# Supervisor & Project Information Form

Please complete and return via email ONLY to gdip.hres@utoronto.ca by Monday, November 2, 2020

**Supervisor Information**

*MUST have unrestricted University of Toronto School of Graduate Studies (SGS) appointment (to independently supervise graduate students)*

<table>
<thead>
<tr>
<th>Name:</th>
<th>Tereza Martinu</th>
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<tbody>
<tr>
<td>Email:</td>
<td><a href="mailto:Tereza.martinu@uhn.ca">Tereza.martinu@uhn.ca</a></td>
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<tr>
<td>SGS Department:</td>
<td>IMS, Immunology</td>
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<tr>
<td>Field of Research:</td>
<td>Lung transplantation, immunology</td>
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<td>Research Institution affiliation (if applicable):</td>
<td>TGHRI</td>
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<td>Location of Work:</td>
<td>PMCRT (MARS)</td>
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<tr>
<td>Student contact time (number of hours per week YOU are available to the student for any concerns or to review progress):</td>
<td>2h + lab meetings</td>
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TITLE:
Validation of a Novel Biomarker Signature for Early Identification of Gastroesophageal Reflux and Allograft Dysfunction in Lung Transplantation

DESCRIPTION (MAX 500 WORDS):

Chronic lung allograft dysfunction (CLAD), or chronic rejection, occurs in 50% of lung transplant recipients at 5 years post-surgery and is the main limitation to long-term survival. The pathophysiology of CLAD remains poorly understood but is thought to involve the combination of alloreactivity and recurrent injuries to the lung graft. Multiple risk factors have been associated with CLAD, including gastroesophageal reflux disease (GERD), which is common in patients with end-stage lung disease and is exacerbated after transplantation. As a result of significant reflux, exposure of the lung allograft to gastric contents may promote and perpetuate chronic inflammation and injury, leading to chronic rejection.

GERD is diagnosed using an esophageal probe that is introduced intranasally and monitors changes in pH and electrical conductance over a 24-hour period. However, the intranasal probe and long test duration are highly uncomfortable for the patient, leading to poor overall compliance. Furthermore, this test is unlikely to detect patients with sporadic or transient GERD and also does not inform us about actual aspiration of gastric contents.

Given the limitations of GERD testing, other methods to diagnose microaspiration have relied on the detection of bile acids in the airways of the lung. Our group has now established the utility of a specific lung fluid sample called the large airway bronchial wash (LABW), which is obtained in proximal airways during bronchoscopy, as a superior sampling technique to detect aspiration and inflammation. Specifically, we found that a signature comprised of biomarkers representing inflammation (IL-6, IL-8, CCL5, and CCL2) and bile acid salts (taurocholic acid, TCA and glycocholic acid, GCA) was predictive of death or CLAD within three years of transplantation. This data utilized mass spectrometry to detect bile acids and multiplex antibody-based detection platforms for protein analysis.

We hypothesize that, in a large retrospective cohort of lung transplant recipients, our LABW biomarker signature will correlate with GERD and graft dysfunction, and will enable accurate and cost-effective testing for GERD and microaspiration. The following objectives will be tested in this proposal:

Aim 1: Validation of the LABW microaspiration signature in lung transplant recipients with GERD and graft dysfunction. Bile acids TCA and GCA will be measured by mass spectrometry and inflammatory proteins (CCL5, IL-6, IL-8, CCL2) by multiplex assay in LABW fluid obtained 3 months after transplantation in a large retrospective cohort. Associations with clinical measurements of GERD and graft dysfunction will be assessed.

Aim 2: Translation of the LABW microaspiration signature for rapid clinical adoption.
Aim 2.1: Validation of the feasibility of the enzyme-linked immunosorbent assay (ELISA) as an equivalent to mass spectrometry for the analysis of bile acid levels in LABW in lung transplant recipients.

Aim 2.2: Evaluation of the financial and quality of care implications of the LABW microaspiration signature.

Significance:
We anticipate that implementation of our LABW microaspiration biomarker signature will enable early detection of GERD and microaspiration, resulting in earlier intervention and triage for anti-reflux surgery. Ultimately, this strategy will reduce lung injury, prevent CLAD, and lead to better outcomes in all lung transplant recipients.

If human subjects are involved, have the appropriate Research Ethics Board approvals been obtained?
☐ Yes    ☐ No    ☐ Application Submitted (Date: ________________)

Do you expect this work will be published within the 20 months?
☐ Yes    ☐ No    ☐ Uncertain / Other
**Student Roles & Responsibilities (please be as specific as possible)**

The specific aims are outlined above. The student will be expected to acquire general knowledge about lung transplantation, transplant immunology, and the role of GERD and microaspiration. The student will perform data queries and clinical chart reviews in order to assemble the patient cohort for this study based on reflux testing and availability of bronchoscopic samples for analysis. The student will prepare and organize LABW samples for analysis. They will learn several analytic techniques, including ELISA and multiplex assays for cytokine detection. They will also learn the concepts of mass spectrometry. Significant focus will be placed on data analysis and interpretation in the context of the clinical scenario. The student will work directly with clinicians as well as the PI to perform the clinical review of patients. The student will also get direct technical supervision by a PhD-level research associate and a technician on the team. The student will be expected to interact with the project statistician and understand the concepts behind the statistical analyses. Finally, the student will be expected to present the research results in lab meetings and at an international conference, as well as prepare a manuscript for publication.

*Indicate who will serve as the student’s direct report for daily oversight (PI, PhD student, technician, etc...)*

PI: Tereza Martinu
Co-PI: Shaf Keshavjee
Additional supervision of clinical portions of the project: Juan Fernandez (MD)
Additional supervision of laboratory work: Andrew Sage (PhD), Jenna Fortunato (technician)

*Indicate to what extent the student’s research activities could, if necessary, be completed remotely.*

A large component of the project is a retrospective clinical data collection and analysis, which can be done remotely, using our lung transplant clinical research database. I hope that this portion of the project will lead to an additional manuscript of its own.