PI: **Al-Adra, David**	Title: Targeting Donor Regulatory Dendritic Cells During Normothermic Ex Vivo Liver Perfusion to Overcome Rejection after Liver Transplant	
Received: 11/04/2020	Opportunity: PA-20-203	Council: 05/2021
Competition ID: FORMS-F	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08 Independent Clinical Trial Not Allowed)	
1K08AI155816-01A1	Dual:	Accession Number: 4514379
IPF: 578503	Organization: UNIVERSITY OF WISCONSIN-MADISON	
Former Number: 1K08AI155816-01	Department: SURGERY	
IRG/SRG: AITC	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Early Stage Investigator:

Senior/Key Personnel:	*Organization:*	*Role Category:*
David Al-Adra	The Board of Regents of the University of Wisconsin System	PD/PI
Paul Sondel	The Board of Regents of the University of Wisconsin System	Other (Specify)-Mentor
JOSHUA MEZRICH	The Board of Regents of the University of Wisconsin System	Other (Specify)-Mentor
Christian Capitini	The Board of Regents of the University of Wisconsin System	Other (Specify)-Mentor
WILLIAM BURLINGHAM PhD	The Board of Regents of the University of Wisconsin System	Other (Specify)-Mentor

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier [redacted]

b. Agency Routing Number

2. DATE SUBMITTED 2020-11-04

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: The Board of Regents of the University of Wisconsin System
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: First Name*: CHRISTY Middle Name: R Last Name*: SCHULZ Suffix:
Position/Title: ADMIN PRGM SPEC
Street1*: [redacted]
Street2:
City*:
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*:
Phone Number*: Fax Number: Email:

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Targeting Donor Regulatory Dendritic Cells During Normothermic Ex Vivo Liver Perfusion to Overcome Rejection after Liver Transplant

12. PROPOSED PROJECT
Start Date* 09/01/2021
Ending Date* 08/31/2026

13. CONGRESSIONAL DISTRICTS OF APPLICANT
WI-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: David Middle Name: Last Name*: Al-Adra Suffix:
Position/Title: ASSISTANT PROFESSOR
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: SURGERY
Division: Medicine and Public Health
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* $[redacted]
b. Total Non-Federal Funds* $[redacted]
c. Total Federal & Non-Federal Funds* $[redacted]
d. Estimated Program Income* $[redacted]

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:
b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: BRENDA Middle Name: A Last Name*: EGAN Suffix:
Position/Title*: Managing Officer
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Research & Sponsored Programs
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative* [redacted] **Date Signed*** [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_letter1039337534.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Board of Regents of the University of Wisconsin System
Duns Number:	[redacted]
Street1*:	[redacted]
Street2:	[redacted]
City*:	[redacted]
County:	[redacted]
State*:	WI: Wisconsin
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	[redacted]
Project/Performance Site Congressional District*:	[redacted]

Additional Location(s)

File Name:

Funding Opportunity Number: PA-20-203. Received Date: 2020-11-04T09:42:09.000-05:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1039337535.pdf
8. Project Narrative*	Public_Health_Statement1039337536.pdf
9. Bibliography & References Cited	Al_Adra_K08_References1039438883.pdf
10.Facilities & Other Resources	Facilities1039337824.pdf
11.Equipment	Lab_Equipment___Al_Adra1039337826.pdf

Funding Opportunity Number: PA-20-203. Received Date: 2020-11-04T09:42:09.000-05:00

ABSTRACT

This proposal presents a five-year research career development program focused on targeting donor liver-resident cells with regulatory properties to decrease rejection after transplantation. I am an Assistant Professor of Surgery at the University of Wisconsin-Madison, with previous research and clinical experience in transplant immunology and transplant surgery involving normothermic *ex vivo* machine perfusion (NEVLP), whereby an organ is housed under physiologic conditions. The present project will advance the field of transplant immunology by using NEVLP technology to modify the immune cells within the liver prior to transplantation. I have assembled an outstanding mentorship team of investigators with expertise in transplant immunology, dendritic cell biology, and extracellular vesicle biology. The proposed training will guide and enhance my development in core competencies, including transplant immunology, communication, biostatistics, and ethical research design that will enable me to transition to research independence as a surgeon-scientist dedicated to reducing organ rejection in the field of transplant surgery.

