Plasma induced transcription analysis in pediatric heart transplantation as an assessment of rejection

Alexander Raskin, MD; Steven Kindel, MD; Ann Punnoose, MD; Martin Hessner, PhD

Children's Hospital of Wisconsin

Abstract

Background: Development of graft rejection after cardiac transplantation is a key clinical issue. Acute cellular rejection affects 20% to 40% of patients in the first year after transplantation, and acute antibody-mediated rejection occurs in 10% of cases. Chronic rejection is a multifactorial process that manifests as cardiac allograft vasculopathy and accounts for 30% of recipient deaths. Cardiac catheterization with coronary angiography and myocardial biopsies is currently the gold standard for diagnosing and classifying rejection. However, catheterization may underdiagnose rejection, and it is an invasive procedure with associated complications. Gene expression studies have been investigated in adults for rejection surveillance, which yielded poor sensitivity or required invasive tissue samples. Furthermore, there is limited reported data on transcriptional analysis as a marker of rejection in the pediatric heart transplant population. New measures are needed to define the immune states associated with pediatric heart transplantation, how they relate to immunosuppressive regimens, and whether they predict onset of rejection. We have developed a sensitive bioassay where subject plasma is used to induce transcription in a well-controlled peripheral blood mononuclear cell population. This technique has successfully been used to investigate inflammatory states in pediatric disease, such as Type 1 diabetes mellitus and inflammatory bowel disease.

Methods: Institutional Review Board approval has already been obtained, and subjects are actively being recruited. Subjects ages 0-17 undergoing cardiac catheterization after heart transplantation will have plasma samples collected. Patient plasma will be used to induce a transcriptional response in commercially available peripheral blood mononuclear cells. An analysis performed on 54,000 inflammatory and regulatory genes will determine if plasma induced transcriptional signatures can identify rejection, stratify risk of developing rejection, and demonstrate responsiveness to immune intervention.

Anticipated Results: We hypothesize that plasma induced transcriptional signatures will be able to detect both acute cellular and antibody mediated rejection. Additionally, this technique will be able to identify individuals at risk of rejection, demonstrate response to treatment of rejection, and will produce signatures that are specific to the immunosuppressive regimen used. Based on previous clinical trends, enrolling >100 subjects over the upcoming year is attainable.

Summary

Children undergoing heart transplantation are at risk of developing rejection. Rejection is the process by which the body's immune system attacks the donor heart, which may lead to heart failure and sometimes death. To help reduce the risk of rejection, all transplant recipients have to take medications to suppress their immune system. Currently, the best way of diagnosing rejection is with a heart catheterization procedure that involves taking a heart biopsy (collecting tissue samples). Catheterization based procedures are costly and have associated risks. Our goal is to develop a blood test that reliably identifies the presence of rejection in children without the need for a catheterization procedure. Our proposal focuses on collecting blood at the time of a heart biopsy, and using that blood sample to perform an analysis on genes regulating the immune system. The results of the analysis are then compared to the results of the heart biopsy. The presence of rejection should be identified by a specific pattern of activation in immune related genes. Other advantages of our proposed study is that it may help identify children at risk of developing rejection, it may help monitor the treatment of rejection, and it may expand our knowledge and understanding of the mechanisms involved in rejection.

Specific Aims

Acute cellular rejection affects approximately 20% to 40% of heart transplant recipients in the first 6 months after transplantation, and acute antibody-mediated rejection occurs in 10% of heart transplant recipients[1]. Chronic rejection is a multifactorial process that manifests as cardiac allograft vasculopathy (CAV), and it accounts for 30% of recipient deaths post heart transplantation[2]. Endomyocardial biopsy is the current gold standard for diagnosing rejection, but recently biomarkers and bioassays have become the focus of investigation in the field of transplant medicine[3-8]. These studies have led to the development of clinically available commercial products, such as AlloMap® and Molecular Microscope®. However, there are limitations to these approaches. AlloMap® has high negative predictive value but has poor sensitivity for detection of cellular medicated rejection. This test is also unable to assess for antibody mediated rejection[4,6,7]. Molecular Microscope® can detect both cellular and antibody mediated rejection, but it requires invasive myocardial biopsy samples to perform microarray analysis[8]. Furthermore, to date, no studies have assessed transcriptional analysis as a marker of rejection in the pediatric heart transplant population. New measures are needed to define the immune states associated with pediatric heart transplantation, how they relate to immunosuppressive regimens, and whether they predict onset of rejection. We have developed a sensitive bioassay where subject serum or plasma is used to induce transcription in a well-controlled peripheral blood mononuclear cell (PBMC) population[9-12]. This technique has successfully been used to investigate inflammatory states in pediatric disease, such as Type 1 diabetes mellitus and inflammatory bowel disease[13,14].

Our preliminary data demonstrates that pediatric heart transplant patients have unique transcriptional signatures when compared to healthy controls. These signatures cluster according to the immunosuppressive regimen of the patients, are consistent with the described mechanisms of actions of the drugs used, and could represent targets for monitoring drug response/effectiveness. Despite immunosuppression in the transplant patients, our data identified up-regulation of pro-inflammatory genes, which may play a role in the development of rejection. Given these findings, and our work in other disease settings where we are able to predict progression rate and responsiveness to therapeutic intervention[14,15], we hypothesize that plasma induced signatures will be specific to underlying immunosuppressive regimens, can stratify risk of rejection, accurately identify onset of rejection, and demonstrate responsiveness to immune intervention.

Aim 1: To test the hypothesis that plasma induced signatures measured on transplant patients will be different from healthy controls and will be specific to the immunosuppressive regimen at the time of sampling.

We will analyze samples from >100 pediatric heart transplant patients at the time of their cardiac catheterization. We will determine if various immunosuppressive regimens produce unique and specific transcriptional signatures that are different from healthy controls. Additionally, we will process longitudinally collected samples from >10 patients to measure changes in signatures with concurrent changes in immunosuppressive regimens.

Aim 2: To test the hypothesis that plasma induced signatures can A) identify patients at risk of rejection B) produce signatures specific to acute cellular and antibody mediated rejection and C) demonstrate reversion to quiescence after induction of immune intervention for treatment of rejection.

Using collected samples we will compare plasma induced signatures of patients with rejection vs those without rejection. Presence of rejection will be established by right ventricular endomyocardial biopsy histology obtained at the time of sample collection. Samples will be grouped together for analysis based on the immunosuppressive regimens of the patients. Additionally, longitudinally collected samples will be analyzed for changes in plasma induced signatures prior to onset of rejection, during rejection, and after treatment of rejection to determine if certain signatures are predictive of developing rejection and if plasma induced signatures can be used to show effective response to clinical treatment of rejection.

Pilot data from this study may aid in the development of a non-invasive test for rejection, which will reduce healthcare costs and morbidity. Furthermore, improved understanding of the immunological mechanisms of rejection has significant implications in the development of more sensitive diagnostic modalities, individualized risk stratification, improved monitoring of therapeutic interventions, and development of novel drugs.

Research Strategy

A. Significance

The primary threat to patients after undergoing heart transplantation is development of rejection. Rejection may manifest as hyperacute, acute, or chronic. Hyperacute rejection occurs within minutes to hours after transplantation due to preformed recipient antibodies against donor vascular endothelium. Acute cellular rejection is mediated by T-cells and most commonly occurs in the first 6 months after transplantation. In the first year post transplant, approximately 20% to 40% of heart transplant recipients will experience acute cellular rejection. Additionally, acute antibody-mediated rejection occurs in 10% of heart transplant recipients[1]. Chronic rejection is a multifactorial process that targets the epithelium, arteries, and capillaries and manifests as cardiac allograft vasculopathy (CAV). CAV accounts for 30% of recipient deaths post heart transplantation[2]. Myocardial biopsy obtained during catheterization is currently considered the gold standard for diagnosing and classifying the severity and type of rejection[3-5]. During catheterization coronary angiography is routinely performed to assess for the development of CAV. However, cardiac catheterization is an invasive test with associated complications. Additionally, the diagnostic yield of myocardial biopsies maybe limited based on the sample obtained and may underestimate the presence of rejection[4-7].

Recent research has focused the role of noninvasive testing modalities in post-transplant rejection surveillance. Echocardiography may demonstrate both diastolic and systolic dysfunction, but these are late findings that have low sensitivity and poor positive predictive value. Cardiac Magnetic Resonance Imaging (MRI) may have better sensitivity, but currently there is paucity of data to suggest routine cardiac MRI utilization[3]. More recently, biomarkers such as cell free DNA and gene expression studies have been investigated for their potential role in rejection surveillance after solid organ transplantation[3-8,16-20]. U.S. based (CARGO) and European based (CARGO II) studies assessed gene expression from patient collected peripheral blood mononuclear cells to quantify expression levels in 20 preselected genes. A gene expression profiling logarithmic score from 0 to 39 was calculated and then commercialized as AlloMap®, which was found to discriminate acute cellular rejection from quiescence in heart transplant recipients. AlloMap® has been shown to have a high negative predictive value but poor sensitivity. In CARGO II, the sensitivity of AlloMap >6 months post-transplant was 25%. Additionally, another limitation of AlloMap® is its inability to assess for antibody mediated rejection[4,6,7]. The Invasive Monitoring Attenuation Through Gene Expression (IMAGE) study showed when gene expression was used to identify nonrejection, in low risk patients, there was no difference in clinical outcomes with decreased use of endomyocardial biopsies[5]. Tissue based measurements obtained from biopsy samples could be used to accurately discriminate patients with antibody mediated rejection from those without antibody mediated rejection based on transcripts reflecting Natural Killer cell burden, endothelial activation, macrophage burden, and interferon gamma effects[19]. Additionally, gene expression profiling from myocardial tissue was predictive of acute cellular rejection one month prior to the onset of histological changes[20]. Microarray based transcriptional analysis performed on biopsy tissue samples has been shown to detect both cellular and antibody mediated rejection. This test will be commercially available as Molecular Microscope®[8]. However, these studies are limited by invasive procedures requiring tissue samples.

