

THE LUNG CENTRE

The Canadian Mesothelioma Foundation Professorship in Mesothelioma Research


AN IMPACT REPORT PREPARED FOR
CANADIAN MESOTHELIOMA FOUNDATION

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Table of contents



Thank you	2
Summary of Dr. Marc de Perrot's research progress....	3
Training future leaders in mesothelioma research and care	11
Contact information	13

All photos were taken either before the COVID-19 pandemic
or following appropriate physical distancing guidelines.

Thank you

To the Board of the Canadian Mesothelioma Foundation,

I would like to thank you once again for your generous foundational support of the Canadian Mesothelioma Foundation Professorship in Mesothelioma Research at University Health Network (UHN). Your philanthropy enables me and my colleagues to develop new approaches to treating mesothelioma, ensuring patients have access to leading-edge care today as we develop the treatments of tomorrow.

In this report, we have included updates about our most recent work in mesothelioma research. The SMART trial, the results of which were described to you in our previous report, has led to additional exciting research opportunities. We have included a description of a study that uses SMART as a launching pad to explore genetic alterations that appear to be connected to the immune response triggered by the short course of radiation we deliver in SMART. The SMARTER trial has also progressed substantially: we have completed treatment for all patients participating in the trial and are currently analyzing the significant amount of data that this trial has generated. Finally, the SMARTTEST trial has now launched. This trial incorporates everything we have learned throughout SMART and SMARTER, and includes the use of three drugs to optimize the immune response triggered by radiation. The first eight patients enrolled in SMARTTEST are currently undergoing treatment.

We have also been active in engaging mesothelioma researchers internationally. This report includes updates about some of our research collaborations with Dr. Isabelle Opitz, who was previously supported by the Canadian Mesothelioma Foundation during her fellowship at UHN. Your support has helped Dr. Opitz develop into the exceptional researcher she is today, enabling our continued collaboration.

Finally, we have included an interview with a member of our team, Fatemeh Zaeimi, Clinical Research Manager. Fatemeh supports all the mesothelioma research initiatives that our team engages in, including connecting patients with opportunities to participate in trials like SMART, SMARTER and SMARTTEST. I trust you will enjoy learning more about this critical work going on behind the scenes.

Thank you for the Canadian Mesothelioma Foundation's continued trust, support and partnership, which has made possible all of the progress described in this report.

Sincerely,



Dr. Marc de Perrot
Canadian Mesothelioma Foundation Professor in Mesothelioma Research,
Director, Toronto Mesothelioma Program,
University Health Network
Professor of Surgery and Immunology,
University of Toronto

Summary of Dr. Marc de Perrot's research progress

Mesothelioma remains associated with very poor outcomes, with most patients surviving less than a year after their diagnosis. Incidences of this devastating disease are on the rise, with 600 new cases reported every year in Canada, a number that has doubled over the past two decades.

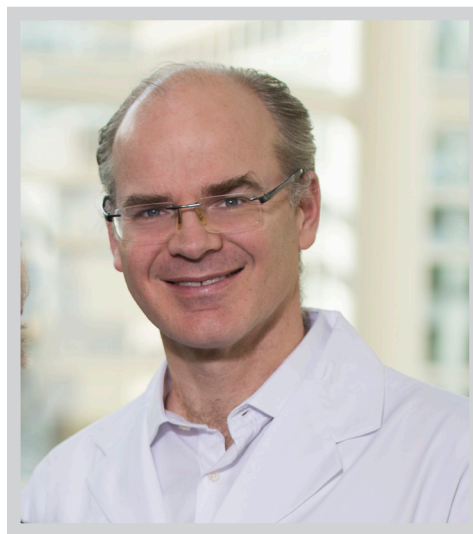
With globally-leading expertise spanning Toronto General Hospital and Princess Margaret Cancer Centre, the team at UHN sees the majority of early stage mesothelioma patients from all across Canada. Through the Toronto Mesothelioma Program at UHN, patients have access to mesothelioma-specific surgical, radiation and medical oncology expertise under one roof, facilitating multidisciplinary treatment recommendations and access to clinical trials that are often not available in patients' local cancer centres.

