Grant Title: Cell-free DNA for the Diagnosis of Rejection after Heart Transplantation

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Lay Summary

Since starting this project in September 2017, we have made substantial progress towards addressing our Aims and overall project goal. We have completed sequencing using the Illumina platform and two separate panels of markers. Unfortunately, the algorithm that we developed previously cannot be used with this new data so we are in the process of developing a new method. We anticipate completion by April 2020. Ms. Sabrina Pattar successfully defended her MSc degree on August 14, 2019 and is now enrolled in medical school at the University of Alberta. From her thesis work she identified several potential biomarkers for rejection after heart transplantation based on DNA methylation. One of these markers showed good correlation with the severity of rejection and represents a novel type of assay for rejection that could undergo further testing and validation in the future. She also identified highly variable cell death in biopsy samples from adult heart transplant patients that were graded as having mild acute cellular rejection (ACR 1R). This insight suggests that not all mild rejection episodes should be treated the same (since some are more severe than others) and that further refinement of the grading system for mild rejection (perhaps based on the degree of cell death) could potentially be beneficial for the longterm health of the transplanted heart. We are preparing a manuscript describing this work for submission to *The Journal of Heart and Lung Transplantation* by December 2019. A second publication describing our analysis of the Illumina data is anticipated with submission in summer 2020.

Original Project Abstract

Monitoring for rejection after heart transplantation (HT) currently requires an endomyocardial biopsy (EMB) which is an expensive and invasive procedure that has serious limitations. A test allowing doctors to assess the donated heart for damage frequently and non-invasively would be a major improvement in post-transplant care. It is known that tissues normally release fragments of DNA into the circulation as cells die and this cell-free DNA (cfDNA) is found in the blood of all individuals. After HT, cfDNA comes from both the donated heart and the recipient tissues. An increase in the amount of donor cfDNA found in the blood of the recipient has been associated with damage to the transplanted heart therefore cfDNA is potentially a new and exciting marker for injury due to rejection after HT. We and others have found that donor and recipient cfDNA can be distinguished based on differences in DNA sequence (single nucleotide polymorphisms, SNPs). Another but untested approach relies upon differences in DNA methylation. We have identified novel heart-specific differentially methylated regions (DMRs) that potentially can be identified in patient cfDNA. It is an unknown but important question as to which cfDNA marker will make the most precise and accurate test for the non-invasive detection of rejection after HT. The **objective** of this project is to compare the two cfDNA-based strategies (SNPs or DMRs) and identify the best assay for further development. From our recent work (funded by Enduring Hearts) we have found our SNP-based assay to be too inaccurate for clinical use. For this project, we are proposing to improve this assay and test an alternative strategy based on DNA methylation. This work will build upon our accumulated expertise and resources. By identifying a non-invasive biomarker for rejection after HT we could reduce the frequency of EMB and enable increased allograft monitoring to individualize and improve patient care.

Project Aims

Specific Aim #1. Improve our SNP-based cfDNA assay. Automating the cfDNA extraction process and switching to the more accurate Illumina sequencing platform using a custom panel of polymorphic SNPs will potentially address the errors we have noted.

Specific Aim #2. Validation of candidate ventricle-specific DMRs. We have identified *in silico* 14 candidate DMRs that are unique to human ventricle and potentially detectable in cfDNA. Using methods that we have developed, we will validate a subset of these ventricle-specific DMRs using genomic DNA from normal human left ventricle and human cfDNA.

Specific Aim #3. Correlation of ventricle-specific cfDNA levels with rejection grade and evaluation of performance relative to our SNP-based assay. Since November 2014 we have been collecting blood from adult recipients undergoing EMB performed for routine surveillance or clinical suspicion of rejection after HT. Plasma levels of donor cfDNA will be determined using our improved SNP-based assay and our novel DMR-based assay. Results will be correlated with rejection grade assigned from the EMB, the current gold standard for the diagnosis of rejection, to identify the best cfDNA-based biomarker.

Progress to Date

Funds from Enduring Hearts supported the salary for graduate student Sabrina Pattar who has now completed her Master of Science degree in my lab. In April 2019 she presented her work in a talk at the International Society for Heart and Lung Transplantation in Florida (acknowledging the support provided by Enduring Hearts).

For Aim 1, sequencing on the Illumina platform has been completed using two different SNP panels. Development of a new algorithm to process this data is in progress. We anticipate completion of this Aim by April 2020.

With regards to Aim 2, Sabrina identified three DMRs unique to human ventricle and that appeared promising as biomarkers for rejection. She went on to test them using our collection of samples from adult heart transplant recipients with either grade 0R, 1R or 2R acute cellular rejection. One of the biomarkers showed promising correlation with the severity of rejection and represents a promising target for future validation.

We also completed a collaboration with Qiagen to test the Pyromark platform for the ability to measure methylation in our samples. Unfortunately, data from this platform showed poor correlation with rejection severity.

Our most interesting data resulted from experiments examining cell death (apoptosis) in endomyocardial biopsy samples. We have found that levels of apoptosis are low in the absence of rejection (ACR 0R) and increased in the presence of moderate rejection (ACR 2R) as would be expected. However, we have found that the degree of apoptosis is highly variable in samples with mild rejection (ACR 1R) with levels approaching those seen in moderate rejection in some cases. This suggests that the current rejection grading method does not capture this heterogeneity and these differences in myocardial cell death may have important implications for the longterm health of the transplanted heart.

Conclusions and Knowledge Translation

In conclusion, with the generous support of Enduring Hearts, we have made substantial progress in completing our research proposal. Sabrina has graduated and we have almost completed all of our initital research aims as originally outlined in our proposal. We are in the

process of writing our first manuscript from this data. With the preliminary data generated from this project we are planning submission of a grant application to the Canadian Institutes of Health Research (CIHR) in February 2020. This grant will further explore our novel biomarker and assess cell death in additional biopsy samples.