Letter of Intent

Dear Research Committee,

I am writing to apply for the Give A Breath Research Award for our project aimed at developing a clinically-relevant platform to optimize ablation protocols for advanced lung cancer patients. Despite recent advances in lung cancer treatment, patients with stage III/IV disease who progress beyond first-line therapy face limited options, and while cryoablation shows promise as a local therapy option, particularly when combined with immunotherapy, we currently lack robust pre-clinical models to optimize treatment protocols.

Our team is developing a three-dimensional hydrogel-based tumor surrogate system, aimed at testing ablation and immunotherapy combinations; specifically, cryoablation. Our preliminary work includes a CHEST-submitted meta-analysis of cryoablation protocols, cellular mapping of 18 chemonaive lung tumors, establishment of a 3D cell culture platform, and an approved protocol for mechanical analysis of fresh tumor specimens.

We propose to:

1. Develop a biomimetic 3D tumor model that accurately reflects the physical and biological properties of human lung adenocarcinoma
2. Create high-resolution 3D thermal maps of different cryoablation protocols, correlating temperature gradients with cell death patterns
3. Evaluate the immunogenic potential of various cryoablation protocols to inform future combination therapy approaches

This work directly addresses the needs of patients beyond first-line therapy by establishing a platform to systematically optimize and potentially personalize cryoablation protocols. The translational nature of our research aims to impact patient care through evidence-based refinement of cryoablation approaches.

Our technical approach will involve optimizing gelatin methacrylate and alginate-based hydrogel matrices to match the mechanical and thermal properties of human lung tumors. We will systematically characterize the thermophysical and biomechanical properties (including stiffness, density, thermal conductivity) using patient-derived specimens as reference standards. The cellular component will incorporate both cancer cells (A549) and stromal elements (MRC5 fibroblasts) at ratios informed by our pathological analyses of human specimens.

The platform will enable precise mapping of temperature gradients and live cell imaging during freeze-thaw cycles, allowing correlation between thermal parameters and biological outcomes. This systematic approach will help identify optimally immunogenic and cytotoxic cryoablation protocols.

The Give A Breath Research Award would enable us to accelerate the development of this platform and advance more effective therapeutic strategies for those beyond first-line therapy.

Sincerely,

Dr. Moishe Liberman, MD, PhD

Project Description

Research Summary: Physically Relevant Cell-Laden Hydrogels for Cryoablation and Immunotherapy Combinations

Lung cancer remains the leading cause of cancer-related deaths worldwide, with patients diagnosed with stage III/IV disease who progress beyond first-line therapy facing particularly limited treatment options. While recent advances in immunotherapy, particularly PD-1/PD-L1 checkpoint inhibitors, have shown promise, approximately 60-70% of patients with advanced NSCLC either fail to respond or develop resistance to first-line immunotherapy, highlighting the critical need for more effective combination approaches for this patient population. Cryoablation (CA) has emerged as a promising local therapy option, particularly for patients with oligometastatic disease or those unsuitable for surgical resection. When combined with immunotherapy, CA demonstrates potential synergistic effects through both the abscopal effect and the release of tumor-associated antigens (TAAs) and damage-associated molecular patterns (DAMPs). This immunomodulatory effect is temperature-dependent, with optimal cryoactivation occurring in the -20°C to -40°C range, where sub-lethal freezing induces immunogenic cell death rather than immediate necrosis seen at lower temperatures. However, the development and optimization of these combination approaches have been hampered by the lack of suitable pre-clinical models that accurately recapitulate both the physical properties and microenvironment of human lung tumors. Current *in vitro* models fail to capture the complex interplay between mechanical properties, thermal gradients, and cellular responses that characterize clinical CA procedures, significantly impeding our ability to optimize treatment protocols for patients who have progressed beyond first-line therapy.

Our team has established a strong foundation for this research through several key accomplishments. We have completed a comprehensive meta-analysis of cryoablation protocols (currently under review at CHEST), synthesizing current clinical practices and identifying key parameters for optimization (see **Figure 1**). Additionally, we have conducted detailed cellular mapping of 18 chemonaive lung tumors (see **Figure 2**), providing critical insights into tumor composition and cellular distributions. This work has been complemented by the development and validation of initial 3D cell culture protocols for lung cancer cells (see **Figure 3**), along with an approved protocol for analyzing mechanical properties of fresh tumor specimens. These preliminary studies have provided crucial baseline data for the development of our biomimetic tumor model and have informed our approach to protocol optimization.