Liver transplantation is the only treatment option for patients with end-stage liver disease; however, rejection of the transplant can decrease liver and patient survival. In addition, patients still require lifelong use of anti-rejection medications that suppress the immune system. Modification of the donor liver, and the immune cells within it, has the potential to promote acceptance of the liver and minimize the need for anti-rejection drugs. Advances in an innovative technique called normothermic *ex vivo* liver perfusion (NEVLP) offer a unique opportunity to benefit significantly the 25% of liver transplant recipients that develop acute rejection, as well as many more transplant recipients who would benefit from using fewer anti-rejection drugs. Recent studies have demonstrated the importance of regulatory dendritic cells (DCregs) for prolonging transplant survival. My central hypothesis is that expansion of the number of liver-resident DCregs during NEVLP will promote a regulatory environment for the organ after transplant. Using a rat model of NEVLP and liver transplantation that my research group has optimized, I expect NEVLP to expand DCregs potently, leading to an increase in immune checkpoint molecule expression and production of anti-inflammatory extracellular vesicles and cytokines that can reduce immune-mediated rejection. This innovative approach of expanding graft-resident DCregs to decrease rejection could be used in deceased donor liver transplantation as well as translated to other types of solid organ transplants. To achieve these objectives, I propose the following scientific aims: 1) Determine the dominant regulatory function of liver-resident DCregs after NEVLP, and 2) Measure the impact of expanded liver-resident DCregs generated by combination cytokine therapy during NEVLP on liver graft rejection *in vitro* and *in vivo*.

PUBLIC HEALTH RELEVANCE STATEMENT

Transplant recipients must take lifelong anti-rejection medications that cause side effects and decrease their ability to defend against infections and cancers. We will use a novel method of liver storage in a rat model of liver transplantation to examine the impact of interventions aimed at increasing regulatory cells within the liver, thereby making the entire organ less likely to cause an immune reaction in the recipient. This exploration of strategies to decrease patient reliance on harmful drugs holds tremendous promise not only for liver transplantation, but all solid organ transplants.

REFERENCES

1. Kwong A, Kim WR, Lake JR, et al. OPTN/SRTR 2018 Annual Data Report: Liver. *Am J Transplant.* 2020;20 Suppl s1:193-299.
2. Raich-Regue D, Glancy M, Thomson AW. Regulatory dendritic cell therapy: from rodents to clinical application. *Immunol Lett.* 2014;161(2):216-221.
3. Steinman RM, Nussenzweig MC. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc Natl Acad Sci U S A.* 2002;99(1):351-358.
4. Levitsky J, Goldberg D, Smith AR, et al. Acute Rejection Increases Risk of Graft Failure and Death in Recent Liver Transplant Recipients. *Clin Gastroenterol Hepatol.* 2017;15(4):584-593 e582.
5. Nacif LS, Pinheiro RS, Pecora RA, et al. Late acute rejection in liver transplant: a systematic review. *Arq Bras Cir Dig.* 2015;28(3):212-215.
6. Li L, Luo Z, Song Z, Zheng L, Chen T. Pre-transplant infusion of donor-derived dendritic cells maintained at the immature stage by sinomenine increases splenic Foxp3(+) Tregs in recipient rats after renal allotransplantation. *Transpl Immunol.* 2017;45:22-28.
7. Li X, Li JJ, Yang JY, et al. Tolerance induction by exosomes from immature dendritic cells and rapamycin in a mouse cardiac allograft model. *PLoS One.* 2012;7(8):e44045.
8. Ezzelarab MB, Zahorchak AF, Lu L, et al. Regulatory dendritic cell infusion prolongs kidney allograft survival in nonhuman primates. *Am J Transplant.* 2013;13(8):1989-2005.
9. Ma B, Yang JY, Song WJ, et al. Combining Exosomes Derived from Immature DCs with Donor Antigen-Specific Treg Cells Induces Tolerance in a Rat Liver Allograft Model. *Sci Rep.* 2016;6:32971.
10. Song J, Huang J, Chen X, et al. Donor-derived exosomes induce specific regulatory T cells to suppress immune inflammation in the allograft heart. *Sci Rep.* 2016;7:20077.
11. Zahorchak AF, Macedo C, Hamm DE, Butterfield LH, Metes DM, Thomson AW. High PD-L1/CD86 MFI ratio and IL-10 secretion characterize human regulatory dendritic cells generated for clinical testing in organ transplantation. *Cell Immunol.* 2018;323:9-18.
12. Vogel T, Brockmann JG, Friend PJ. Ex-vivo normothermic liver perfusion: an update. *Curr Opin Organ Transplant.* 2010;15(2):167-172.
13. Barbas AS, Goldaracena N, Dib MJ, Selzner M. Ex-vivo liver perfusion for organ preservation: Recent advances in the field. *Transplant Rev (Orlando).* 2016;30(3):154-160.
14. Ceresa CDL, Nasralla D, Coussios CC, Friend PJ. The case for normothermic machine perfusion in liver transplantation. *Liver Transpl.* 2018;24(2):269-275.
15. Goldaracena N, Spetzler VN, Echeverri J, et al. Inducing Hepatitis C Virus Resistance After Pig Liver Transplantation-A Proof of Concept of Liver Graft Modification Using Warm Ex Vivo Perfusion. *Am J Transplant.* 2017;17(4):970-978.
16. Machuca TN, Cypel M, Bonato R, et al. Safety and Efficacy of Ex Vivo Donor Lung Adenoviral IL-10 Gene Therapy in a Large Animal Lung Transplant Survival Model. *Hum Gene Ther.* 2017;28(9):757-765.
17. Nouri-Shirazi M, Guinet E. Direct and indirect cross-tolerance of alloreactive T cells by dendritic cells retained in the immature stage. *Transplantation.* 2002;74(7):1035-1044.
18. Lu L, Li W, Zhong C, et al. Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation.* 1999;68(6):747-757.
19. Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity.* 2003;18(5):605-617.
20. Boks MA, Kager-Groenland JR, Haasjes MS, Zwaginga JJ, van Ham SM, ten Brinke A. IL-10-generated tolerogenic dendritic cells are optimal for functional regulatory T cell induction--a comparative study of human clinical-applicable DC. *Clin Immunol.* 2012;142(3):332-342.
21. Kryczanowsky F, Raker V, Graulich E, Domogalla MP, Steinbrink K. IL-10-Modulated Human Dendritic Cells for Clinical Use: Identification of a Stable and Migratory Subset with Improved Tolerogenic Activity. *J Immunol.* 2016;197(9):3607-3617.
22. Torres-Aguilar H, Aguilar-Ruiz SR, Gonzalez-Perez G, et al. Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory CD4+ T cells. *J Immunol.* 2010;184(4):1765-1775.
23. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell.* 2008;133(5):775-787.