One of the other major limitations in the gene expression studies conducted in the heart transplant population is that all of them have exclusively focused on adult subjects, but none studied pediatric heart transplant recipients. This is certainly an area in need of further scientific exploration, as pediatric patients are unique in many aspects. An additional benefit to the proposed study is that it will utilize a novel sample sparing genomics approach not previously assessed in the heart transplantation population that only requires 200 uL of plasma. As described above, previous studies relied on gene expression from patient collected peripheral blood mononuclear cells or patient collected tissue. Patient collected tissue has the same limitations as routine catheter based biopsy as it requires an invasive procedure. The limitation of sampling patient peripheral blood mononuclear cells is that the cells circulating in the periphery may not be indicative of activity at the site of interest, i.e. heart. We propose utilizing a novel technique to the transplant population that uses patient plasma to induce a transcriptional response in commercially available “reporter” cell population (peripheral blood mononuclear cells). **The fundamental basis of this proposal is that measures enabling comprehensive assessment of the immune state will improve our understanding of the transplant population, including pathogenesis of rejection**. **Additionally, the proposed work will also foster the development of biomarkers to stratify risk of developing risk of rejection, improve diagnostic yield, and monitor therapeutic response.**

B. Innovation

Immunological responses are local events, and therefore participating immune cells may not be abundant in the periphery. As such, it is not surprising that immune mediators associated with heart transplant rejection are generally too dilute in the periphery for detection by traditional methods. Examining one or a few mediators may be uninformative due to important combinatorial effects. For these reasons, we utilize an innovative bioassay whereby patient plasma or serum is co-cultured with a well-controlled 'reporter' cell population (Fig. 1). The induced transcriptional response is then globally measured with a microarray; Ontological analysis of the transcripts differentially induced by plasma is conducted with Ingenuity Pathway Analysis (IPA) and other software to identify potential mediators underlying the immune signature of a given subject or cohort[13,14]. **This approach enables an integrated functional assessment of the many plasma components (cytokines, chemokines, lipids, metabolites, microbial antigens).** Additionally, it offers a powerful tool to sensitively and comprehensively detect and differentiate immune states. This approach offers advantages over the common approach of directly profiling patient PBMC. The Hessner Lab[9], and others[21], have observed that expression differences measured in PBMCs incubated with case vs control plasma are more robust than those observed with direct profiling of case vs control PBMCs. This strategy has been used to study juvenile rheumatoid arthritis[21], and the Hessner lab has applied it to Type 1 Diabetes, H1N1 influenza, bacterial pneumonia, cystic fibrosis, ulcerative colitis, Crohn's disease, and cervical cancer[9,11,12,14,22].

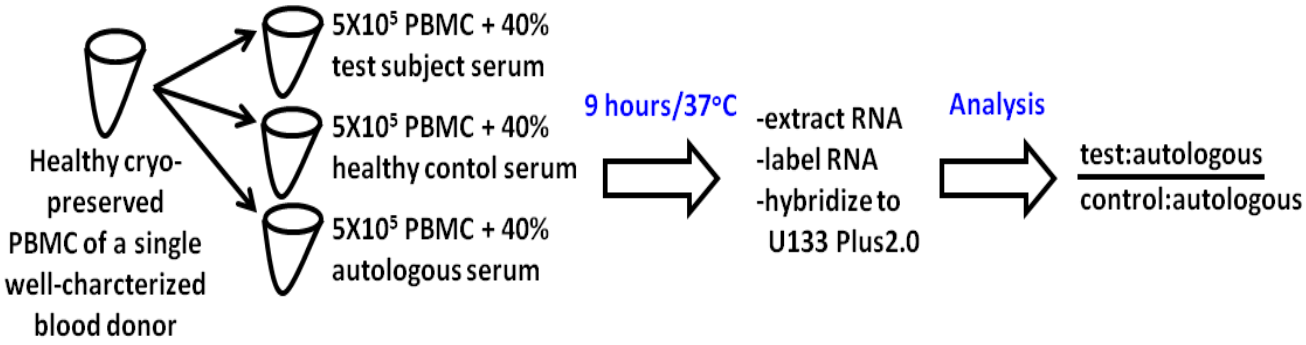

Fig. 1 Serum/plasma induced transcription. Our optimized assay uses commercially supplied PBMC of a single draw of a **single well-characterized healthy person representative of the normal population**. In 500ml, PBMC (Cellular Technology Ltd., Shaker Heights, OH) are cultured with **200ml** of patient serum/plasma. Purified RNA (~100ng) from PBMC is labeled/hybridized to the Affymetrix U133+2.0 array as described in [9-14]. The conditioned media is retained for other analyses. Signal intensities are normalized with Robust Multichip Analysis (www.bioconductor.org/). Responses of the PBMCs are normalized with those induced by autologous sera.

The Hessner laboratory has been applying this technique to Type 1 Diabetes for over a decade. Based on those previous experiences the proposed bioassay allows for a functional assessment of the numerous plasma components and offers an unprecedented means to sensitively differentiate immune states[12,14,23]. When applied to the pediatric heart transplant population, this novel technique offers many innovative aspects:

- The proposed study will be one of the first to look at immune related gene transcriptional profiling in pediatric heart transplant recipients.
- Application of a sensitive and comprehensive bioassay never previously applied to any transplant population. The methodology is ***well-developed, well-validated[9-13,24] and reproducible*** in blinded replicate testing[25].
- The use of algorithms that express the immune state as a quantitative inflammatory index ($I.I._{com}$) that is readily integrated with other clinical measures, such as histological scores, plasma levels of therapeutic agents, and laboratory markers of myocardial injury.

C. Approach

C1. Preliminary Data

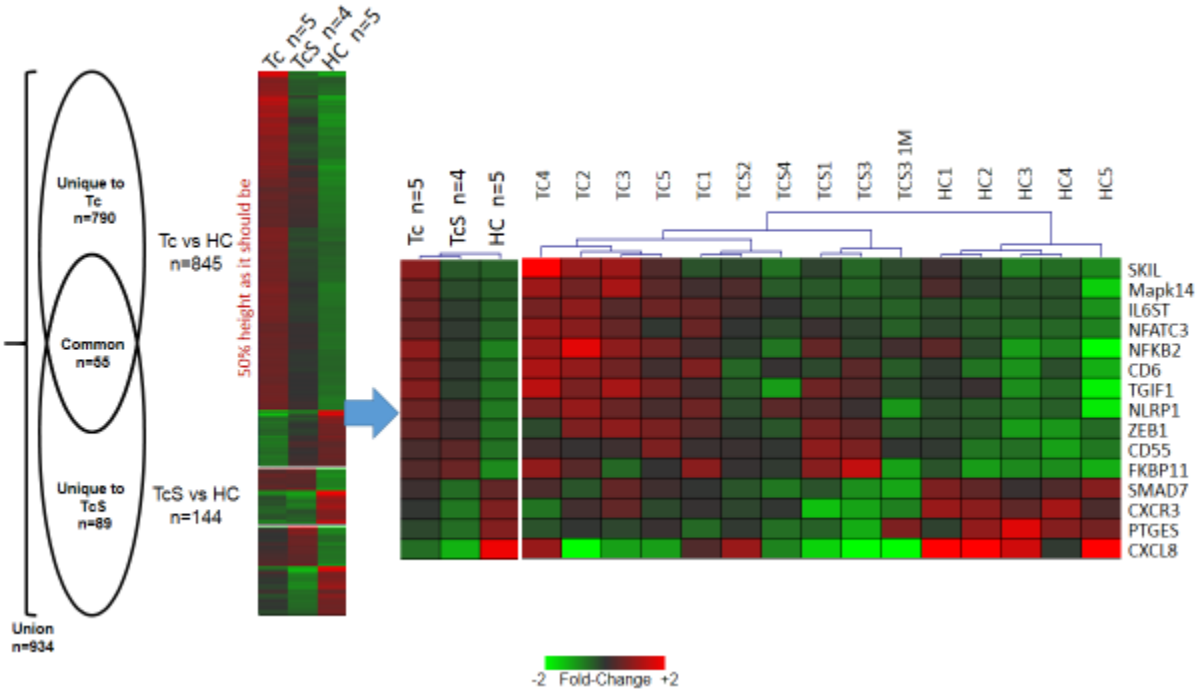

Fig. 2: Transcriptional heat map comparing Tacrolimus+Cellcept (TC) and Tacrolimus+Cellcept+Steroids (TCS) vs Healthy Controls (HC). Total of 934 gene probe sets met statistical significance with 790 probe sets unique to TC, 89 unique to TCS, and 55 common to both TC and TCS. Blue arrow indicates examination of select identified possible candidate genes.

Key: Red indicates transcriptional up-regulation and green indicates down-regulation.