At the head of this groundbreaking research and care is Dr. Marc de Perrot, Canadian Mesothelioma Foundation Professor in Mesothelioma Research. In addition to working as an expert surgeon, Dr. de Perrot leads the team's translational research lab within the Latner Thoracic Surgery Research Laboratories and performs world-leading clinical research. With dual expertise in the clinic and the lab, Dr. de Perrot and his team can capitalize on the benefits of both, using insights from the clinic to inform studies in the lab and vice versa.

Dr. de Perrot and his colleagues within the Toronto Mesothelioma Program – a team that includes other leading experts in mesothelioma such as Dr. John Cho, radiation oncologist – are working to change the devastating toll of mesothelioma, developing new treatments so that patients can live longer, healthier lives.

The SMART Trial: The foundation of a paradigm shift in treatment

As reported to you previously, Dr. de Perrot, Dr. Cho and their colleagues completed a novel clinical trial called Surgery for Malignant Pleural Mesothelioma After Radiotherapy (SMART). Through the SMART trial, the team demonstrated the effectiveness of combining



Dr. Marc de Perrot

pneumonectomy with radiotherapy to improve patient outcomes. They administered radiation daily for five days and the following week, patients underwent a pneumonectomy. This treatment protocol resulted in the best results reported so far in the world in a large prospective clinical trial for pleural mesothelioma, both in terms of disease-free and overall survival. This trial also uncovered the novel insight that the short course of radiation triggers an immune response, which continues to be explored and refined to enhance treatment success.

USING SMART SAMPLES TO EXPLORE GENETIC ALTERATIONS THAT IMPACT SURVIVAL IN MESOTHELIOMA

As reported to you previously, Dr. de Perrot and his colleagues have been exploring genetic alterations that impact two proteins – BAP1 and p16 – that appear to be important factors in the development of malignant pleural mesothelioma. The loss of BAP1 and p16 are the two most common alterations to the genome in mesothelioma. They are both known to affect the environment immediately surrounding the tumour, influencing how a tumour hijacks and avoids the immune system's defenses. The impact of BAP1 and p16 loss on patient outcomes is currently unknown, but a better understanding of

these proteins could uncover new therapeutic targets for mesothelioma.

To deepen our understanding of the role of these two critical proteins, Dr. de Perrot and his colleagues leveraged the biobanked tumour samples that they had previously collected for the SMART trial. Because the SMART trial's use of a short course of radiation, activating an immune response, was a world-first achievement, this was a unique opportunity to analyze the impact of BAP1 and p16 loss on the immune response, characterize the mechanism of immune activation and determine the impact of these genetic alterations on survival after a short course of radiation.

They found that BAP1 loss had limited impacts on survival; however, p16 loss appeared to be much more impactful. Low p16 was associated with worse overall survival, even when accounting for other factors. This analysis demonstrated the importance of p16 – and the gene which encodes it, called CDKN2A – on the immune response after radiation and on overall survival.

For the first time in the world, the study also demonstrated that radiation amplifies type-1 interferon (IFN) signaling, which is associated with the best outcomes in mesothelioma. Type-1 IFN is a class of proteins that play an essential role in activating inflammation, tumour-cell recognition by the immune system and the immune response to the tumour. Higher levels of p16 (and expression of CDKN2A, the gene associated with p16) was associated with an enriched type-1 IFN response. These findings indicate that p16 and type-1 IFN may play a combined role in generating a stronger immune response to mesothelioma.

The team also identified an additional genetic pathway that may be of particular importance in controlling the patient's immune response after radiation. This pathway was highly upregulated after radiation, particularly in tumours with high levels of p16, and may play a key role in amplifying the immune response.

Finally, the team identified three genes that may be potential therapeutic targets to augment treatment for patients whose tumours are high in p16. This research also identified the need to explore different treatment strategies to target tumours that are low in p16.

Overall, as the first study of its kind in the world, this research supported previous observations about the impact of radiation on the immune response when treating pleural mesothelioma. It also validates the importance of p16 (and its associated gene CDKN2A) for patient outcomes. Future studies will be important to determine how best to amplify and maintain the anti-tumour response from p16 to achieve long-term benefit for patients with pleural mesothelioma.