24. Kim WR, Lake JR, Smith JM, et al. OPTN/SRTR 2017 Annual Data Report: Liver. *Am J Transplant.* 2019;19 Suppl 2:184-283.
25. Palmer WC, Taner CB, Keaveny AP, Nakhleh RE, Nguyen JH, Rosser BG, Jr. Antithymocyte Globulin Use for Corticosteroid Nonresponsive Rejection After Liver Transplantation. *Transplant Proc.* 2018;50(10):3606-3614.
26. Libby P, Pober JS. Chronic rejection. *Immunity.* 2001;14(4):387-397.
27. Lodhi SA, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant.* 2011;11(6):1226-1235.
28. Xia S, Guo Z, Xu X, Yi H, Wang Q, Cao X. Hepatic microenvironment programs hematopoietic progenitor differentiation into regulatory dendritic cells, maintaining liver tolerance. *Blood.* 2008;112(8):3175-3185.
29. Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol.* 2007;7(8):610-621.
30. Beriou G, Peche H, Guillonneau C, Merieau E, Cuturi MC. Donor-specific allograft tolerance by administration of recipient-derived immature dendritic cells and suboptimal immunosuppression. *Transplantation.* 2005;79(8):969-972.
31. Peche H, Trinite B, Martinet B, Cuturi MC. Prolongation of heart allograft survival by immature dendritic cells generated from recipient type bone marrow progenitors. *Am J Transplant.* 2005;5(2):255-267.
32. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature.* 2018;557(7703):50-56.
33. Goldaracena N, Echeverri J, Spetzler VN, et al. Anti-inflammatory signaling during ex vivo liver perfusion improves the preservation of pig liver grafts before transplantation. *Liver Transpl.* 2016;22(11):1573-1583.
34. De Creus A, Abe M, Lau AH, Hackstein H, Raimondi G, Thomson AW. Low TLR4 expression by liver dendritic cells correlates with reduced capacity to activate allogeneic T cells in response to endotoxin. *J Immunol.* 2005;174(4):2037-2045.
35. Ochando J, Ordikhani F, Jordan S, Boros P, Thomson AW. Tolerogenic dendritic cells in organ transplantation. *Transpl Int.* 2020;33(2):113-127.
36. Ono Y, Perez-Gutierrez A, Nakao T, et al. Graft-infiltrating PD-L1hi cross-dressed dendritic cells regulate anti-donor T cell responses in mouse liver transplant tolerance. *Hepatology.* 2017.
37. Bracamonte-Baran W, Florentin J, Zhou Y, et al. Modification of host dendritic cells by microchimerism-derived extracellular vesicles generates split tolerance. *Proc Natl Acad Sci U S A.* 2017;114(5):1099-1104.
38. Kink JA, Forsberg MH, Reshetylo S, et al. Macrophages Educated with Exosomes from Primed Mesenchymal Stem Cells Treat Acute Radiation Syndrome by Promoting Hematopoietic Recovery. *Biol Blood Marrow Transplant.* 2019;25(11):2124-2133.
39. Thomson AW, Humar A, Lakkis FG, Metes DM. Regulatory dendritic cells for promotion of liver transplant operational tolerance: Rationale for a clinical trial and accompanying mechanistic studies. *Hum Immunol.* 2018;79(5):314-321.
40. He W, Chen L, Zheng L, Luo L, Gao L. Prolonged survival effects induced by immature dendritic cells and regulatory T cells in a rat liver transplantation model. *Mol Immunol.* 2016;79:92-97.
41. Marino J, Babiker-Mohamed MH, Crosby-Bertorini P, et al. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. *Sci Immunol.* 2016;1(1).
42. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. *Int Rev Cell Mol Biol.* 2012;298:229-317.
43. Quesnelle KM, Bystrom PV, Toledo-Pereyra LH. Molecular responses to ischemia and reperfusion in the liver. *Arch Toxicol.* 2015;89(5):651-657.
44. Harper IG, Gjorgjimajkoska O, Siu JHY, et al. Prolongation of allograft survival by passenger donor regulatory T cells. *Am J Transplant.* 2019;19(5):1371-1379.
45. Scheuermann U, Zhu M, Song M, et al. Damage-Associated Molecular Patterns Induce Inflammatory Injury During Machine Preservation of the Liver: Potential Targets to Enhance a Promising Technology. *Liver Transpl.* 2019;25(4):610-626.
46. Hashimoto K, Cypel M, Juvet S, et al. Higher M30 and high mobility group box 1 protein levels in ex vivo lung perfusate are associated with primary graft dysfunction after human lung transplantation. *J Heart Lung Transplant.* 2017.