Our preliminary data supports the hypothesis that transplant patients have unique signatures from healthy controls. Furthermore, the transcriptional signature of transplant patients seems dependent on the immunosuppressive regimen used (Fig. 2). When comparing transplant patients on Tacrolimus+Cellcept (TC) and Tacrolimus+Cellcept+Steroids (TCS) vs Healthy Controls (HC) there were statistically significant differences in expression of 934 gene. Of those, 790 genes were unique to the TC group, 89 unique to the TCS group, and 55 in common between TC and TCS when compared to HC. Furthermore, the differently induced genes are consistent with the known mechanisms of action for the used drugs. For example, ZEB1, a transcriptional repressor of IL-2, was up-regulated in patients taking tacrolimus, which is known to suppress IL-2 production. Tacrolimus functions by binding FK Binding Protein (FKBP)[26]. FKBP was up-regulated in patients on tacrolimus. Patients on steroids demonstrated down-regulation of NFK-Beta2, chemokine receptor CXCR3, and glutathione-dependent prostaglandin E synthase but up-regulation in CD55 complement decay accelerating factor. These findings are consistent with previously described mechanisms of action of steroids[27,28]. Both the TC and TCS significantly down regulated CXCL8, a chemotactic factor, when compared to HC. At this point it is not entirely clear whether this represents measured response from the patients or direct effect of dissolved drug on PBMCs during co-culture. To eliminate this confounding variable in future experiments, we plan to “spike in” drug with PBMCs to directly measure drug effect. However, the data does suggest that the assay did actually measure patient response, which demonstrated an underlying pro-inflammatory state in post-transplant patients. Specifically, markers of T-cell activation and proliferation such as: Mapk14, NFATC3, and CD6 were upregulated. Additionally, pro-inflammatory genes IL6ST and NLRP1 Inflammasome were also up-regulated in transplant patients. Addition of steroids to the pharmacological management of transplant patients seemed to either mitigate or even reverse some of these pro-inflammatory states possibly through IL10/TGFB pathways, which is suggested by decreased induction of TGFB repressors such as TGIF1.

C2. Strategy

Subjects will include pediatric heart transplant recipients (ages 0-17 years) at Children’s Hospital of Wisconsin undergoing a cardiac catheterization post-transplant during the 2019-2020 calendar years. During the

catheterization procedure, subjects will have blood collected to undergo plasma induced transcription analysis. The results of the transcriptional analysis will be compared to the obtained endomyocardial biopsies, coronary angiography, labs, and most recent echocardiography. To investigate **Aim 1 (testing hypothesis that plasma induced signatures measured on transplant patients will be different from healthy controls and will be specific to the immunosuppressive regimen used)** samples will be grouped together for analysis based on the immunosuppression regimen of the patient at the time of sample collection. Based on clinical trends from previous years it is expected that >100 pediatric heart transplant recipients will undergo cardiac catheterization over the course of the year. Most commonly patients are on Tacrolimus/Cellcept, Tacrolimus/Cellcept/Steroid, or Tacrolimus/Sirolimus drug combinations. These drug classes will be compared against each other and healthy controls. For the purposes of investigating Aim 1, no samples with evidence of rejection will be included in this analysis. CMV and EBV status at the time of sample collection will also be analyzed. Additionally, to account for the possibility of the therapeutic in the subject's plasma acting directly on the sample, we will "spike" healthy control plasma with therapeutic. If the treatment signature is simply a direct consequence of carry-over drug present in the plasma, it should be possible to largely recapitulate that signature in healthy control samples. In our studies of IL-1 antagonism and CTLA4-Ig trials in Type 1 Diabetes, we successfully used this approach to differentiate the in-vitro versus ex-vivo effect of therapeutics [13,15]. The Children's Hospital of Wisconsin Pharmacy has the means and expertise to provide our team with the necessary drugs diluted down to the concentrations found in patient plasma. Additionally, our study design allows for longitudinal data analysis as >10 patients will have more than one cardiac catheterization with concurrent medication regimen changes. Using the subjects as self-controls, we plan to analyze these longitudinally collected samples to appreciate the difference in the transcriptional signatures between different drug regimens.

To investigate **Aim 2 (testing the hypothesis that plasma induced signatures can identify patients at risk of rejection, produce signatures specific to acute cellular and antibody mediated rejection, and demonstrate reversion to quiescence after induction of immune intervention for treatment of rejection)** collected samples of patients with rejection will be compared vs those without rejection. Presence of rejection will be established by right ventricular endomyocardial biopsy histology obtained at the time of sample collection. Samples will also be grouped together for analysis based on the immunosuppressive regimens of the patients. Analysis will take account of CMV/EBV status, troponin I, NT pro BNP, most recent ejection fraction by echocardiography, presence of donor specific anti-HLA antibodies, and coronary vasculopathy seen by angiography at the time of catheterization. Additionally, longitudinally collected samples will be analyzed for changes in plasma induced signatures prior to onset of rejection, during rejection, and after treatment of rejection to determine if certain signatures are predictive of developing rejection and if plasma induced signatures can be used to show effective response to clinical treatment of rejection. To foster these analyses, the Hessner Laboratory has developed a gene ontology-based scoring algorithm to quantitatively measure temporal changes in immune balance. A composite inflammatory index (I.I.com) is determined by calculating the ratio between the mean log intensity of the inflammatory genes versus the mean log intensity of the regulatory genes. A high score reflects greater inflammatory bias and a low score reflects greater regulatory bias. I.I.com is easily integrated with other clinical measures through regression modelling[12]. I.I.com will be calculated on longitudinally collected samples that have evidence of rejection. Based on previous trends, it is estimated that a 1 year study period will capture >10 patients with rejection, all of which will undergo multiple follow up catheterizations after the diagnosis of rejection.

C3. Methodology

The Hessner Laboratory has been employing plasma induced transcriptional analysis for over a decade. A minimum of 1 mL of blood must be collected in a yellow top tube. Two hundred microliters of plasma will be centrifuged from 1 mL whole blood collected in an ACD Solution A Vacutainer tube. The plasma will be used to stimulate gene expression responses in peripheral blood monocytes (PBMCs) from previously characterized and prequalified healthy blood provided by a commercial vendor. After co-culture, RNA will be isolated using TRIzol (Invitrogen) and hybridized to the Affymetrix U133+2.0 gene chip, which is employed to generate plasma induced transcriptional signatures. Commercial providers now offer highly viable, cryopreserved PBMC where in a single draw of a healthy well-characterized donor, billions of cells, sufficient for thousands of assays are collected by aphaeresis. Our human studies have used UPN727 PBMC (Cellular Technology Ltd) as they closely mimicked the mean response of the fresh PBMC of multiple donors previously used[15]. ***We purchased***

the entire UPN727 lot and possess enough cells to complete the proposed aims. The future identification of highly equivalent PBMC is not an obstacle, as in our donor assessments, the response of UPN524 PBMC correlated well with UPN727. We observe >90% post-thaw viability with UPN727 cells, and flow cytometry finds immunocyte subpopulations within the normal expected ranges for fresh PBMCs[11]. The assay is now highly reproducible. We have passed blinded replicate testing conducted through the JDRF Core for Assay Validation[25].

C4. Analysis

All samples and data associated with Aims 1 and 2 will be de-identified. A secure, password-protected relational database will be used for data storage and management. All analyses will be supported by the statistical expertise of Ms. Jia (a MS level analyst that has been with the Hessner Laboratory since 2003). For Aims 1 and 2, we will use summary statistics to examine distributions and relationships within and between groups for all laboratory and clinical measures. When necessary, for parametric assumptions, we will employ appropriate transformations with justifications. When appropriate, nonparametric tests will be applied (e.g. Mann Whitney and Kruskal Wallis). Inverse probability of censoring weighing will be used to incorporate censoring when present. We will utilize SAS v9.4 or newer, the R library, including SIMCA, SPSS v18.0, and Salford Systems Random Forest analysis. All analyses will consider age and sex as a variables. The study will be adequately powered with >100 collected patient samples with longitudinal data from 10-20 patients.

C5. Caveats, Anticipated Outcomes, and Alternatives

Our pilot data supports the hypothesis that different drug regimens will produce unique transcriptional signatures. Furthermore, our preliminary data suggests the presence of a pro-inflammatory state after transplantation that may play a pivotal role in the development of rejection. Our proposed study should be able to accurately and reliably detect the presence of rejection while also identifying those at risk of developing rejection and monitoring therapeutic response to rejection treatment through novel applications such as the inflammatory index (I.I.com). An alternative to induced transcriptional analysis is analysis directly from patient tissue/cells, such as Molecular Microscrope®. Molecular Microscrope® is not yet commercially available and has not been validated in pediatrics. However, future work can directly compare the results of plasma induced transcriptional analysis with those of Molecular Microscrope® to determine if our assay reliably identifies the same target genes.

References

(1) Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. J Heart Lung Transplant 2010 Aug;29(8):914-95.

(2) Costello JP, Mohanakumar T, Nath DS. Mechanisms of chronic cardiac allograft rejection. Tex Heart Inst J 2013;40(4):395-399.

(3) Crespo-Leiro MG, Barge-Caballero G, Couto-Mallon D. Noninvasive monitoring of acute and chronic rejection in heart transplantation. Curr Opin Cardiol 2017 Mar 16.

(4) Deng MC, Eisen HJ, Mehra MR, Billingham M, Marboe CC, Berry G, et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant 2006 Jan;6(1):150-160.