A SMARTER treatment protocol

Dr. de Perrot and his colleagues have now completed patient enrollment and treatment for the Surgery for Mesothelioma After Radiation Therapy using Extended pleural Resection (SMARTER) trial. As reported to you previously, the goal of SMARTER is to explore the beneficial immune reaction triggered by a short course of radiation in patients with epithelioid mesothelioma. In these patients, the radiation causes the immune system to produce T cells that target and help eradicate the tumour.

Rather than the daily radiation used in SMART, in SMARTER, Dr. de Perrot and his colleagues alternated three days of radiation with “rest” days. This process is less toxic but still produces a beneficial immune reaction. Lower toxicity means that the full lung does not need to be removed in all cases, giving surgeons more choice in the most appropriate surgery for a given patient. This also expands the pool of patients who are eligible for this procedure to include those who could not tolerate such drastic surgery.

Treatment for all patients participating in the SMARTER trial has been completed and the team has demonstrated



Dr. John Cho

that the radiation regimen successfully activated an immune response. Dr. de Perrot and his colleagues are currently analyzing the trial's results in detail. In particular, the team is analyzing the genetic markers previously identified through the SMART trial in order to determine if these markers correlate with the immune response seen in the SMARTER trial.

Building on the results of the study mentioned in the previous section, they are performing single-cell RNA sequencing on tumour cell samples from patients in the SMARTER trial. Single-cell RNA sequencing is a cutting-edge technology that allows researchers to study the biology and function of each individual cell within a tissue sample, generating highly detailed and valuable data. They are examining p16 expression and interleukin-15 expression – another key component of the immune system identified as important in previous research – and correlating these expression levels with the rate of immune response. Once this analysis is complete, the team will submit a manuscript for publication in a high-impact journal.

Synthesizing knowledge: The SMARTEST Trial

Leveraging insights gained through SMART, SMARTER and related research, Dr. de Perrot, Dr. Cho and their team developed the Surgery for Mesothelioma After Radiation Therapy using Exquisite Systemic Therapy (SMARTEST) trial. As reported to you previously, this randomized trial will investigate the use of three drugs to optimize the immune response triggered by radiation by boosting the proliferation of cancer-fighting T cells. This will help consolidate the immune system's "memory" of the cancer, so that T cells can continue to target recurrent cancer long after treatment, analogous to a vaccine. The team will be targeting key pathways in the immune system to optimize the long-term benefits of treatment, aiming to delay or prevent recurrence.

The team will also use single-cell RNA sequencing to analyze tumour samples in order to identify biomarkers that are potentially associated with patient prognosis and response to therapy. This research and treatment protocol is the first of its kind in the world, and has the potential to have a major impact on the treatment of mesothelioma.

Dr. de Perrot, Dr. Cho and their colleagues are pleased to report that the SMARTEST trial is now underway. All necessary safety testing has now been completed, and the first eight trial participants have been recruited and are undergoing treatment.

The team has also begun creating molecular profiles for all patients undergoing treatment for mesothelioma at UHN, including those patients participating in the SMARTEST trial. These profiles use biomarkers identified in the SMART trial (such as BAP1 and p16) and enable the team to make better-informed treatment decisions for patients with this devastating disease, further personalizing treatment.

Uncovering factors that drive mesothelioma growth and development

In mesothelioma, tumours generate a microenvironment, a sort of protective “shield” that helps the tumour grow. One of the key components of a tumour microenvironment are cells called tumour-associated macrophages (TAM). TAM are particularly important because they help the tumour hijack the immune system, preventing the tumour from being detected and destroyed by the body’s natural defenses. They also enable the tumour to develop blood vessels that deliver nutrition, helping the tumour grow, and facilitate the migration of cancer cells to other areas of the body. This also means that TAM create barriers that prevent effective immunotherapy.

Other types of TAM seem to play different roles, though these are not yet well characterized. Within these varieties, there are multiple subtypes of TAM and the development process and immunosuppressive roles for each are not currently well understood. Uncovering the different types of TAM and their characteristics may help with the development of therapeutic targets for mesothelioma.

In a study using a pre-clinical model, Dr. de Perrot and his team analyzed two distinct TAM populations called SPM and LPM. SPM are white blood cell-derived TAM, while LPM are derived from tissue. The team investigated the dynamics, role and gene expression profiles of SPM and LPM during mesothelioma development.