47. Shimizu A, Ishii E, Kuwahara N, et al. Chronic antibody-mediated responses may mediate chronic rejection in rat orthotopic liver transplantation. *Transplant Proc.* 2013;45(5):1743-1747.
48. Kamada N. Transplantation tolerance and immunosuppression following liver grafting in rats. *Immunol Today.* 1985;6(11):336-342.
49. Trajkovic K, Hsu C, Chiantia S, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science.* 2008;319(5867):1244-1247.
50. Lin CH, Hunig T. Efficient expansion of regulatory T cells in vitro and in vivo with a CD28 superagonist. *Eur J Immunol.* 2003;33(3):626-638.
51. Sullivan JA, Tomita Y, Jankowska-Gan E, et al. Treg-Cell-Derived IL-35-Coated Extracellular Vesicles Promote Infectious Tolerance. *Cell Rep.* 2020;30(4):1039-1051 e1035.
52. Yoon H, Kim TS, Braciale TJ. The cell cycle time of CD8+ T cells responding in vivo is controlled by the type of antigenic stimulus. *PLoS One.* 2010;5(11):e15423.
53. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol.* 1994;12:991-1045.

FACILITIES AND OTHER RESOURCES

The proposed projects will be conducted at the University of Wisconsin-Madison in the laboratory of David Al-Adra, MD, PhD.

Institutional

The University of Wisconsin-Madison has long been recognized as an institution that excels in research and training. Student enrollment continues to grow, with over 45,000 students in 2019. The university offers one of the largest graduate degree programs in the US, with 162 master's degree programs and 125 doctoral degree programs. According to the National Science Foundation 2018 rankings, UW-Madison placed 8th in the nation for research and development expenditures. The university is a research powerhouse, with more than $[redacted] billion in annual expenditures for research, about half of which comes from federal awards. UW-Madison consistently ranks in the top 10 amidst public institutions in the nation for federal funding and research expenditures. Twenty Nobel Prizes and 38 Pulitzer Prizes have been awarded to UW faculty or alumni.

UW-Madison Libraries: The UW-Madison has the 11th largest research library collection in North America. The campus library collections include more than 8 million printed volumes, 55,000 serial titles, 6.2 million microforms, 160 linear feet of manuscripts and over 7 million items in other formats, including government documents, maps, musical scores and more. There is also access to over 20,000 electronic journals, as well as e-books. The UW-Madison Library system is a member of the Committee on Institutional Cooperation (CIC), comprised of Big Ten institutions plus the University of Chicago and the University of Illinois – Chicago; the Council of University of Wisconsin Libraries (CUWL); and the Wisconsin E-Book Consortium. The UW-Madison Libraries supplement our on-site collections with the resources of the nationally recognized Center for Research Libraries (CRL). Thus, we can conduct the necessary literature searches and access publications for this project.

Division of Information Technology (DoIT): The Division of Information Technology (DoIT) at the UW-Madison provides support to the campus community in selection, purchase, and use of computers and electronic communications, including networks, printing, telephones, Internet connectivity, security, instructional technology, and related services. Nearly 900 employees at DoIT are dedicated to supporting the academic, research, and administrative missions of UW-Madison. DoIT's data center houses over 500 servers operating AIX, Sun Solaris, and Linux versions of UNIX, as well as MAC OS X and Windows. It also hosts two IBM mainframes, a large enterprise Storage Area Network, and performs 24x7 monitoring for all of the above as well the Campus Backbone Network and various other educational and research networks. As such, we have the ability to easily communicate, store, and protect the research activities in this project.