(5) Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, et al. Gene-expression profiling for rejection surveillance after cardiac transplantation. N Engl J Med 2010 May 20;362(20):1890-1900.

(6) Crespo-Leiro MG, Stypmann J, Schulz U, Zuckermann A, Mohacsi P, Bara C, et al. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J 2016 Sep 1;37(33):2591-2601.

(7) Crespo-Leiro MG, Zuckermann A, Bara C, Mohacsi P, Schulz U, Boyle A, et al. Concordance among pathologists in the second Cardiac Allograft Rejection Gene Expression Observational Study (CARGO II). Transplantation 2012 Dec 15;94(11):1172-1177.

(8) Halloran PF, Potena L, Duong Van Huyen JP, Bruneval P, Leone O, Kim DH, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: the heart molecular microscope MMDx. J Heart Lung Transplant 2017;36(11):1192-200.

(9) Wang X, Jia S, Geoffrey R, Alemzadeh R, Ghosh S, Hessner MJ. Identification of a molecular signature in human type 1 diabetes mellitus using serum and functional genomics. J Immunol. 2008;180(3):1929-1937.

(10) Jia S, Kaldunski M, Jailwala P, et al. Use of transcriptional signatures induced in lymphoid and myeloid cell lines as an inflammatory biomarker in Type 1 diabetes. Physiol Genomics. 2011;43(11):697-709.

(11) Levy H, Wang X, Kaldunski M, et al. Transcriptional signatures as a disease-specific and predictive inflammatory biomarker for type 1 diabetes. Genes Immun. 2012;13(8):593-604.

(12) Chen YG, Cabrera SM, Jia S, et al. Molecular signatures differentiate immune States in type 1 diabetic families. Diabetes. 2014;63(11):3960-3973.

(13) Cabrera SM, Chen YG, Hagopian WA, Hessner MJ. Blood-based signatures in type 1 diabetes. Diabetologia 2016 Mar;59(3):414-425.

(14) Gurram B, Salzman NH, Kaldunski ML, Jia S, Li BU, Stephens M, et al. Plasma-induced signatures reveal an extracellular milieu possessing an immunoregulatory bias in treatment-naive paediatric inflammatory bowel disease. Clin Exp Immunol 2016 Apr;184(1):36-49.

(15) Cabrera, SM, Engle, S, Kaldunski, M. et al. Diabetologia (2018) 61: 2356.

(16) Spivey TL, Uccellini L, Ascierto ML, Zoppoli G, De Giorgi V, Delogu LG, et al. Gene expression profiling in acute allograft rejection: challenging the immunologic constant of rejection hypothesis. J Transl Med 2011 Oct 12;9:174-5876-9-174.

(17) Kurian SM, Williams AN, Gelbart T, Campbell D, Mondala TS, Head SR, et al. Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling. Am J Transplant 2014 May;14(5):1164-1172.

(18) Flechner SM, Kurian SM, Head SR, Sharp SM, Whisenant TC, Zhang J, et al. Kidney transplant rejection and tissue injury by gene profiling of biopsies and peripheral blood lymphocytes. Am J Transplant 2004 Sep;4(9):1475-1489.

(19) Loupy A, Duong Van Huyen JP, Hidalgo L, Reeve J, Racape M, Aubert O, et al. Gene Expression Profiling for the Identification and Classification of Antibody-Mediated Heart Rejection. Circulation 2017 Mar 7;135(10):917-935.

(20) Bodez D, Hocini H, Tchitchek N, Tisserand P, Benhaiem N, Barau C, et al. Myocardial Gene Expression Profiling to Predict and Identify Cardiac Allograft Acute Cellular Rejection: The GET-Study. PLoS One 2016 Nov 29;11(11):e0167213.

(21) Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med. 2005;201(9):1479-1486.

(22) Palatnik A, Ye S, Kendziorski C, et al. Identification of a serum-induced transcriptional signature associated with metastatic cervical cancer. PloS one. 2017;12(8):e0181242.

(23) Cabrera SM, Wang X, Chen YG, et al. Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset. Eur J Immunol. 2016;46(4):1030-1046.

(24) Cabrera SM, Henschel AM, Hessner MJ. Innate inflammation in type 1 diabetes. Transl Res. 2016;167(1):214-227.

(25) Speake C, Odegard JM. Evaluation of Candidate Biomarkers of Type 1 Diabetes via the Core for Assay Validation. Biomark Insights. 2015;10(Suppl 4):19-24.

(26) Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther Drug Monit. 1995 Dec;17(6):584-91.

(27) Newton R Molecular mechanisms of glucocorticoid action: what is important? Thorax 2000;55:603-613.

(28) Agrawal A, Sinha A, Ahmad T, et al. Maladaptation of critical cellular functions in asthma: bioinformatic analysis. Physiological Genomics. 2009 40:1,1-7

Program Director/Principal Investigator (Last, First, Middle): Steven J. Kindel

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 04/01/2019	THROUGH 04/01/2020

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Steven J. Kindel	PD/PI	0.24			189,600	3,792	557	4,349
Alexander Raskin	Co-PI	0.24			68,000	0	0	0
Martin J. Hessner	Co-I	0.12			189,600	1,896	279	2,175
Ann Punnoose	Co-I	0.12			189,600	1,896	279	2,175
Jia Shuang	Project Analyst	1.20			81,960	8,196	2,393	10,589
Mary Kaldunski	Technician	1.20			78,376	7,838	2,289	10,127
SUBTOTALS →						23,618	5,797	29,415

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* 130 assay samples at $350/sample	45,500
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 74,915
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 74,915

CHILDREN'S RESEARCH INSTITUTE
V - BUDGET JUSTIFICATION

Personnel: **Steven J. Kindel, MD (Program Director/Principal Investigator; 2% effort, 0.24 calendar months).** Dr. Kindel is an Associate Professor in the Division of Cardiology, Department of Pediatrics at the Medical College of Wisconsin (MCW). He currently serves as the medical director of advanced heart failure and heart transplantation at the Children's Hospital of Wisconsin. He has been actively involved in multiple collaborative multi-center programs including the Pediatric Heart Transplant Study Foundation (PHTS). Dr. Kindel's research interest lies in improving outcomes for children after cardiac transplantation. He has served as either PI or Co-PI in multi-center trials, including a study of donor-specific cell free DNA as a non-invasive marker of transplant rejection (RO1 funded). To date, his efforts have led to numerous peer-reviewed publications in the field of pediatric heart failure and transplantation. Dr. Kindel will oversee and lead all aspects of the project, including analyzing data, writing manuscripts, and presenting data at meetings. He will coordinate all project meetings

Alexander Raskin, MD (Co-Principal Investigator; 0% effort, 0 calendar months). Dr. Raskin is a Pediatric Cardiology Fellow employed by MCW Affiliated Hospitals (MCWAH). His effort is being provided by MCWAH, and no funding is needed from the grantor. Dr. Raskin is preparing for a career in Pediatric Heart Failure and Transplant medicine. He has a particular interest in translational research. This study will help prepare Dr. Raskin for scientific independence in research. He will assist in recruitment of subjects, interpretation of data, and in publication/presentation.

Martin J. Hessner, Ph.D. (Co-Investigator; 1% effort, 0.12 calendar months). Dr. Hessner is a tenured Professor in the Division of Endocrinology, Department of Pediatrics and the Department of Microbiology, Immunology and Molecular Genetics at MCW, and Director of the Max McGee Research Center. He previously conducted clinical research on genetics/genotyping of hematologic antigen systems. He is the PI of the McGee Center Family Genetics Study. To date, his efforts have led to more than 100 peer-reviewed publications and highly collaborative projects using genomics tools he has developed. Dr. Hessner will help support all laboratory aspects of the project. He will also assist in analyzing data.

Ann Punnoose, MD (Co-Investigator; 1% effort, 0.12 calendar months). Dr. Punnoose is an Assistant Professor in the Division of Cardiology, Department of Pediatrics at MCW, specializing in heart failure and transplantation. Her research interest lies in improving morbidity and mortality in pediatric heart transplant patients. She currently is a co-investigator in the multicenter TEAMMATE trial of everolimus in children post transplant. For this study, she will be instrumental in recruitment of study subjects, interpretation/integration of clinical data, as well as data publication and presentation.

Shuang Jia, M.S., Project Analyst (10% effort; 1.2 calendar months). Ms. Jia is a bioinformatics specialist. She is an expert programmer, proficient in R and MatLab, as well as specialized tools for gene expression data analysis, including Partek, STEM, Genesis, IPA and others. She will perform data analysis for the project using in-house as well as commercially available software tools.

Mary Kaldunski, B.S., Hessner Laboratory Manager (10% effort; 1.2 calendar months). Ms. Kaldunski is highly proficient in Affymetrix GeneChip analysis, flow cytometry/FACS analysis, ELISA, and tissue culture. She will manage all laboratory aspects of the project

Fringe Benefits: The Medical College of Wisconsin's institutionally identified fringe benefit rate is 14.7% for faculty and 29.2% for staff.