While they found that both SPM and LPM contributed to mesothelioma development, they each played different roles. SPM appears more likely to play a key role in the development of mesothelioma itself, while LPM appears to contribute more to the immune response, including the activation of T cells in response to the tumour.

They identified five genes that could potentially be therapeutic targets and warrant additional research. Deleting one of these genes led to reduced levels of SPM,

“Mesothelioma is a complex, incredibly challenging disease. Your support is helping us uncover the mechanisms behind it, which will lead to new treatment options and allow us to adapt our treatment specifically for each patient.”

– Dr. Marc de Perrot

a compensatory increase in LPM, and slower tumour growth overall. This indicates that SPM plays a more prominent role in tumour progression. Therefore, SPM may be an important target for immunotherapy in clinical trials. Further research in this area could provide significant benefits for the treatment of mesothelioma.

Refining international definitions of mesothelioma

Dr. de Perrot and his colleagues have been working with other international experts, including the World Health Organization (WHO), to update the standard international definitions of mesothelioma. These definitions are important to ensure patients are accurately diagnosed and receive optimal treatment strategies that incorporate the most up-to-date knowledge about the disease.

Mesothelioma is typically diagnosed using biopsy. Biopsy results help clinicians determine the prognosis and make treatment decisions. Additionally, the diagnosis that a patient receives may impact their eligibility to participate in clinical trials.

The standard tools used to determine the prognosis for a case of mesothelioma include: histologic subtype (epithelioid, biphasic or sarcomatoid); nuclear grade (how closely the nuclei of cancer cells look like the nuclei of normal lung cells); and necrosis (levels of cell or tissue death). In one study, Dr. de Perrot and his collaborators examined the accuracy of these tools. By comparing samples from patients' biopsies at the time of diagnosis and the time of surgery, they aimed to determine whether these tools accurately predict how the disease progresses.

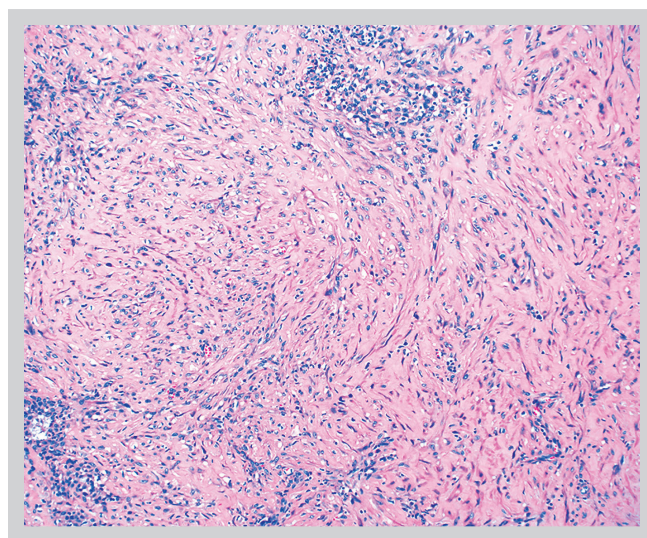
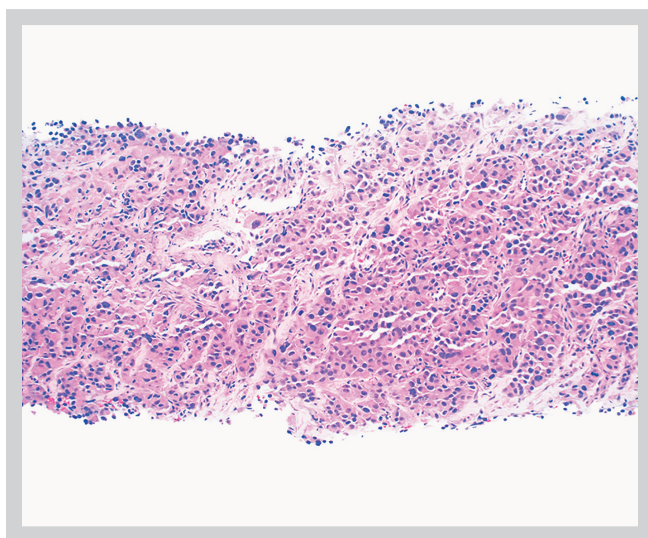
This study included 19 different institutions from around the world, and a total of 429 paired biopsies. This was the largest study of its kind in the world for pleural mesothelioma, and the first to study nuclear grade and necrosis using paired biopsies. It was also the first study to describe the impacts of neoadjuvant therapy (treatment such as radiation or chemotherapy to shrink a tumour prior to surgery) on histologic subtype.

