that are planned for curative surgery, and if patients have ctDNA detected in their bloodstream, whether they would benefit from additional treatment after surgery (chemotherapy and immune therapy). Currently we estimate that we are missing approximately half of patients that may need further treatment, and we need a more sensitive assay. New technologies have been developed such as PhasED-Seq which is over 90 times more able to detect very low levels of ctDNA than the current leading method. This assay has been developed by a team at Stanford University, USA. We will try this approach in our patient blood samples that are banked to see if we can detect ctDNA where we were not previously able to detect ctDNA. We will try this in patients where ctDNA is detectable 12 months following surgery, and in those where ctDNA was not detected in any sample. Particular samples of interest include patients who never had detectable ctDNA but had their cancer return, suggesting that the current test may not be perfect.

If we are able to detect ctDNA in our group of patients' samples, it suggests that these patients would be eligible for treatment whereas previously they were not. Should this approach be successful, we hope to use this test for the remainder of the clinical trial. Our overall aim is to help identify patients who are at high risk of their lung cancers returning after surgery and offer chemotherapy and immunotherapy to them, thereby increasing cure rates, whilst sparing those who do not need it. Ultimately, this means we are able to cure more patients with lung cancer and reduce toxicity in those who do not need treatment, thus improving quality of life.

5. Budget

The funding received from the Ogram grant will be put toward a subset of 50 patients (Appendix Table 1). The Ontario Institute for Cancer Research (OICR) will cover the medical oncology fellow. This portion of the project is not covered by these sources. Please see Appendix Figure 2 for timeline of activity.

Detailed Budget	Source of Funding	Cost/Unit	Number of patients	$ (CAD)	Justification
PERSONNEL:					
Medical Oncology Fellow	OICR	-	-		Lead on study - PMH will cover
Senior Biostatistician		salary 125000; benefits 30000; 0.02 FTE		$ 3,100.00	Data analysis
Project Manager		salary 87000; benefits 20880; 0.05 FTE		$ 5,394.00	Coordination of sample manifest and data analysis
SERVICES:					
Princess Margaret Cancer Biobank	GOMRG	22.05	per timepoint per patient (preop, postop, 1yr postop)	$ 6,615.00	Sample retrieval fee (plasma and buffy coat)
	GOMRG	20	per shipment	$ 20.00	Tissue slides shipment preparation (ambient); batch shipment
	GOMRG	50	per shipment	$ 50.00	Plasma/buffy coat frozen shipment preparation; Batch shipment
Pathology Clinical Research Program - PRP Lab	GOMRG	169.76		$ 8,488.00	Slide creation required from tissue blocks
	GOMRG	900		$ 900.00	Batch shipment through courier
Diehn lab assay		in kind	50		Assay and analysis costs
TOTAL					**$24,567**

The Princess Margaret Cancer Centre (PMCC):
The PMCC Thoracic Oncology Group has the largest comprehensive thoracic oncology program in Canada and is an internationally recognized centre of excellence for clinical care, research, and teaching in thoracic oncology. Dr. Natasha Leighl is Site Lead of the Medical Oncology Thoracic Group at PMCC and Professor of Medicine at the University of Toronto. The research team under the supervision of Dr. Leighl includes senior biostatistician, Lisa Le, MSc, and project manager, Mary Rabey, BMBS. Ms. Le will provide statistical support and analysis for this study. Dr. Rabey will manage project activities including obtaining all institutional approvals, monitoring, and reporting key metric progress pertaining to objectives and milestones for this study.

Princess Margaret Cancer Biobank (PMCB):
For over 20 years, PMCB has been a large-scale repository for human biospecimens ranging from blood components to formalin fixed paraffin-embedded tissue and associated clinical data. They provide a quality service of ethical collection, processing, annotation, storage, and distribution of patient samples.

UHN Pathology Laboratory:
The Pathology Lab, a part of UHN's larger Laboratory Medicine Program, processes tissue and performs immunohistochemistry.

Diehn Laboratory
This laboratory was founded in 2020 by Dr. Max Diehn, Stanford, to develop high-performing ctDNA assays by improving LOD95, accuracy, and MRD measurement. Their team have established an automated and scalable CLIA-registered lab in Boulder, CO, USA.

6. Names of investigators

PI: Dr. Natasha Leighl – please see separate uploaded CCV.

7. Statement of support

Dr. Amit Oza, Department of Medical Oncology Hematology Head – please see attached.

Dr. Max Diehn, Stanford University-Diehn Laboratory/Foresight Diagnostics – please see attached.