Personnel Requested: Requested salaries and fringe benefits for faculty are compliant with the budget guidelines (not to exceed 10% of overall budget). Amount requested for Faculty and Staff salary support including fringe benefits: **$29,415**

Supplies: Plasma induced transcription analyses (Aim 1 & Aim 2). Estimated 130 assays total consisting of 100 transplant patient assays, including longitudinally collected samples, and 30 healthy controls at $350/assay. **Supplies requested: $45,500**

Total Amount Requested: $74,915

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kindel, Steven J

eRA COMMONS USER NAME (credential, e.g., agency login): SKINDEL

POSITION TITLE: Associate Professor Pediatrics, Section of Pediatric Cardiology, Medical College of Wisconsin, Milwaukee, WI

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Washington University St. Louis, MO	BS	06/1999	Biomedical Engineering
The Ohio State University College of Medicine Columbus, OH	MD	06/2003	Medicine
Children's Memorial Hospital Northwestern University Feinberg School of Medicine Chicago, IL	Residency	06/2007	Pediatrics
Cincinnati Children's Hospital Medical Center Cincinnati, OH	Fellowship	06/2010	Pediatric Cardiology
Children's Memorial Hospital Northwestern University Feinberg School of Medicine Chicago, IL	Fellowship	06/2011	Advanced Heart Failure & Heart Transplantation

A. Personal Statement

I am well-qualified to participate as a member of the investigative team for the Plasma induced transcription analysis in pediatric heart transplantation as an assessment of rejection. I have extensive clinical and research experience in the field of pediatric heart failure and heart transplantation. I currently serve as the medical director of advanced heart failure and heart transplantation at the Children's Hospital of Wisconsin and in this role I direct the care of a panel of approximately 120 post transplant patients and another 50 children with cardiomyopathy and varying stages of heart failure. In addition I continue to serve multiple regional and national leadership roles in the field of pediatric heart transplantation and heart failure. I am actively involved in teaching and mentoring medical students, residents, and fellows daily through my various clinical and research roles. Finally, I have become involved in multiple collaborative multi-center programs including the Pediatric Heart Transplant Study Foundation (PHTS) where I have served as a PI for a multi-center research project, a writing group member for others, and have served a role on the research steering committee. Our center is involved in three multi-center clinical trials in heart failure and heart transplantation with me either serving as PI or Co-PI including a study of donor-specific cell free DNA as a non-invasive marker of transplant rejection (RO1 funded), the PANORAMA study of Entresto in pediatric heart failure (industry sponsored), and the TEAMMATE trial of everolimus in children post transplant (Military Grant funded). These opportunities have allowed me to lead a recent multi-center study on cardiac allograft vasculopathy in children as well as to contribute to state-of-the-art review and preparation of a new guideline statement on the evaluation and management of heart failure in children. For these reasons, I am qualified to be the principal investigator in the proposed research looking at plasma induced transcriptional analysis as an assessment of rejection a pediatric heart transplant population.

1. **Kindel SJ**, Law Y, Chin C, Burch M, Kirklin J, Naftel D, Pruitt E, Carboni M, Arens A, Atz A, Dreyer J, Mahle W, Pahl E. Assessment of functional parameters improves detection of significant

cardiac allograft vasculopathy in children following heart transplant: A multi-institutional analysis of 3120 pediatric heart transplant recipients. JACC Vol 66, No 5 4Aug15; 547-557
2. **Kindel SJ**, Everitt MD. A Contemporary Review of Pediatric Heart Transplant and Mechanical Circulatory Support. Cardiol Young. 2016 Mar 16:1-9.
3. Etiology and Pathophysiology of Heart Failure. In: Kirk R, Dipchand I, Rosenthal D ISHLT Monograph Series Volume 8: ISHLT Guidelines for the Management of Pediatric Heart Failure. University of Alabama, Birmingham. Birmingham, AL 4/2014

B. Positions and Honors

Positions and Employment

2007 - 2010 Member, Graduate Medical Education Committee Fellow Representative, Cincinnati Children's Hospital Medical Center
2010 - 2013 Member, Heart Center Research Committee, Ann & Robert H. Lurie Children's Hospital of Chicago
2010 - 2013 Member, PTLD Multidisciplinary Working Group, Ann & Robert Lurie Children's Hospital of Chicago
2011 - 2013 Member, Transplant Outcomes Improvement Committee, Ann & Robert H. Lurie Children's Hospital of Chicago
2011 - 2013 Member, NPC-QIC Project Site Leader, Ann & Robert H. Lurie Children's Hospital of Chicago
06/2011 - Present Member, Junior Faculty and Trainee Committee, International Society of Heart & Lung Transplantation
06/2011 - Present Member, Standards and Guidelines Committee, International Society of Heart & Lung Transplantation
09/2011 - 07/2013 Assistant Professor of Pediatrics, Division of Pediatric Cardiology, Ann & Robert H. Lurie Children's Hospital / Northwestern University Feinberg School of Medicine, Chicago, IL
07/2013 - 06/2015 Medical Director, Advanced Heart Failure & Transplantation, Children's Hospital and Medical Center, Omaha, NE
07/2013 - 06/2015 Assistant Professor of Pediatrics, Pediatric Cardiology, Children's Hospital and Medical Center, University of Nebraska College of Medicine, Omaha, NE
2013 - Present Member, Pediatric Heart Failure Work Group, International Society for Heart and Lung Transplantation
10/2014 - 06/2015 Assistant Professor of Clinical Pediatrics, Creighton University School of Medicine, Omaha, NE
07/2015 - Present Assistant Professor of Pediatrics, Medical College of Wisconsin, Milwaukee, WI
2015 - Present Member, At Large Representative Pediatric Transplant, United Network for Organ Sharing, Program Committee Representative
2015 - Present Member, Pediatric Heart Transplant Study Foundation Research Steering Committee

Other Experience and Professional Memberships

2007 - Present American Heart Association (Member)
2010 - Present International Society of Heart & Lung Transplantation (Member)
2011 - Present Heart Failure Society of America (Member)

Honors and Awards

03/2009 Becoming a Young Investigator - Travel Grant Award, Pediatric Heart Network

C. Contribution to Science

My research has focused on two primary areas in the care of the children with end stage heart failure and heart transplantation.

1. Identification and management of cardiac allograft vasculopathy (CAV) in children following heart transplantation: While heart transplantation has become a well accepted and successful approach to the management of children with end-stage heart failure with 1 year survival approaching 90-95% at many centers. Further, one year survival has improved progressively over the last 3 decades when comparing early to late eras. However, the despite several advances in the care of these children there has been minimal improvement in the long term hazard for graft loss or death following the first year when comparing between eras. The most common cause of late graft loss in children after the fifth year post-transplant is cardiac allograft vasculopathy (CAV) which is a progressive immune and non-immune mediated process that leads to graft dysfunction. We recently performed an analysis of the rich data set collected through the Pediatric Heart Transplant Study to evaluate the incidence, monitoring and outcomes of children with CAV and specifically to analyze how hemodynamic profiles may help better understand the degree of CAV in these children. This data revealed a powerful association of graft loss to changes in hemodynamics and allowed determination of pediatric specific cut-points for more severe disease. I have also led a single center review of eosinophilic disease in children as a marker of immunesuppression status and its correlation to outcomes which demonstrated that eosinophilic infiltrative disease of the GI tract is relatively common post-heart transplantation and indicates a more significant degree of immunesuppression along with increased risk of post-transplant lymphoproliferative disease and lower rates of rejection. The Targeted, Highly Sensitive, Non-Invasive Cardiac Transplant Rejection Monitoring project is an important continuation of this work as it could potentially allow a better monitoring approach for these children post-transplant and further may improve our understanding of the ongoing process of cardiac injury following hear transplantation with correlation to outcomes and management strategies.

    a. Kindel SJ, Pahl E. “Cardiac allograft vasculopathy in children - Treatment challenges”; Progress in Pediatric Cardiology Vol. 32(1) August 2011: 37-42.
    b. Kindel SJ, Pahl E. “Current therapies for cardiac allograft vasculopathy in children.” Congenital Heart Disease. 2012 Jul-Aug;7(4):324-35
    c. Kindel SJ, Law Y, Chin C, Burch M, Kirklin J, Naftel D, Pruitt E, Carboni M, Arens A, Atz A, Dreyer J, Mahle W, Pahl E. Assessment of functional parameters improves detection of significant cardiac allograft vasculopathy in children following heart transplant: A multi-institutional analysis of 3120 pediatric heart transplant recipients. JACC Vol 66, No 5 4Aug15; 547-557
    d. Kindel SJ, Joy BF, Pahl E, Wald EL. Eosinophilic esophagitis in children following cardiac transplantation: association with post-transplant lymphoproliferative disorder and other transplant outcomes. Pediatric Transplantation 2014 Aug;18(5):491-6.