University of Wisconsin School of Medicine and Public Health (UWSMPH)

The UWSMPH is the nation's only combined school of medicine and public health, and is recognized as an international, national, and statewide leader in educating physicians, investigating the causes of disease, exploring innovative solutions to medical problems and translating research into compassionate patient care. UWSMPH is home to over 1500 faculty who hold appointments in 27 departments, 17 in the clinical sciences and 10 in the basic and applied sciences. Faculty members in the clinical sciences serve on the medical staff of UW Hospital and Clinics and/or American Family Children's Hospital, the former of which is consistently ranked by *U.S. News & World Report* as the #1 best hospital in Wisconsin. The faculty includes many National Medal of Science recipients and National Academy of Science honorees. The faculty routinely garners national distinction in biomolecular chemistry, physiology, cancer, medicine, neuroscience, ophthalmology, surgery, and many other fields.

The UWSMPH consistently ranks among *U.S. News & World Report's* best medical schools for research, and has the largest research commitment of any school or college on the UW-Madison campus, receiving more than $[redacted] million in extramural research support in 2017-18. Medical school facilities such as the Wisconsin Institutes for Medical Research (WIMR) ensure that the University of Wisconsin-Madison will remain at the forefront of basic, clinical, translational and public health research, ultimately improving the health of the residents of Wisconsin and beyond. The UWSMPH has recently identified Population Health as one of the top seven priority areas for research.

The UWSMPH is home to the Ebling library. Located in the Health Sciences Learning Center, adjacent to UW Hospital and the Wisconsin Institutes for Medical Research for easy access by researchers in the health

sciences end of campus, the library is part of the UW campus library system. The Health Sciences (Ebling) Library occupies over 53,000 square feet and contains 375,231 print volumes, 3,642 online journals, 4,090 electronic books, and 125 databases. Ebling library offers a number of services useful to researchers and students, including a free of charge electronic document delivery system, which allows user to access articles throughout and beyond the UW campus electronically. FindIt allows users to efficiently locate full-text versions of citations with a click of a button through the library website.

UW Health Delivery System

UW Health is the integrated health system of the University of Wisconsin-Madison, serving more than 600,000 patients each year in the Upper Midwest and beyond. UW Health consists of 6 hospitals and 80 outpatient sites, including University Hospital, UW Health at The American Center, American Family Children's Hospital and UW Carbone Cancer Center. UW Health has major programs in trauma, organ transplant, heart and vascular care, cancer care, neurology and neurosurgery, pediatrics, and orthopedics and rehabilitation. UW Health partners with UW School of Medicine and Public Health to fulfill their shared patient care, research, education and community service missions. Our expert doctors, nurses and staff serve the health needs of Wisconsin, and beyond. The UW Health Delivery System thus provides the clinical volume to support the research proposed for this research.

Department of Surgery

The UW Department of Surgery (DOS) has over 100 full-time faculty members and its portfolio of research funding exceeds $[redacted] million annually, with approximately two-thirds of this support coming from the National Institutes of Health. In 2018, the department ranked eighth in NIH funding in Departments of Surgery across the United States. The department's basic science and translational research is complemented by an increasing focus on clinical outcomes research. The Department of Surgery has over 20,000 square feet of research space within the UW hospital complex. The department employs 6 full-time biostatisticians and programmers who consult with faculty on protocol design and planning, data collection, data analysis and interpretation, publication and presentation of data, file manipulation and maintenance, as well as grant and manuscript review. The Department's Office of Clinical Research provides comprehensive support for interventional clinical research trials including the activities of: activation, budgeting and contract negotiation, IRB submissions, enrollment/consenting, data collection and subject-follow-up. All clinical research staff obtain certification in Human Subjects Research, Good Clinical Practice, and HIPAA. The office is currently staffed with a Director, 3 regulatory specialists, 6 clinical research coordinators, 2 clinical research nurses and one research assistant. Currently, the office coordinates and provides regulatory support for over 40 active federally sponsored or industry-sponsored clinical trials. The Departmental Accounting Office staffs 5 full-time accountants and financial specialists who provide post-award grant accounting and purchasing services. The Research Office provides assistance in preparing grant administrative pages and fulfilling University as well as agency specific requirements for assurances and certifications. In addition, an editor and graphic designer in the Communications Office provide a broad range of services including editing, slide preparation, poster presentation development, and medical illustration. There are 1.5 full-time writers to assist faculty and large multi-PI grants with proposal and manuscript preparation. Nine full-time information technologists maintain the extensive network and computer system within the department and provide user support to all employees, including research staff. The Department of Surgery will provide the administrative support for this research project.