They found that, while biopsy and surgical specimens were concordant in approximately 80 per cent of cases, classifying the biphasic subtype presented challenges.

A large number of the cases in which the biopsy classification and surgical specimen did not match were due to epithelioid cases later being reclassified as biphasic. Based on this, they have highlighted the need for the international community to revisit the classification standards for biphasic mesothelioma. They further identified the need for optimizing the biopsy process to achieve the highest quality samples for determining prognosis.

The team also demonstrated that both nuclear grade and necrosis were useful prognostic tools that should be included in pathology reports. Standardizing the use of these tools would aid in accurate prognosis. This research was published in the high-impact journal *American Society for Clinical Pathology*.

Dr. de Perrot and his colleagues are working with other international partners to share these results and encourage physicians around the world to consider these factors when analyzing biopsies from patients recently diagnosed with mesothelioma.



The biopsy sample (left) was classified as epithelioid mesothelioma. However, the resected tumour (right) showed both epithelioid and sarcomatoid features, leading to a diagnosis of biphasic mesothelioma. Samples such as this one demonstrated the importance of redefining the parameters for diagnosing mesothelioma subtypes.

In addition to this important work refining how prognostic tools are used in mesothelioma care, Dr. de Perrot is participating in the WHO's revised classification strategy for mesothelioma. This work involves using our deepened understanding of the genetic markers related to mesothelioma – such as BAP1 mutation – to refine the description of mesothelioma and its subgroups in a more clinically accurate way. This work will also help with earlier diagnosis of mesothelioma, allowing clinicians to make a diagnosis of mesothelioma based on a patient's genetic profile. For example, mesothelioma in situ can be diagnosed genetically, indicating that a patient has a 70-80 per cent chance of developing mesothelioma within five years. Early diagnosis will enable clinicians to treat mesothelioma sooner, and to connect patients with clinical trials.

International Mesothelioma Interest Group conference

Dr. de Perrot is a member of the Board of Directors and Scientific Committee of the International Mesothelioma Interest Group (IMIG). IMIG is a community of physicians, researchers, scientists, care providers and social advocates engaged in understanding the mechanisms and factors associated with mesothelioma. It is the world's only society dedicated solely to the study of mesothelioma.

This year, IMIG's international conference will be held June 26-28 in France. Dr. de Perrot is on the program committee, involved in the planning and organization of this globally impactful meeting.

Impacts of your support: International research collaborations

Your generous and longstanding support has had far-reaching impacts across the field of mesothelioma research, here in Toronto and around the world. As one example, Dr. de Perrot and his colleagues have been collaborating with Dr. Isabelle Opitz, a researcher who was

supported by the Canadian Mesothelioma Foundation during her fellowship over a decade ago at UHN. In 2012, Dr. Opitz returned to the University Hospital of Zurich. Since becoming established in Zurich, she and Dr. de Perrot have established a fruitful and longstanding research collaboration. Two recently published studies, developed as a result of this collaboration, are described below. Research collaborations like these amplify the impacts of your support, expanding study sizes and research capabilities that Dr. de Perrot and his colleagues can draw upon.

UNCOVERING A PROGNOSTIC TOOL AND POTENTIAL THERAPEUTIC TARGET FOR SARCOMATOID MESOTHELIOMA

While the histologic subtype (epithelioid, biphasic and sarcomatoid types) and stage in mesothelioma are important prognostic factors, patient outcomes vary significantly, even among patients with similar characteristics. Enhanced prognostic tools are needed to more accurately predict patient outcomes.