2. The evaluation and management of children with significant cardiac dysfunction: Unlike adults, the etiologies of pediatric heart failure are diverse and multitudinous with genetic, familial, metabolic, syndromic, arrhythmogenic, and infectious causes all playing a major role along with a significant number of children with cardiac dysfunction secondary to the effects of surgical repair or palliation. The etiology of heart disease in these children is an important prognostic indicator and in many cases targeted therapies are available to improve outcomes. I have researched the etiologic causes of cardiac dysfunction in children at both the clinical and basic science level working on projects related to the development of a massively parallel sequencing platform for pediatric Cardiomyopathy as well as the application of this testing at the clinical level. Further I continue to focus on the development of acute heart failure diagnostic pathways and management approaches both in the ICU and clinic setting. This work includes assessment of patients with end-stage congenital heart disease requiring transplantation with focus on high risk groups such as single ventricle patients who have worse outcomes historically following heart transplantation. Through this work I have been invited to participate in production of a review chapter on acute heart failure in the pediatric patient in the ICU through the Society of Critical Care Medicine for their board review materials and for the most recent guidelines in the evaluation and management of pediatric heart failure as produced by the International Society for Heart and Lung Transplantation. In addition our site has recently been chosen as a site for a multi-center randomized controlled drug trial on LCZ696 heart failure compound in pediatric patients to begin this summer for which I will serve as site PI.

    a. Kindel SJ, Miller EM, Gupta R, Cripe LC, Hinton RB, Spicer RL, Towbin JA, Ware SM. “Pediatric cardiomyopathy: importance of genetic and metabolic evaluation”; J Card Failure. May 2012; 18(5): 396-403

b. Tariq M, Le T, Putnam P, Kindel S, Keddache M, Ware SM. “Targeted capture and massively parallel sequencing in pediatric cardiomyopathy: development of novel diagnostics” Cardiogenetics. Vol 2, No 1 (2012)
c. Backer CL, Russell HM, Pahl E, Gambetta K, Kindel SJ, Gossett JG, Hardy C, Costello JM, Deal BJ. Heart transplantation for the failing Fontan. Ann Thorac Surg. 2013 Oct;96(4):1413-9
d. Etiology and Pathophysiology of Heart Failure. In: Kirk R, Dipchand I, Rosenthal D ISHLT Monograph Series Volume 8: ISHLT Guidelines for the Management of Pediatric Heart Failure. University of Alabama, Birmingham. Birmingham, AL 4/2014
e. Heart Failure. In:. Stockwell JA, Preissig CM, Kane JM, eds. Comprehensive Critical Care Review: Pediatric. Society of Critical Care Medicine. Mount Prospect, IL 8/2012

Ongoing Research Support

NIH R01 HL119747-01 Mitchell (PI) 07/23/13 – 05/31/18

Targeted, Highly Sensitive, Non-Invasive Cardiac Transplant Rejection Monitoring

The major goal of this project is to evaluate donor specific DNA as a biomarker for rejection in a multicenter clinical study.

Role: Site Principal Investigator

Novartis Pharmaceuticals Corporation 01/01/17 – 09/30/21

Protocol LCZ696B2319 Multicenter, open-label, study to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics of LCZ696 followed by a 52-week randomized, double-blind, parallel group, active-controlled study to evaluate the efficacy and safety of LCZ696 compared with enalapril in pediatric patients from- 1 month to < 18 years of age with heart failure due to systemic left ventricle systolic dysfunction

The purpose of this study is to determine whether pediatric HF patients will derive greater clinical treatment benefit assessed by a global rank endpoint with LCZ696 compared to enalapril over 52-weeks treatment duration. This study includes two parts. Part 1 will determine the dose for Part 2. Part 2 will be used to assess the efficacy and safety of LCZ696 compared to enalapril

Role: Site Principal Investigator

Completed Research Support
None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Hessner, Martin John

eRA COMMONS USER NAME (credential, e.g., agency login): mhessner

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Carroll College, Waukesha, WI	B.S.	05/1985	Biology
University of Wisconsin-Milwaukee, Milwaukee, WI	Ph.D.	12/1990	Micro/Molecular Biology
The BloodCenter of Wisconsin, Milwaukee, WI	Post-doc	12/1993	Molecular Biology

A. Personal Statement

My interest and experience in developing novel genetic/genomic applications for human disease spans nearly 30 years. As a post-doctoral trainee at the BloodCenter of Wisconsin, I focused on developing and demonstrating clinical utility of new molecular methods in the bone marrow transplant setting, specifically nucleic acid based HLA typing/sequencing methods, and approaches for monitoring residual disease and donor engraftment. This led to directing a group focused on further applications, including hematological genotyping assays for detecting maternal/fetal incompatibilities and assessing genetic risk for coagulopathy. In 2001, I joined the faculty of the Medical College of Wisconsin (MCW) to apply my skills as a molecular biologist. I am experienced at managing projects, budgets, laboratories, and now am applying these experiences programmatically in directing a diabetes research center. I have served as PI or Co-Investigator on many successful intra- and extramural-funded projects that have resulted in relevant publications, a better understanding of human disease, and improved patient care. In T1D, I have made relevant contributions. Using a novel genomics-based bioassay, our group has identified an innate inflammatory state in T1D families that is independent of auto-antibody status, HLA haplotype, or T1D progression. In collaboration with other investigators, I have successfully applied this approach to study other pediatric inflammatory conditions such inflammatory bowel disease and juvenile idiopathic arthritis. My previous works and experiences make me well qualified to apply our novel genomics-based bioassay as part of Dr. Kindel's project assessing plasma induced transcription analysis as an assessment of rejection in a pediatric heart transplant population. With the utilization of this technique, we are uniquely positioned to gain new knowledge regarding the mechanisms, diagnosis, and treatment of heart transplant rejection in a pediatric population.

B. Positions and Honors

Positions and Employment

1993–1996 Associate Director/Product Development Scientist, Cancer Diagnostics Laboratory, The Blood Center of Wisconsin, Milwaukee, WI
1996–2000 Director of Product Development, Diagnostic Laboratories, The Blood Center of Wisconsin, Milwaukee, WI
1995–2000 Lecturer, Department of Health Sciences, The University of WI-Milwaukee, Milwaukee, WI
2001–2004 Assistant Professor, Department of Pediatrics, The Medical College of WI, Milwaukee, WI
2004–2010 Associate Professor, Department of Pediatrics, The Medical College of WI, Milwaukee, WI
2001–present Director, Microarray Laboratory within The Human and Molecular Genetics Center, The Medical College of WI, Milwaukee, WI
2007–2010 Associate Professor, Department of Microbiology and Molecular Genetics (Secondary Appointment), The Medical College of WI, Milwaukee, WI

2008–present Director, The Max McGee National Research Center for Juvenile Diabetes, Department of Pediatrics, The Medical College of WI, Children's Hospital of Wisconsin, Milwaukee, WI
2010–present Professor (tenured), Department of Pediatrics, (Primary Appointment), Department of Microbiology and Molecular Genetics (Secondary Appointment), The Medical College of WI, Milwaukee, WI

Other Experience and Professional Memberships

2007–present Member, The American Diabetes Association
2003 NIH Study Section: SEP Genomic Technologies ZRG1 GNM 90S.
2010 NIH Study Section: SEP Vaccines Against Microbial Diseases. ZRG1 IMM-N (02) M
2011 NIH Study Section: Hypersensitivity, autoimmune, and immune-mediated diseases (Ad Hoc)
2013 NIH Study Section: Special Emphasis Panel ZDK1-GRB-S (M1)
2011–2016 T1D Exchange Biobank Scientific Review Committee
2012–2015 American Diabetes Association Research Grant Review Committee
2014-2016 NIH/NIDDK TrialNet Biomarkers and Mechanisms Panel
2015-2019 NIH Study Section: Hypersensitivity, autoimmune, and immune-mediated diseases; standing member
2015-2017 Co-chair, Juvenile Diabetes Research Foundation Biomarker Working Group
2018-present NIH/NIDDK TrialNet Collaborative Mechanistic Studies Panel

Honors

2009 Invited Speaker: Ninth Annual Rachmiel Levine Diabetes and Obesity Symposium
2009 Invited Speaker: 10th International Congress of the Immunology of Diabetes Society
2010 Invited Speaker: American Diabetes Association's 70th Annual Meeting
2014 Recipient: Advancing a Healthier Wisconsin (AHW) Excellence Award in Innovation
2014 Recipient: Juvenile Diabetes Research Foundation Living and Giving Award
2015 Recipient: Carroll University Distinguished Alumni Award for Professional Achievement
2015 Recipient: American Diabetes Association, Wisconsin Chapter, LEARN Award
2018 University of Washington Type 1 Diabetes Poll Visiting Scholar Lecture
2018 Invited Speaker: American Diabetes Association's 78th Annual Meeting

C. Contribution to Science

1. When I entered the field of functional genomics in 2001, data quality issues with microarray analyses truly impaired their application. As reflected by my publications as a junior faculty member, my clinically focused molecular genetics background served me in developing a robust, quality controlled, technical and analytical infrastructure focused on the generation of reliable gene expression data.
    a. **Hessner M.J.**, X. Wang, K. Hulse, L. Meyer, Y. Wu, S. Nye, S.W. Guo, S Ghosh. (2003). Three color cDNA microarrays: Quantitative assessment through the use of fluorescein-labeled probes. Nucleic Acids Research, 31:e14. PMID: 12582259.
    b. **Hessner, M.J.**, X. Wang, S. Khan, L. Meyer, M. Schlicht, J. Tackes, M.W. Datta, H. J. Jacob, S. Ghosh. (2003). Use of a three-color cDNA microarray platform to measure and control support-bound probe for improved data quality and reproducibility. Nucleic Acids Research, 31:e60. PMCID: PMC156737.
    c. **Hessner, M.J.**, V.K. Singh, X. Wang, S. Khan, M.R. Tschannen, T.C. Zahrt. (2004). Utilization of a labeled tracking oligonucleotide for visualization and quality control of spotted 70-mer arrays. Genomics, 5:12. PMCID: PMC362869.