The proposed research follows a systematic three-step approach. **First**, over the first quarter of 2025, we will conduct comprehensive characterization of thermophysical and biomechanical properties from 20 resected human lung adenocarcinoma specimens. Our analysis will establish baseline parameters including tissue stiffness (Young's modulus range expected 2-20 kPa), compressive strength, density (typically 1.02-1.07 g/cm³), elasticity, and thermal conductivity (ranging 0.45-0.55 W/m·K). These parameters vary significantly between tumor core and peripheral regions, necessitating spatial mapping for accurate model development. **Secondly**, over the following 2 months, using this data as our target parameters, we will tailor our three-dimensional hydrogel matrix system utilizing gelatin methacrylate and alginate as extra-cellular matrix surrogates. These materials were selected for their biocompatibility, cost-effectiveness, and

highly tunable properties. Through iterative optimization of synthesis protocols and concentrations, including strategic integration of polyethylene glycol diacrylate (PEG-DA), we will create matrices that precisely match the patient-derived parameter values. **Finally**, in the last third of 2025, we will subject these biomimetic matrices to various cryoablation protocols, enabling systematic evaluation of treatment parameters in a physically relevant model system.

Building on this foundation, we will create high-resolution 3D thermal maps of various cryoablation protocols and correlate temperature gradients with biological outcomes. Thermal mapping will employ an array of 22-gauge thermocouples placed in a three-dimensional grid pattern, with particular focus on the critical isotherms (-20°C and -40°C) that define the ablation zones. The biological response assessment will integrate A549 lung adenocarcinoma cells and MRC5 lung fibroblasts at clinically relevant ratios, enabling monitoring of cell viability during cryoablation procedures. Cell viability monitoring will utilize a combination of calcein-AM and propidium iodide staining, enabling real-time assessment of both immediate and delayed cell death patterns. Additionally, we will monitor key hypoxia markers (HIF-1α) and stress response proteins (HSP70, HSP90) during the freeze-thaw cycles.

The final phase will evaluate the immunogenic potential of optimized cryoablation protocols and their combination with immunotherapy. We will characterize immunogenic responses through analysis of damage-associated molecular pattern expression and quantification of immunogenic cell death markers such as calreticulin and HMGB1. This will be complemented by assessment of immune cell infiltration and activation. The optimization of combination therapy will involve evaluation of synergistic effects with immune checkpoint inhibitors, analysis of treatment timing and sequencing, and development of standardized protocols for combination approaches.

This research will deliver three key outcomes in 2025: **(1)** by July, a validated biomimetic 3D tumor model for pre-clinical testing of combined ablation and immunotherapy platforms; **(2)** by October, optimized cryoablation protocols ready for clinical implementation planning; and **(3)** by December, a first attempt at testing combined approaches with immunotherapy in a *in vitro* 3D platform. These deliverables are designed for rapid clinical translation, with preparation for a pilot clinical trial to begin by mid 2026. The platform will provide immediate translational value for patients with advanced lung cancer who have progressed beyond first-line therapy, particularly benefiting those with oligometastatic disease or who are unsuitable for surgical intervention.

See appendix for cited figures.