Department of Surgery Statistical Analysis/Research Programming Core: The Statistical Analysis/Research Programming Core, located in an office next to the Wisconsin Surgical Outcomes Research Program (WiSOR), provides faculty, researchers, and residents with comprehensive statistical support for their research activities. The department employs 6 full-time biostatisticians and programmers who assist with study design and grant application through selection of outcome variables, statistical analysis planning, power analysis and sample size calculation, and random assignment of treatment conditions.

- *Statistical Analysis and Results Interpretation:* Statisticians perform research hypothesis testing and data analysis using a variety of statistical methods. The choice of statistical method depends on the study design, type of outcome measure, and research question. The statistical methods they are able to apply include but are not limited to: Analysis of Variance (ANOVA), Linear Regression, Logistic Regression, Generalized Linear Model, Survival Analysis (Kaplan-Meier, log-rank test, Cox proportional hazards models), Nonparametric Statistics, Meta-Analysis, Longitudinal Data Analysis, Multilevel

Modeling, Structural Equation Modeling, Data Mining, Machine Learning, Model Selection and Variable Selection. The statisticians also interpret the analysis results and translate statistical significance to clinical implications.

- *Evaluation of Measurement:* Statisticians will evaluate psychometric properties of measurement instruments including survey questionnaires. They can assess reliability, validity, and factor structure of the instruments, and perform item and test analysis.
- *Database Management and Preparation:* Statisticians work closely with the investigators in WiSOR on research projects that utilize large national health outcome databases. They provide database management, maintenance, patient cohort extraction, and analytic data set preparation.
- *Manuscript and Presentation Assistance:* The statistical analysis core can provide feedback for peer-reviewed publications and oral and poster conference presentations. Their collaboration and contribution can include the description of statistical analysis methods, generation of results summary tables and figures, and reviewing and editing of manuscripts, abstracts, and presentations.
- *Consultation and Training:* Statisticians provide consultation and training on study design, data creation and management (for both in house and large national databases), statistical analysis methods, and statistical software programs (including SASTM, SQL, Stata, R, SPSS, and Matlab).

Department of Surgery Computing Hardware/Software Infrastructure
The Department of Surgery's network features over 890 endpoints, which include modern Windows-based computer devices with access to the Department's fully managed, secure, and high-speed infrastructure. These resources are physically secured within the SMPH's state-of-the-art data center and logically secured with a next generation firewall. All data are protected using an off-site secure data storage facility. Data access is controlled via user and group access control lists that are centrally managed and monitored for compliance. Full data logging is available including access times, modifications, and any other requirements necessary to meet specifications.

- *Physical security:* Only members of the SMPH IT staff have physical access to the secure data center. Access is controlled by individual key card access and pin number. All entries are logged and cameras are in place to record activities. These files can be examined under secure circumstances with cybersecurity and facilities personnel if required.
- *Identity and authorization:* Users must apply for and be approved for access by Department of Surgery human resources. Accounts are fully HIPAA compliant, which includes password complexity requirements and expiration policies.
- *Access management:* Users have unique accounts that allow them to access the data for which they have been authorized. Users access the data via managed and secured workstations or via our secure Citrix services for remote access. Rights to files and folders can be audited by Surgery IT staff. Access control lists are only editable by Department of Surgery IT staff and changes are only made under the authorization of the Principal Investigator or data custodian.
- *Audit controls:* In collaboration with Surgery's IT staff, Principal Investigators and data custodians can perform regular review of access to systems and data storage folders and files.

The statistical application server hosts SAS, STATA, and SPSS applications with flexibility to add other products as needed. This server's access is protected using the same access and audit control processes defined for Surgery's infrastructure.

University of Wisconsin Department of Biostatistics and Medical Informatics
The Biomedical Computing Group (BCG) within the Department of Biostatistics and Medical Informatics has developed a centralized state-of-the-art computing facility for the support of statistical and medical informatics research, and for the management and analysis of clinical, genomic and other biological data. Access to the facility is provided by workstations running Linux or UNIX. The BCG, a highly skilled group of 20 computing professionals within the Department, administers this facility and its desktop clients. The core of this environment is a secured, integrated, fault tolerant network of 40+ computational servers, 6 database servers, 16 Web application servers and 62 additional servers running Solaris, Linux, Microsoft and MacOS operating systems. 100+ UNIX, 80+ Macintosh and over 150 Microsoft desktop computers on campus are fully integrated with this facility. Storage for this facility is provided by a pair of redundant file servers in multiple server rooms

(2161 HSLC and K4/564 CSC) providing over 120TB of mirrored, RAID storage augmented by a LTO-5 tape backup system with offsite storage as required.

This computing facility adheres to the HIPAA policies, and helps enforce IRB clinical trial management and data integrity determinations. A member of the BCG is also the HIPAA security coordinator for the Medical School, and works closely with the University of Wisconsin Hospital and Clinics (UWHC), the University of Wisconsin Madison's Division of Information Technology, and the campus CIO's office in this and other areas.