One potential prognostic marker is called the PI3K pathway. This is a signaling pathway, which refers to a process where a cell receives a signal such as a hormone, and a reaction is triggered causing the cell to carry out a certain function. Activation of this pathway is related to cell growth and proliferation, which can also promote the survival and proliferation of cancer cells. Therefore, the team anticipated this pathway could potentially be both a prognostic biomarker and therapeutic target.

Dr. de Perrot, Dr. Opitz and their colleagues collaborated with researchers from seven other institutions to complete a study that included records from more than 350 patients. This is almost five times larger than any other study related to this biomarker.

An area of particular interest for the research team was a protein called pS6, which is activated by the PI3K pathway. In previous studies, low levels of pS6 were significantly correlated with longer progression-free survival and

overall survival in patients with pleural mesothelioma.

Interestingly, the team found that pS6's use as a prognostic marker for survival was more nuanced than those previous studies had shown. They found that pS6 expression levels were a prognostic marker of survival only in non-epithelioid types of mesothelioma. This finding indicates that for sarcomatoid mesothelioma, pS6 and the PI3K pathway are of particular importance, and could represent a therapeutic target for this specific subtype of mesothelioma. Given that sarcomatoid features in mesothelioma are associated with worse prognosis and higher resistance to chemotherapy, finding new ways to treat this subtype is highly important.

A GENE FAMILY THAT MAY CONTRIBUTE TO MESOTHELIOMA DEVELOPMENT

Mesothelioma is a heterogeneous cancer, meaning that there can be differences in the DNA and RNA between different tumours within the same patient, and even between different cancer cells within the same tumour. This heterogeneity can impact diagnosis as well as treatment response, as treatments may be less effective against the DNA and RNA patterns found in some cells or tumours compared to others within the same patient.

One of the potential contributors to this heterogeneity and the development of mesothelioma overall is a family of enzymes and related genes called ADAR. ADAR can "edit" the RNA of other cells, and mutations in ADAR genes are associated with other types of cancer. Additionally, in a previous pre-clinical study, the team observed higher levels of ADAR-dependent RNA during mesothelioma development. In that previous research, the team found that one type of ADAR, called ADAR1, increased expression upon exposure to asbestos. ADAR2 expression increased only upon tumour formation.

To elucidate more about the relationship between ADAR and mesothelioma development, as well as its relationship to a patient's BAP1 status, Dr. de Perrot, Dr. Opitz and their colleagues designed a retrospective study. Using

samples of tumour tissue collected from 193 patients over a decade-long period as well as a pre-clinical model, the team performed detailed analyses to uncover more about the interactions between ADAR and BAP1, and mesothelioma development overall.

They successfully uncovered a number of interesting results. First, the researchers demonstrated that tumours and mesothelioma cell cultures have higher levels of ADAR-mediated editing compared to healthy cells in the same part of the lung. They also demonstrated that the RNA editing caused by ADAR contributes to the heterogeneity of mesothelioma. Importantly, they observed that ADAR2 plays an important role in the growth of mesothelioma, chemotherapy response and regulation of the inflammatory response.

ADAR can prevent type-1 interferon (IFN) production, and reduce its ability to remove dead or damaged cells. As previous research has shown, type-1 IFN signaling is associated with BAP1 mutations, but the underlying mechanisms of this relationship are unknown. In this study, the team found that levels of ADAR2 expression are higher in mesothelioma cell lines with higher levels of BAP1, which is associated with the worst survival outcomes. There also appeared to be differences in how RNA was edited according to a given patient's BAP1 status. This indicates an association between ADAR2 and BAP1, which may lead to new understandings about the relationship between BAP1 and type-1 IFN signaling.

Overall, this study demonstrated that RNA editing caused by ADAR, particularly ADAR2, has wide implications for mesothelioma cell growth, response to therapy and interaction with the tumour microenvironment. This research has also illuminated areas for future research to deepen our understanding of the complex interaction between BAP1, type-1 IFN and ADAR. The results of this research were published in the high-impact journal *Molecular Oncology*.