Appendix

	Including all studies				*Excluding high risk of bias studies*			
Outcome	**Number of studies or subgroups**	**Number of nodules**	**Odds Ratio (95% CI)**	**P**	**Number of studies or subgroups**	**Number of nodules**	**Odds Ratio (95% CI)**	**P**
Size	24	1022	0.29 (0.16 -0.56)	0.0006*	18	611	0.33 (0.16 – 0.69)	0.0057*
Number of cycles	25	1048	2.81 (1.33 -5.95)	0.0091*	19	637	3.70 (1.68 – 8.14)	0.0027*
Margin of ice ball	25	1048	1.03 (0.94 - 1.14)	0.4219	19	637	1.06 (0.95 – 1.17)	0.3036
First freeze duration	25	1048	0.89 (0.84 - 0.96)	0.0029*	19	637	0.89 (0.83 – 0.95)	0.0013*
Second freeze duration	25	1048	0.86 (0.73 - 1.00)	0.0557	19	637	0.84 (0.71 – 1.00)	0.0461*
Third freeze duration	20	873	1.11 (1.02 - 1.21)	0.0133*	14	462	1.15 (1.05 – 1.26)	0.0044*
First thaw duration	21	860	0.86 (0.74 - 1.00)	0.2963	17	583	0.85 (0.69 – 1.06)	0.0461*
Second thaw duration	20	812	1.11 (0.96 - 1.29)	0.1524	16	535	1.15 (0.98 – 1.35)	0.784
Third thaw duration	13	538	1.13 (0.89 - 1.43)	0.2842	9	261	1.11(0.83 – 1.48)	0.4442
Cancer type								
Primary	9	326	1.33 (0.46 -3.87)	0.5950	7	172	1.48 (0.40 – 5.55)	0.5600
Metastasis	8	370			6	200		
Type of first thaw								
Passive	16	641	0.67 (0.22 -2.00)	0.4680	14	473	0.84 (0.22 – 3.15)	0.7940
Active	3	111			2	61		
Final thawing								
Yes	17	817	2.18 (1.03- 4.62)	0.0414*	12	501	2.67 (1.16 – 6.13)	0.0211*
No	8	231			7	136		
Type of final thaw								
Passive	1	33	0.89 (0.29 -2.76)	0.8370	1	33	1.04 (0.34 – 3.21)	0.9440
Active	13	610			10	432		

Figure 1 - Univariate Analysis of the Factors Affecting the Local Control Rate at 1 Year of Cryoablation in Patients with Lung Malignancies including all studies. Sarshoghi, A., Sarshoghi, A., Tetu, M., Ng, C.S.H., Yarmus, L., Bourgouin, P., Solomon, S.B., Herth, F., Liddell, R.P., Liberman, M., "Cryoablation Algorithms for Lung Tumors: A Systematic Review and Meta-Analysis Evaluating Efficacy and Safety of Proposed Protocols for Non-Operative Pulmonary Cryosurgery," under review at CHEST Pulmonary (2025).*

Appendix

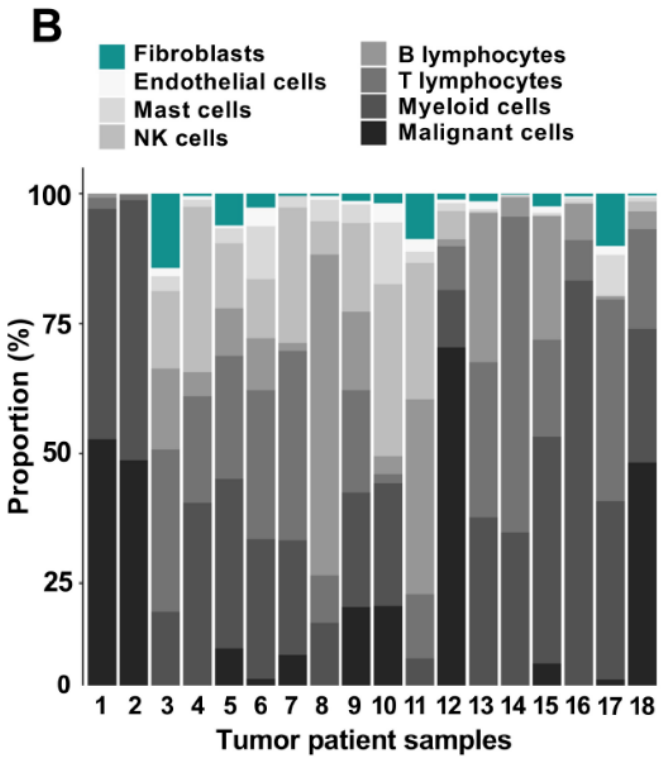

Figure 2 - Patient samples (n=18) proportion of normal vs malignant cells.