Full complements of up-to-date computational tools are available, including Splus, R, SAS and Matlab for statistical exploration, as well as a number of optimizing compilers and a large suite of utilities. LaTex and Word are fully supported for producing publication-quality papers. A centralized storage model allows collaborative data sharing with flexible access controls and the manipulation of very large datasets. Storage centralization also allows large amounts of data to be stored and backed up very reliably. Its disk subsystem consists of industrial quality SAN-based redundant disk arrays that currently hold three terabytes of data and have a total capacity of more than 20 terabytes. Data are backed up each night to a large tape library, and all data are archived monthly, both on-site and offsite.

The Department of Surgery maintains a formal agreement with the Department of Biostatistics and Medical Informatics such that investigators can take advantage of their extensive expertise. Faculty and staff within the Department of Biostatistics and Medical Informatics are collaborators with investigators within the Wisconsin Surgical Outcomes Research Program (WiSOR).

Services to Facilitate Communications

Significant technology and space is available at UW-Madison for teleconferencing and videoconferencing to facilitate effective communications between the Principal Investigator, collaborators and research staff at each institution.

- Teleconferencing: WisLine is an easy and affordable way for researchers to teleconference with colleagues at other institutions and elsewhere on campus. The conference call service operated by the University of Wisconsin-Extension allows multi-user conferencing via a toll-free number. These calls are easily arranged for a nominal fee via an internet request.

- Videoconferencing: Videoconferencing technology is available in the Distance Education Center at the Health Sciences Learning Center adjacent to UW Hospital and Clinics. This center offers state-of-the-art technology to communicate with colleagues at other institutions. The center is fully equipped for teleconferencing and videoconferencing with rooms to accommodate up to 24 participants. The rooms include a full array of audio-visual equipment, including DVD, slide projection, document camera, and x-ray projection. HSLC also provides staff to pre-coordinate connections and provide technical assistance with participating institutions. This service and use of the rooms and equipment is free of charge to UWSMPH faculty and staff.

The Department of Surgery also has a longstanding program of interactive remote educational programs, which is highly effective for advanced videoconferencing should high-resolution communication be required. The equipment supports interactive, bi-directional audio and video relay between conference facilities. The high definition system is portable, to allow flexible use in conference rooms throughout the health sciences buildings, and has the capacity to project multiple streams of video simultaneously.

The Department of Surgery also subscribes to Cisco Webex, which is utilized extensively for internet based videoconferencing and document exchange, and will be highly integrated into communication strategies with investigators at subcontracted institutions.

AL-ADRA LAB FACILITIES

Office: The Principal Investigator has an office on the [redacted] of the [redacted] of the UW Hospital. The hospital is adjacent and connected to WIMR within short walking distances of each other. Within WIMR, all lab personnel will have an office or private 36 sq. ft. cubicle. A fully equipped conference room with presentation and conference call capabilities is immediately adjacent to the lab.

Laboratory: The lab contains all the major equipment and bench space necessary for the proposed project. The lab space is highly collaborative and collegial, with multiple investigators present and willing to offer assistance and/or equipment to assist in the proposed project.

Computer: The PI's lab contains two computer workstations. These computers all have software for imaging, artwork and molecular biology applications, as well as internet access, and are used by lab personnel for data analysis, graphics, and literature searching. An HP Color Laser Jet 4650dn color printer and HP black and white printer are also available. Full software suites are supported by the department. All computers are networked.

Animal: All small animal surgery can be performed in microsurgery laboratories equipped with two stereomicroscopes, in the WIMR Vivarium animal care facilities. Several rooms are available for housing rodents. The animal care facilities are under the direction of three licensed veterinarians and are AAALAC and NIH approved. This facility offers a full range of animal care services, including purchasing, housing, animal care, sentinel monitoring and euthanasia. Animal care and use protocols are in place and have been approved by the UW Animal Care and Use Committee. A one-way 200 sq. ft. procedure room equipped with anesthesia machines and two stereomicroscopes is located immediately adjacent to the PI's lab space in WIMR.

Other: The Department of Surgery employs six full-time biostatisticians, several full-time microcomputer technicians, an editor, and a medical librarian available to assist researchers in their work. The staff in the Surgery accounting office provides post-award grant accounting services. A pre-award and editorial office provides assistance in preparing grant administrative pages and fulfilling University requirements for assurances and certifications. Medical illustration services are located on the third floor of the Clinical Science Center in the Department. The Department maintains copy machines and color printers for the laboratories. The hospital has electronic, machinist, and carpentry shops to support in-house construction needs.