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September 19, 2024

Lung Cancer Canada

RE: Statement of Support for Dr. Natasha Leighl - Geoffrey Ogram Memorial Research Grant (GOMRG)

Dear Award Review Committee,

I am pleased to write this letter in support of Dr. Natasha Leighl's application for the Lung Cancer Canada award entitled "The Geoffrey Ogram Memorial Research Grant." Dr. Leighl is the Lead Thoracic Medical Oncologist of the Lung Cancer Group which is comprised of research experts in the field, Drs. Frances Shepherd, Adrian Sacher, Geoffrey Liu, Penny Bradbury, and Lawson Eng. Since joining our faculty as a member of the Division of Medical Oncology and Hematology at the Princess Margaret Cancer Centre (PMCC) in 2017, she has mentored several fellows in both research and clinical practice including the lead fellows of this project. I am confident this proposal is aligned with the Lung Cancer Group's dedication to innovation for better patient experiences and outcomes.

Dr. Leighl's group is currently conducting a clinical trial for early stage lung cancer patients who have had surgical resection of their tumours and their risk of recurrence. They will use a novel circulating tumour DNA (ctDNA) minimal residual disease detection (MRD) method, PhasED-Seq™ to identify those who are at high risk of relapse. This highly sensitive assay has shown promise in detecting ctDNA in those with early stage lung cancers. Dr. Leighl's work will be comparing a variety of liquid biopsy approaches to further our ability to accurately detect ctDNA in non-small cell lung cancer patients. The presence of MRD suggests heightened risk of recurrence. The team will subsequently aim to intervene with therapeutic regimens to improve outcomes. This study greatly reflects Geoffrey Ogram's legacy of advancing early detection methods and making personalized medicine commonplace.

I have seen the productivity of Dr. Leighl's team ranging from liquid biopsies to real world evidence such as turnaround times for patients. I believe the financial support that this grant can provide would positively impact lung cancer patients' lives. On behalf of the Princess Margaret Cancer Centre, I confirm the proposed research is feasible at PMCC.

Yours sincerely,

Amit M. Oza, BSc, MD, MBBS, FRCPC

Stanford MEDICINE

Department of Radiation Oncology
Division of Medical Physics
Division of Radiation Therapy
Division of Radiation and Cancer Biology

September 23, 2024

Geoffrey Ogram Memorial Research Grant (GOMRG)

GOMRG Committee
Lung Cancer Canada
133 Richmond St. W., Suite 208
Toronto, ON, Canada M5H 2L3

Dear Committee Members,

I am pleased to write this letter in commitment to work collaboratively with Dr. Natasha Leighl and team at the Princess Margaret Cancer Centre (PMCC) on the project detailed in their application to “The Geoff Ogram Memorial Research Grant.”

Dr. Leighl’s work in the field of clinical applications of ctDNA in the management of early-stage non-small cell lung cancer (NSCLC) closely aligns with my research interests in genomics-based biomarkers for personalized medicine. We will be using an assay developed by our group known PhasED-Seq™ or Clarity™ which is an ultra-sensitive and specific method for detection of ctDNA and minimal residual disease (MRD). This will be used to analyze specimens from Dr. Leighl’s investigator-initiated trial ctDNA Lung Detect at PMCC.

Detection of MRD in patients with resected NSCLC remains challenging and it is important to compare the performance of different assays. We have previously demonstrated increased ctDNA detection rates using our PhasED-Seq assay in lung cancer. Analyzing samples retrospectively from Leighl’s cohort will allow us to assess the prognostic and predictive impact of our technology. This will be critical as we move towards personalizing care of early-stage lung cancers. Further, accurate assessment of MRD will empower oncologists and patients to make informed decisions about additional treatment strategies. The ctDNA Lung Detect cohort is a wealth of clinical data and biospecimens that will help advance the field of liquid biopsy in early-stage cancers.

I give my commitment to collaborate with the team at PMCC. With the collective expertise between our groups at PMCC and Stanford, this will be a fruitful collaboration that will expand our ability to implement ctDNA as a biomarker in the management of patients with early-stage lung cancers with the goal to cure even more patients.

Sincerely,

Maximilian Diehn, MD PhD
Jack, Lulu, and Sam Willson Professor
Division Chief of Radiation and Cancer Biology
Vice Chair of Research
Department of Radiation Oncology
Stanford University School of Medicine

Appendix

Figure 1. ctDNA Lung Detect/RCT schema.