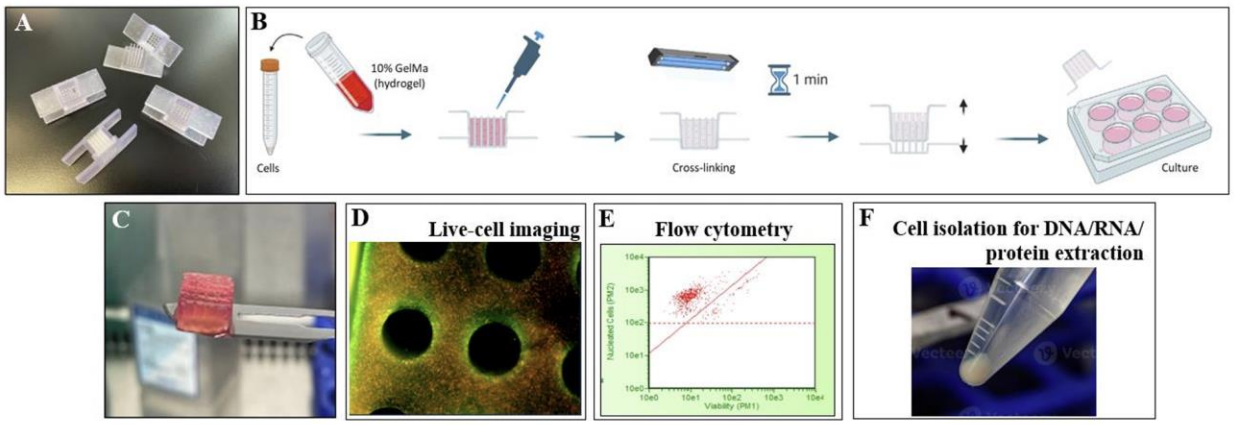

Figure 3 - Illustration of the Cuboid system. A) The Cuboid is a scaffold composed of two parts with 25 pillars to create a capillary system. B) Schematic procedure to produce 3D culture of 1 cm3 tumor using photo-crosslinking and custom-made hydrogel. A simple detachment of scaffold from the mold and removal of the molded hydrogel to be culture in a 6 well-plate. C) Image showing hydrogel structure. D) Images of lung cancer cells and fibroblasts in Cuboid. E) Flow cytometry for cell viability and F) cell isolation for DNA/RNA/protein downstream analysis.

Impact Statement

The development of our biomimetic tumor platform represents **a transformative advance in the treatment landscape**. Cryoablation technology holds exceptional promise as a minimally invasive alternative to surgery, offering distinct advantages over other ablative modalities: the largest ablation radius, minimal bleeding risk, and unique tissue-preservation properties. These mechanical benefits, combined with cryoablation's demonstrated immunomodulatory effects, position it as a particularly promising option for patients who have exhausted standard treatments.

The immunological potential of cryoablation stems from its unique ability to induce immunogenic cell death through the release of damage-associated molecular patterns (DAMPs) and tumor-associated antigens (TAAs). This cryoimmunological effect, particularly prominent in the **-20°C to -40°C temperature range**, can potentially convert local tumor destruction into a systemic anti-tumor immune response, addressing both local and distant disease in stage III/IV patients. However, current pre-clinical models fail to capture the complex thermal and mechanical dynamics of human cryoablation procedures. Existing platforms, such as tumor-on-chip systems exposed to refrigeration, cannot replicate the rapid temperature changes and thermal gradients generated by clinical cryoprobes, nor can they account for the thermal load of surrounding tissue that significantly influences treatment outcomes.

Our platform addresses these limitations by providing**, for the first time, a physically and biologically accurate model** system that enables precise correlation between thermal parameters and biological outcomes. By incorporating both the mechanical properties of lung tissue and the thermal dynamics of clinical cryoablation procedures, our system enables realistic evaluation of treatment protocols that simply cannot be achieved with current in vitro models. This capability will **accelerate the development of evidence-based treatment protocols, drastically** reducing the number of clinical trials needed to establish optimal combinations.