2. The triggering and pathogenesis of T1D remains incompletely defined. Through our functional genomics infrastructure and collaborations with Dr. Åke Lernmark and other investigators, my laboratory has focused on developing a natural history of T1D pathogenesis in the BioBreeding (BB) rat model. We have discovered the presence of an elevated innate inflammatory state associated with T1D susceptibility. Through the study of BBDR+/+ rats, which do not develop spontaneous T1D, we discovered that this innate state is independent of insulitis and diabetes progression. We determined that this innate state is temporally supplanted by an IL-10/TGF-β mediated regulated state that coincides with the inability of virus to trigger T1D progression, offering insight as to the juvenile nature of T1D in this inducible model system. Further we have determined the innate state in BB rats extends to the islet level, as reflected by beta cell

chemokine expression, and that it can be normalized through modulation of the diet and intestinal microbiota.

a. Geoffrey, R., S. Jia, A.E. Kwitek, J. Woodliff, S. Ghosh, Å. Lernmark, X. Wang, **M.J. Hessner**. (2006). Evidence of a functional role for mast cells in the development of type 1 diabetes mellitus in the BioBreeding rat. The Journal of Immunology, 177:7275-86. PMID: 17082646.
b. Kaldunski, M.L., S. Jia, R. Geoffrey J. Basken, S. Prosser, S. Kansra, J.P. Mordes Å. Lernmark, X. Wang, **M.J. Hessner**. (2010). Identification of a serum-induced transcriptional signature associated with type 1 diabetes in the BioBreeding Rat. Diabetes, 59(10):2375-85. PMCID: PMC3279523.
c. Chen, Y.-G., J.P. Mordes, E. Blankenhorn, H. Kashmiri, M.L. Kaldunski, S. Jia, R. Geoffrey, X. Wang, **M.J. Hessner**. (2013). Temporal induction of immunoregulatory processes coincides with age-dependent resistance to viral-induced Type 1 Diabetes. Genes and Immunity (6):387-400. PMCID: PMC4027975.
d. Henschel, A.M., S.M. Cabrera, M.L. Kaldunski, S. Jia, R. Geoffrey, M.F. Roethle, V. Lam, Y.-G. Chen, X. Wang, N.H. Salzman, **M.J. Hessner**. 2018. Modulation of the gastrointestinal microbiota normalizes systemic inflammation and islet immunocyte recruitment potential associated with autoimmune diabetes susceptibility. PLoS One. 13(1):e0190351. PMCID: PMC5749787.

3. New immune measures are needed in human T1D studies for improved disease prediction and monitoring responses to therapeutic intervention. We developed and applied a novel array-based bioassay, whereby subject plasma is used to induce transcriptional responses in a well-controlled reporter cell population. With this sensitive and comprehensive approach, we determined that pre- and recent onset T1D plasma induces a disease-specific, partially IL-1 dependent signature relative to unrelated healthy controls. With it, we also identified an innate inflammatory state among healthy T1D family members that is temporally supplanted by an IL-10/TGF-β mediated regulatory state amongst sibling non-progressors possessing high-risk HLA haplotypes. Emergence of this regulated state parallels peripheral increases in activated CD4+/CD45RA-/FoxP3^{high} regulatory T-cell (Treg) frequencies, suggesting that failures in endogenous regulatory mechanisms that normally manage inherited T1D risk may underlie disease progression. Notably with this approach high inflammation at baseline appears to be predictive of the rate of post-onset disease progression as well as responsiveness to immunotherapy.
    a. Wang, X., S. Jia, R. Geoffrey, R. Alemzadeh, S. Ghosh, **M.J. Hessner**. (2008). Identification of a molecular signature in human type 1 diabetes mellitus using serum and functional genomics. Journal of Immunology, 180:1929-1937. PMID: 18209091.
    b. Levy, H., X. Wang, M. Kaldunski, S. Jia, J. Kramer, S.J. Pavletich, M. Reske, T. Gessel, M. Yassai, M.W. Quasney, M.K. Dahmer, J. Gorski, **M.J. Hessner**. (2012). Transcriptional signatures as a disease-specific and predictive inflammatory biomarker for Type 1 diabetes. Genes and Immunity. 13:593-604. PMCID: PMC4265236.
    c. Chen, Y.-G., S. Cabrerra, S. Jia, M.L. Kaldunski, J. Kramer, S. Cheong, R. Geoffrey, M.F. Roethle, J.E. Woodliff, C.J. Greenbaum, X. Wang, **M.J. Hessner**. (2014). Molecular signatures differentiate immune states in Type 1 Diabetes families. Diabetes. 63:3960–3973. PMCID: PMC4207392.
    d. Cabrera, S.M., S. Engle, M. Kaldunski, S. Jia, R. Geoffrey, P. Simpson, A. Szabo, C. Speake, C.J. Greenbaum, T1D TrialNet CTLA4-Ig Study Group, Y.G. Chen, **M.J. Hessner**. Innate immune activity as predictor of persistent insulin secretion and association with responsiveness to CTLA4-Ig treatment in recent onset T1D Diabetologia, 2018; ***IN PRESS***.

4. In addition to the contributions described above, I have worked closely with others within the field, in collaborative as well as mentoring roles, to advance an understanding of the genetic basis and immunological processes underlying T1D pathogenesis.
    a. Bogdani, M., A.M. Henschel, S. Kansra, J.M. Fuller, R. Geoffrey, S. Jia, M.L. Kaldunski, S. Pavletich, Y.-G. Chen, Å. Lernmark, **M.J. Hessner**. (2013). Biobreeding rat islets exhibit reduced anti-oxidative defense capacity and N-acetyl cysteine treatment delays Type 1 diabetes onset. Journal of Endocrinology. 216(2):111-23. PMCID: PMC4027975.
    b. Chen, Y.-G., M. Forsberg, S. Khaja, A.E. Ciecko, **M.J. Hessner**, A.M. Geurts. (2014). Gene targeting in NOD mouse embryos using zinc-finger nucleases. Diabetes. 63(1):68-74. PMCID: PMC3868049.
    c. Cabrera, S.M., X. Wang, Y.-G. Chen, S. Jia, M.L. Kaldunski, C.J. Greenbaum and the Type 1 Diabetes TrialNet Canakinumab Study Group, T. Mandrup-Poulsen and the AIDA Study Group,

M.J. Hessner. (2016). Interleukin-1 antagonism moderates the inflammatory state associated with Type 1 diabetes during clinical trials conducted at disease onset. European Journal of Immunology, 46(4):1030-46. doi: 10.1002/eji.201546005. PMID: 26692253. PMC4828314.

d. Cabrera, S.M., Y.-G. Chen, W.A. Hagopian, **M.J. Hessner**. (2016). Blood-Based Signatures in Type 1 Diabetes. Diabetologia. 59(3):414-25. PMID: 26699650. PMC4744128

Complete List of Published Work in MyBibliography (>100 publications):
http://www.ncbi.nlm.nih.gov/sites/myncbi/martin.hessner.1/bibliograpahy/40453852/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

JDRF 3-SRA-2018-478-S-B Hessner (PI) 10-01-2017 – 9-30-2020
Prediction of post-onset partial remission duration in new onset T1D
This study is validating the use of plasma induced transcription for prediction of the post-onset partial remission period in new onset T1D patients which will improve post onset management and stratification of patients for clinical trials.
Role: PI

NIH/NIDDK 1R56DK108802 Hessner (PI) 7/1/2016 – 06/30/2018, NCE to 6/30/2019
Plasma Induced Signatures as a Measure of Immunomodulation in T1D Clinical Trials
This study applies serum/plasma induced transcription assay to human clinical trials in new onset T1D
Role: PI

American Diabetes Association (1-18-ICTS-094) Cabrera (PI) 01/01/18 – 12/30/2020
Gluten and amylase trypsin inhibitors (ATI) as nutritional contributors to type 1 diabetes susceptibility
This study investigates a role for gluten and ITI as contributors to the innate state associated with T1D susceptibility in the BB rat model and human T1D families.
Role: Co-I

Advancing Healthier Wisconsin Initiative Sood (PI) 01/01/2018 – 12/30/2019
Immune Cross-talk in Pregnancy and Preterm Birth
This project applies our novel plasma induced transcriptional bioassay to studies of preterm birth.
Role: Co-I

Completed Research Support

NIH/NIDDK DP3DK098161 Hessner (PI) 04/01/2013 – 03/31/2017
Quantitative measurement of T1D risk through molecular signature analysis
The goal of this study is to conduct serum signature analysis on longitudinal samples of progressors and nonprogressors to T1D collected as part of the TrialNet Natural History Study as well as conduct disease specificity studies by examining samples collected from patients with other autoimmune diseases.
Role: PI

JDRF 2-SRA-2015-109-Q-R Hessner (PI) 07/01/2015 – 12/30/2017
Juvenile Diabetes Research Foundation International
Serum/Plasma Induced Transcriptional Signatures as a Measure of Therapeutic Intervention
This study applies serum induced transcription assay to human clinical trials in T1D
Role: PI

NIH/NIDDK DK097605 Chen/Guerts (PI) 12/01/2012 – 11/30/2016
Discovery and Functional Studies of Genes for T1D GWAS Susceptibility Loci
The goal of this project is to generate human GWAS gene knockouts in the NOD mouse using Zn finger nuclease technology followed by immunological and beta cell phenotyping.
Role: Co-Investigator

NIH R01AI078713 Hessner (PI) 07/01/2008 – 12/31/2014
Dissection of cellular interactions in T1DM with integrated functional genomics