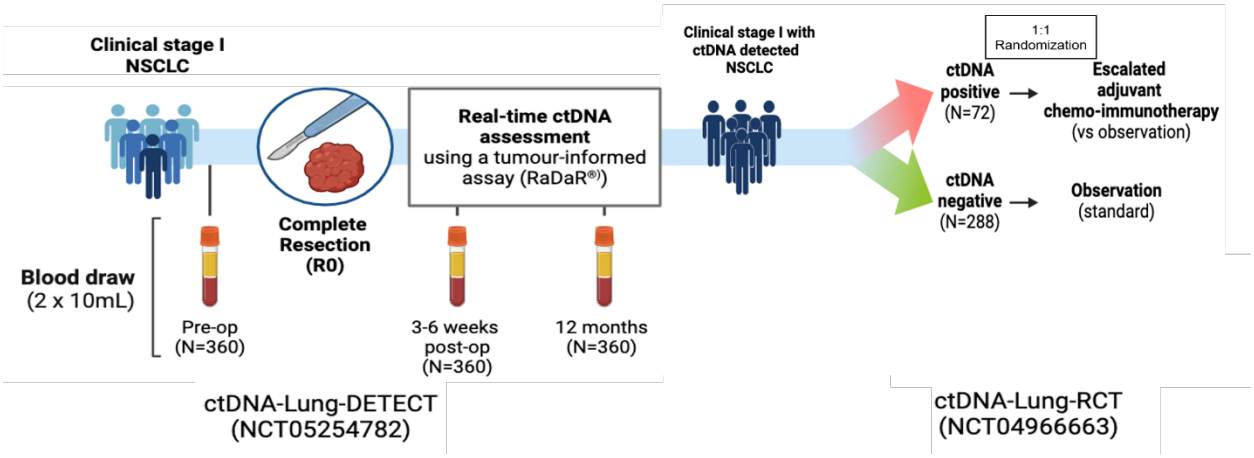
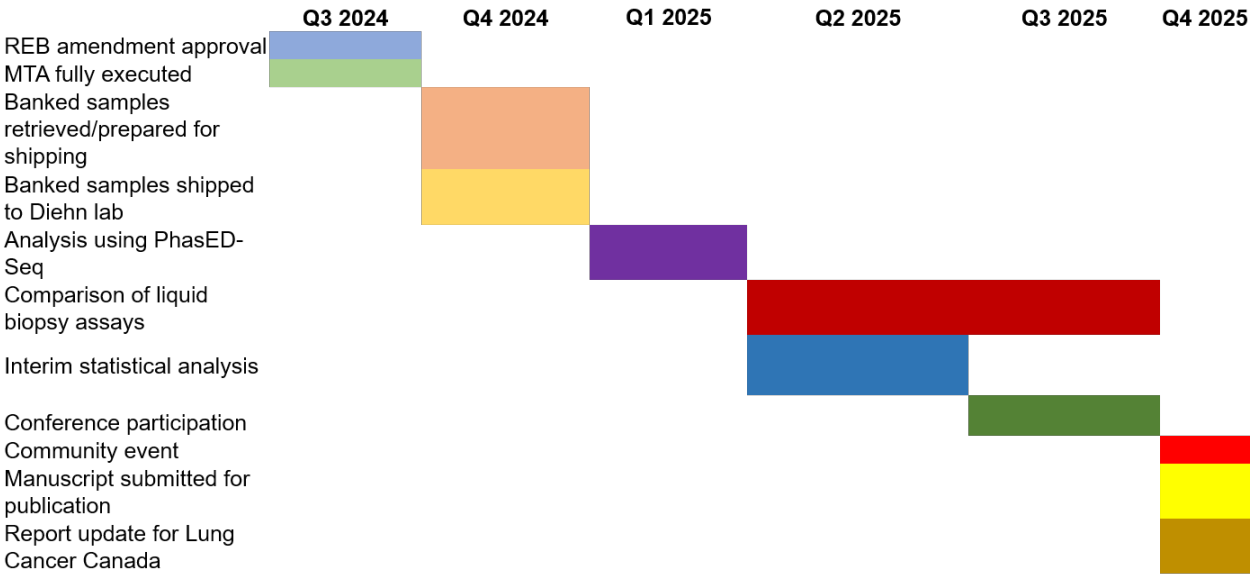


Table 1. Assessment of sensitivity, specificity, positive and negative predictive value.

ctDNA Status (Pre-op or Post-op)	Recurrence	No recurrence by 12 months
+	True positive	False positive
-	False negative	True negative
Total	10 patients	40 patients*

*to be randomly selected from total patients without recurrence at 12 months in the study.

Figure 2. Timeline of study over 12 months.



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