Looking ahead, our platform holds transformative potential for several key areas. First, it provides a foundation for developing **personalized treatment approaches**, allowing clinicians to test patient-specific responses before treatment. Second, it enables more efficient development of endobronchial cryoablation applications - our model's unique ability to be implanted into large animal models while maintaining human-tissue properties addresses a critical gap in the current pre-clinical testing pipeline, where the lack of reliable tumor models in porcine lungs has hindered the development of **bronchoscopic ablation techniques**. Third, it creates a standardized framework for evaluating next-generation immunotherapies in combination with cryoablation, **accelerating the development pipeline for novel treatments**.

In the short term **(2025-2026)**, we anticipate delivering optimized **cryoablation protocols ready for clinical implementation**. In the medium term **(2026-2027)**, our platform will facilitate rapid **evaluation of combination therapies, potentially leading to new treatment strategies** for patients who have exhausted standard options. By providing this crucial translational bridge between laboratory findings and clinical application, our work promises to significantly reduce the burden of advanced lung cancer while improving both survival outcomes and quality of life for patients beyond first-line therapy.

Public – Non Scientific – Statement

When lung cancer spreads or returns after initial treatment, patients often face limited options. Traditional surgery may no longer be possible, and while newer treatments like immunotherapy show promise, many patients either don't respond or develop resistance over time. Our research aims to develop better treatment options for these patients by combining two promising approaches: cryoablation (a freezing technique that destroys cancer cells) and immunotherapy (which helps the body's immune system fight cancer).

Cryoablation works by inserting a special probe that freezes and destroys cancer tissue while largely sparing healthy lung tissue. What makes this approach particularly interesting is that the freezing process can also "wake up" the immune system, potentially helping it recognize and fight cancer cells throughout the body. This effect could be especially powerful when combined with immunotherapy drugs, but finding the right way to combine these treatments has been challenging because we lack good ways to test them in the laboratory.

Our team is developing a new testing platform that recreates the conditions of human lung cancer more accurately than ever before. We're creating a special gel-based material that mimics the physical properties of lung tissue, complete with cancer cells and other important cell types found in real tumors. This "artificial tumor" allows us to test different freezing patterns and combinations with immunotherapy drugs in a way that closely matches what happens in actual patients.

The impact of this research could be significant for several reasons. First, it will help doctors determine the best freezing patterns to not only destroy the tumor but also stimulate the strongest immune response. Second, it will allow us to test various combinations of cryoablation and immunotherapy to find the most effective treatment schedules. Finally, our platform could speed up the development of new treatment combinations by providing a more reliable way to test them before moving to clinical trials.

We expect this research to yield practical benefits for patients within the next two years. Our goal is to develop optimized treatment protocols that doctors can use to provide better outcomes for patients who have limited treatment options. By improving our ability to combine these treatments effectively, we hope to offer new hope to patients with advanced lung cancer while reducing the physical and emotional burden of their treatment journey.

Budget Justification

Personnel ($8,500):

- Medical and master's Student (Primary Researcher) - $8,500 (100% time commitment) Lead investigator responsible for experimental design, protocol optimization, and data analysis. Brings expertise in cell culture and biomaterials engineering.
- Medical and PhD Student (Auxiliary) – covered by other funding (5% time commitment) Supports surgical modelling, for clinical implementation.
- Engineering Student Assistant – covered by other funding (20% time commitment) Supports mechanical testing and mathematical modeling for thermal gradient prediction.

Cell Culture and Biological Materials ($3,000):

- Culture media and supplements: $2,200
- Culture consumables: $200 (Additional supplies needed for extended testing phases)
- Collagen binding materials: $600 (Includes various ECM protein options)

Analytical Reagents and Imaging ($3,200):

- DMSO cytotoxicity kit: $600
- Caspase-3/7 reagents: $800
- Annexin V staining: $800
- Incucyte imaging time: $1,000

Bioengineering Materials ($10,300):

- 3D mold fabrication: $2,000 (Including multiple iterations for optimization)
- Hydrogels and matrices (GEL-MA, PEG-MA, alginate): $2,500
- Temperature monitoring equipment (thermocouples and logger): $4,000
- Compressive force testing rig: $1,800

Total Budget: $25,000

See CCV's of PI and CO-PI's attached on the LCC platform.