The goal of this study is to develop natural history of BB rat T1DM through longitudinal expression profiling of immunocyte expression profiling and serum signature analysis.
Role: PI

JDRF 17-2012-621 Hessner (PI) 10/01/2012 – 03/30/2015
Juvenile Diabetes Research Foundation International
Quantitative assessment of T1D risk though molecular signature analysis
This study applies predictive serum induced transcription assay to human pre-T1D.
Role: PI

ADA 7-12-BS-075 Hessner (PI) 07/01/2012 – 06/30/2015
American Diabetes Association
Serum/plasma induced signatures as T1D biomarkers
This study applies predictive serum induced transcription assay to human T1D intervention.
Role: PI

NIH/NHLBI 1DP2OD007031 Levy (PI) 09/30/2010 – 08/29/2015
Integration of genomics with genetics-molecular phenotypes in CF lung disease
The major goal of this project is to use a combined genetics/genomics strategy to define the pathological mechanisms underlying pulmonary tract infection in CF.
Role: Co-Investigator

NIH HL034708 Bosnjak (PI) 07/01/2010 – 06/30/2014
Anesthetics and Cardiac Signal Transduction
The goal of this study is to apply functional genomics to examine how diabetes might alter the potential efficacy of stem cells for future use in regenerative medicine.
Role: Co-Investigator

NIH CA100030 Johnson (PI) 05/01/2009 – 04/30/2014
Optimizing Immunotherapy for Neuroblastoma after BMT
The goal of this study is to apply functional genomics to better understand the induction of tumor immunity.
Role: Co-Investigator

JDRF 5-2012-220 Hessner (PI) 05/01/2012 – 04/30/2013
Juvenile Diabetes Research Foundation International
Longitudinal Analysis of Pre-Diabetes Using Serum Induced Transcription
This study applies predictive serum induced transcription assay to human pre-T1D.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ann Punnoose

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Assistant Professor of Pediatrics, Children's Hospital of Wisconsin

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge	S.B.	06/2004	Brain & Cognitive Sciences
Penn State University College of Medicine, Hershey, PA	M.D.	05/2008	Medicine
Cohen Children's Medical Center of New York, New Hyde Park	Residency	06/2011	Pediatrics
Journal of the American Medical Association, Chicago	Fellowship	06/2012	Fellowship in Medical Editing
Children's National, Washington	Fellowship	06/2015	Pediatric Cardiology
Ann and Robert H Lurie Children's Hospital of Chicago, Chicago	Fellowship	06/2016	Pediatric Heart Failure & Transplant

A. Personal Statement

I am currently an Assistant Professor of Pediatrics in the division of Cardiology at the Children's Hospital of Wisconsin. I am subspecialized in Pediatric Heart Failure and Transplant. My main career goals are to treat and care for children with cardiomyopathy, but structurally normal hearts as well as those with congenital heart disease who develop heart failure. I also work to provide advanced heart failure treatment with mechanical circulatory support and heart transplantation. I am also interested in medical education for cardiology fellows.

B. Positions and Honors

Positions and Employment

2011-2012 Morris Fishbein Fellow in Medical Editing, Journal of the American Medical Association
2016-present Assistant Professor of Pediatrics, Medical College of Wisconsin

Other Experience and Professional Memberships

2014-present American Heart Association
2015-present International Society of Heart and Lung Transplantation
2017-present Medical College of Wisconsin, First Year Cardiology Fellowship Introductory Bootcamp Course Director

Honors

2000-2004 Lord Swarj Paul Scholarship, Massachusetts Institute of Technology
2003 Honorable Mention for Excellence in Research, Massachusetts Institute of Technology, Department of Bran & Cognitive Sciences
2004 Alumni Scholarship, Penn State University College of Medicine
2014 Best Poster in Basic & Translational Science, Children's National

2014 American Heart Association Woman in Cardiology, Excellence in Training Award
2018 International Society of Heart and Lung Transplantation International Travelling Scholarship

C. Contribution to Science

1. Aicher SA, Punnoose A, Goldberg A. μ-Opioid Receptors often colocalize with the Substance P Receptor (NK1) in the Trigeminal Dorsal Horn. *Journal of Neuroscience* 2000; 20 (11): 4345-54. PMID: 10818170

2. Aydin S, Seiden H, Blaufox A, Parnell V, Choudhury T, Punnoose A, Schneider J. Acute Kidney Injury After Surgery for Congenital Heart Disease. *Annals of Thoracic Surgery*. 2012; 94: 1589-95. PMID: 22884599

3. Punnoose AR, Kaltman J, Pastor W, McCarter R, He J, Spurney C. Cardiac Disease Burden and Risk of Mortality in Hospitalized Muscular Dystrophy Patients. *Pediatric Cardiology*. 2016. PMID: 27914489

4. Greene EA, Punnoose A. Sports-related Sudden Cardiac Injury or Death. In: *AM:Stars:Acute Emergencies in Adolescents.* Vol 26:3. Elk grove Village, IL. Houston Adams; 2015. http://reader.aappublications.org/amstars-acute-emergencies-in-adolescents-vol-26-no-3/3?ajax.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Raskin, Alexander

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Pediatric Cardiology Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of Illinois at Chicago, Chicago, IL	BS	05/2008	Neuroscience
Medical College of Wisconsin, Milwaukee, WI	MD	06/2013	Medicine
Medical College of Wisconsin Affiliated Hospitals, Milwaukee, WI	Resident	06/2016	Pediatrics
Medical College of Wisconsin Affiliated Hospitals, Milwaukee, WI	Fellow	present	Pediatric Cardiology

A. Personal Statement

I am currently a Pediatric Cardiology Fellow at Children's Hospital of Wisconsin, Medical College of Wisconsin Affiliated Hospitals who is currently applying for a subspecialty fellowship in Pediatric Heart Failure and Transplant. My career goal is to treat and care for children with cardiomyopathy and complex congenital heart disease who develop heart failure by providing advanced heart failure treatment with mechanical circulatory support and heart transplantation. I am also interested translational research, particularly in the field of genomics, as it applies to advancing practice and improving outcomes in pediatric heart transplantation patients. Participating in Dr. Kindel's study of plasma induced transcription analysis in pediatric heart transplantation as an assessment of rejection directly aligns with my career goals and will be an invaluable experience in the development of my research career.

1. Raskin A, Loomba RS, Kirkpatric E. Diastolic dysfunction is present by tissue Doppler in acute Kawasaki disease but is not predictive of IVIG responsiveness. EC Cardiology. 2018 August 14; 5(9): 616-620.
2. Loomba RS, Raskin A, Gudausky TM, Kirkpatrick E. Role of the Egami Score in Predicting Intravenous Immunoglobulin Resistance in Kawasaki Disease Among Different Ethnicities. Am J Ther. 2016 Nov/Dec;23(6):e1293-e1299. PubMed PMID: 25611359.
3. Loomba RS, Raskin A, Aggarwal S, Arora RR. Long-term Sequelae of Kawasaki Disease: The Coronary Arteries and Beyond. Ann Paediatr Rheum. 2014; 3(2):49-61.
4. Looba RS, Raskin A, Gudausky TM, Kirkpatrick E. Racial differences in Kawasaki Disease: Comparison of clinical findings, laboratory values, treatment efficacy. Annals of paediatric rheumatology. 2013; 2(4):147-153.

B. Positions and Honors

Positions and Employment

2011 - 2013 Medical Microbiology Tutor, Medical College of Wisconsin Academic Affairs, Milwaukee, WI

Other Experience and Professional Memberships

2010 - Member, American Medical Association
2011 - Member, Medical Society of Milwaukee County
2011 - Member, Wisconsin Medical Society
2012 - Member, Alpha Omega Alpha

2013 -	Member, American Academy of Pediatrics

Honors

2007 - 2008	Adam Kuhn Scholarship for pre-med students, University of Illinois at Chicago
2007 - 2008	Department of Biological Sciences student leader of the year award, University of Illinois at Chicago
2008	Highest Departmental Distinction in Neuroscience, University of Illinois at Chicago
2013	Standing Ovation Award for Homeless Outreach in Medical Education (HOME) Project, Medical College of Wisconsin
2015 - 2016	Outstanding Medical Student Teacher , Medical College of Wisconsin
2016	Larrie Sarff Teaching Award, Medical College of Wisconsin

C. Contribution to Science

a. Raskin A, Loomba RS, Kirkpatric E. Diastolic dysfunction is present by tissue Doppler in acute Kawasaki disease but is not predictive of IVIG responsiveness. EC Cardiology. 2018 August 14; 5(9): 616-620.

b. Loomba RS, Raskin A, Gudausky TM, Kirkpatrick E. Role of the Egami Score in Predicting Intravenous Immunoglobulin Resistance in Kawasaki Disease Among Different Ethnicities. Am J Ther. 2016 Nov/Dec;23(6):e1293-e1299. PubMed PMID: 25611359.

c. Loomba RS, Raskin A, Aggarwal S, Arora RR. Long-term Sequelae of Kawasaki Disease: The Coronary Arteries and Beyond. Ann Paediatr Rheum. 2014; 3(2):49-61.

d. Looba RS, Raskin A, Gudausky TM, Kirkpatrick E. Racial differences in Kawasaki Disease: Comparison of clinical findings, laboratory values, treatment efficacy. Annals of paediatric rheumatology. 2013; 2(4):147-153.