Looking ahead

Dr. de Perrot and his colleagues' research plans in the near term will focus on the SMARTTEST trial, the continued analysis of data from the SMART and SMARTER trials, new insights about BAP1 and tools for early diagnosis and screening. One particular project of interest is the development of a "liquid biopsy" – a blood test – for mesothelioma. Using knowledge of the loss of BAP1 as a risk factor for mesothelioma, Dr. de Perrot and his colleagues are working to find a way to detect the earliest stages of mesothelioma through a blood test. This tool could then be used to screen individuals at high risk of developing mesothelioma, such as the family members of a person currently diagnosed with the disease.

Training future leaders in mesothelioma research and care

Introducing Fatemeh Zaeimi

Fatemeh Zaeimi is a Clinical Research Manager in the Division of Thoracic Surgery at UHN. Her educational background is in medical physics. Previously, she worked as a Clinical Research Coordinator in rheumatology at Toronto Western Hospital. In 2017, Fatemeh moved to Toronto General Hospital as a Clinical Research Coordinator within the Mesothelioma Screening Program. In 2022, she was promoted to Clinical Research Manager.

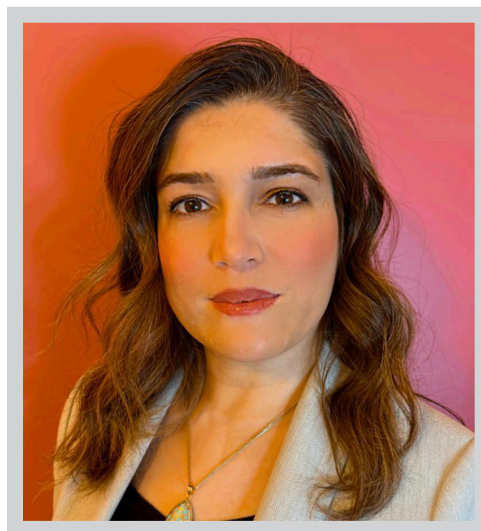
What does your role as clinical research manager entail?

I am responsible for the management, operational, and organizational effectiveness of the clinical research program for mesothelioma in the Division of Thoracic Surgery. I manage and oversee all day-to-day administrative functions and the Division's human resources (such as managing the research office budget, supervising the staff, and fostering a positive, collaborative working environment). I manage all aspects of research ethics approvals such as protocol submission, preparation of study documents and managing study data by designing and organizing computer databases.

I work closely with the researchers and the multidisciplinary healthcare team to recruit patients to participate in clinical research. I facilitate the research consent process by identifying, screening/reviewing eligibility, and enrolling patients. I am also a delegate for Dr. de Perrot's research lab, where I supervise lab activities and research.

Can you describe the importance of this work?

By engaging patients in research opportunities, they will better understand the research underway in mesothelioma and find new hope through participation in this research. We hope that this involvement will provide them with direct access to new treatments. By sharing their personal experiences in health care, these patients play an integral role in improving care for others.



Fatemeh Zaeimi

What is something that you are proud of that you and the team have achieved?

I am proud to have enabled more studies and collaborative work in the program. When I joined the program in 2017, we only had two active prospective studies (SMART and Screening). Currently, we have multiple national and international projects and we are able to collaborate with many teams across UHN and University of Toronto on mesothelioma and lung research. I was able to assist Dr. de Perrot and Dr. Cho to develop a transplant oncology study to benefit our lung transplant patients, based on the knowledge we have gained in mesothelioma research.

What is something that inspires you about this clinical research and the environment on Dr. de Perrot's team?

I am inspired by the opportunity for this research to be applied in other areas of medicine; by new technologies and by our collaborations with researchers across the world. There are new challenges that come with every project, and this makes every day interesting. The experimental nature of this research and the opportunity to see the research impact patients in real time has motivated me.

“I cannot express enough gratitude to the Canadian Mesothelioma Foundation for supporting our research. Seeing the impacts of new discoveries for patients within the Toronto Mesothelioma Program at UHN has been incredibly rewarding. With your support, we have been able to grow our research to an international level, which has allowed us to reach even more people.”

– Fatemeh Zaeimi

Contact information

Thank you for your visionary support of the Canadian Mesothelioma Foundation Professorship in Mesothelioma Research, supporting the groundbreaking research of Dr. Marc de Perrot and his colleagues. Your generosity is helping build the future of mesothelioma treatment and care.

For more information, please contact:

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