Principal Investigator: Dr. Moishe Liberman, MD, PhD, Université de Montréal Clinical Professor of Surgery, Université de Montréal Marcel et Rollande Chair of Thoracic Surgical Oncology, CRCHUM
Email: moishe.liberman@umontreal.ca

Dr. Liberman brings extensive clinical expertise in thoracic surgical oncology and leadership in developing minimally invasive therapeutic approaches for lung cancer.

Co-Principal Investigators:

Dr. Noël J-M Raynal, PhD, Associate Professor, Université de Montréal Full Professor in the Department of Pharmacology and Physiology, Hôpital Sainte-Justine
Email: noel.raynal@umontreal.ca

Dr. Raynal's expertise in tridimensionnal cellular biology will be crucial for characterizing the immunological responses to cryoablation and developing optimal combination strategies with immunotherapeutic agents.

Dr. Thomas Gervais, PhD, Université de Montréal Full Professor in the Department of Engineering Physics, Polytechnique Montréal, CRCHUM
Email: thomas.gervais@polymtl.ca

Dr. Gervais's background in engineering physics and expertise in microfluidics and biomechanical systems will be essential for developing and characterizing the physical properties of the hydrogel-based tumor model.

Key Collaborators:

Dr. Charles Leduc, MD, Université de Montréal Assistant Clinical Professor in the department of pathology, CHUM Chief of Lung Pathology, CRCHUM
Email: charles.leduc.chum@ssss.gouv.qc.ca

Dr. Leduc's expertise in lung pathology will be crucial for validating the biological relevance of our tumor model and ensuring accurate representation of tumor microenvironment characteristics.

Arman Sarshoghi, MD-MSc Student at Université de Montréal, Technology, Innovation and Development Lab, CRCHUM
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Arman Jafari, PhD Candidate at Université de Montréal, Savoji Lab, Hôpital Sainte-Justine
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This multidisciplinary team combines expertise in thoracic surgery, cancer biology, engineering, pathology, and translational research, ensuring comprehensive coverage of all aspects of the proposed research program. The collaboration between clinical and basic science investigators will facilitate rapid translation of findings into clinical application.

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

Université de Montréal

January 7, 2025

Research Committee
Lung Cancer Canada
133 Richmond Street West, Suite 208
Toronto, Ontario M5H 2L3

OBJECT: Institutional support for Dr Liberman application to the Give a Breath Research Award

Dear Committee Members,

I hereby wish to express my strong support for Dr. Moishe Liberman application for the Give A Breath Research Award competition. The proposed research program, which focuses on developing a biomimetic platform for optimizing cryoablation-immunotherapy combinations, aligns perfectly with the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) commitment to advancing innovative cancer treatments through translational research. The multidisciplinary nature of this project, which brings together expertise from thoracic surgery, cancer biology, engineering, and pathology, exemplifies the type of collaborative research we foster at the CRCHUM.

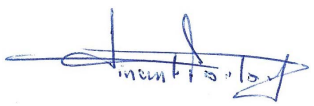
Dr Liberman joined the CHUM in 2009 and has internationally recognized expertise in minimally invasive thoracic surgery, leading him to head the Minimally Invasive, Robotic and Endoscopic Surgery and holds the Research Chair of Thoracic Surgical Oncology. Dr Liberman and his multidisciplinary team, have already demonstrated their capability to conduct this research through their preliminary studies and high impact publication record.

I confirm that the proposed research is feasible within our institution and that Dr. Liberman's team will have access to state-of-the-art facilities essential for the project, including cell culture rooms, common specialized equipment and the CRCHUM core facilities (Cellular imaging, Biobanks, Microfluidics) along with technical support. Dr Liberman' full research status at our institution ensures a minimum of 50% of protected time for research and remuneration for research time, access to a computer, storage space on the CRCHUM servers, benefit from identified spaces within our institution, usage of shared instruments and services of 19 available platforms for which the services are subsidized up to 40%. Additionally, our clinical research infrastructure will support the eventual translation of their findings into clinical applications.

We are particularly excited about the potential impact this research program could have on patients with advanced lung cancer who have progressed beyond first-line therapy.

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

Director of Research and Innovation - CHUM and Scientific Director - CRCHUM,



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