University of Wisconsin Institute for Clinical and Translational Research (www.ictr.wisc.edu)
The Institute for Clinical and Translational Research (ICTR), supported by an NIH Clinical and Translational Science Award, was established to create an environment that transforms research into a continuum from investigation through discovery to translation into real-life community practice. ICTR's mission is to link even the most basic research to evidence-based policies and practices that will foster practical improvements in human health. Through the interdisciplinary nature of the ICTR (Schools of Medicine and Public Health, Nursing, Veterinary Medicine, and Pharmacy) and the College of Engineering, the mission is to change the UW culture from "silos" to collaborations among ICTR members and within the entire university. ICTR is located on the 4th floor of the Health Sciences Learning Center and offers many research and educational resources such as research methods courses and workshops, informatics tools, biostatistical and regulatory support, research software, and core technologies.

The Department also provides a **Histology Core Service** with equipment for paraffin-embedding and sectioning, and cryosectioning) for shared use, as well as a dedicated computer for Metamorph analyses.

In addition, we have access to the **Waisman Center Confocal Core Facility**, which is located next to both the hospital and WIMR. This facility contains a two-laser FACSCalibur, a three-laser Nikon C1 Confocal Microscope, and a Zeiss Photomicroscope with AxioVision and Stereo Investigator Software.

UW Carbone Cancer Center Flow Cytometry Facility is located two floors above the Al-Adra Lab in the WIMR building. It is a cutting-edge facility that provides researchers the technical and educational support for fluorescence-based single cell analysis and isolation to further the characterization and understanding of cellular functions. Equipment includes: two ThermoFisher Attune NxT Flow cytometers, a BD LSRII, a BD LSR Fortessa, a MACSQuant10, a Imagestream, and two BD FACSArias. iLab software is used for sign up and billing. Computers are loaded with a variety of analysis packages including FlowJo, ModFit, WinList, and IDEAS ImageStream analysis software, Microsoft Office, and Adobe Illustrator.

EQUIPMENT

Access to equipment and resources to facilitate research is rarely an obstacle in achieving research goals due to the abundant quantity of shared equipment in the Department of Surgery and on the UW campus, including NIH-funded shared equipment and facilities as described here.

In addition to a wealth of temperature-controlled storage units and traditional laboratory furniture and equipment, the department offers a large variety of shared equipment, allowing many users to avoid expensive purchases to meet part-time or infrequent needs. A network of experienced researchers provide training on use of the equipment. Shared equipment includes the following: Applied Biosystems 384 well qPCR, numerous centrifuges, numerous CO2 incubators, Nikon E600 compound microscope with 3 color fluorescence, Olympus DP70 camera Nikon Ti100 inverted microscope with fluorescence, monochrome and color camera with software, Molecular Devices flex Station 5 function multiplate reader, Nanodrop spectrophotometer, BD FACS flow cytometer (5 color), BD Accuri flow cytometer, Leica 1850 cryostat, several Nikon S10 stereomicroscopes, 1-6 channel anesthesia machine, Molecular Devices Vmax ELISA plate reader, AID ELISPOT reader, Biotek EL800 microplate ELISA reader UUP imaging systems for gel and western blot imaging, and biosafety cabinets.

Other major equipment within the Department includes: laminar flow hoods, dual carbon dioxide incubators, Zeiss microscopes, a dual-head microscope for microsurgery training, refrigerators, liquid nitrogen tanks, water baths, centrifuge and microfuge, electrophoresis equipment, pH meter, UV transilluminator, autoclaves, dishwashing equipment and services, and balances.

Surgical Facilities: All small animal surgery can be performed in microsurgery laboratories equipped with two stereomicroscopes, in the WIMR Vivarium animal care facilities. In additon, a one-way 200 sq. ft. procedure room equipped with anesthesia machines and two stereomicroscopes is located immediately adjacent to the PIs lab space in WIMR.

Major Equipment- Al-Adra Lab

The lab contains all the major equipment and bench space necessary for the proposed project. This includes: One laminar flow biocontainment hood; a fume hood; P75 low range blood pressure transducer; Ecoline II drive roller perfusion pump; CO2 water-jacketed incubator; Nikon inverted phase contrast microscope with computer-assisted digital imaging system; 2 Forma Series II (Dual gas) copper lined CO2 incubators; an Applied Biosystems 7500 Quantitative Real-Time PCR System; PCR enclosure; 2 Stratagene DNA Thermal Cyclers; 1 Stratagene Robocycler 96 Gradient Temperature Cycler; MJ Research PTC-100 thermal cycler; Beckman GS-GR table top refrigerated centrifuge; multiple microfuges; Fuji Mini 4000 Gel imaging system a Biotek Synergy II microplate ELISA reader; pH meter; balances; 1 refrigerator; 2 full-size -20^{o}C and -80^{o}C freezers; 2 water baths; 2 heat blocks; a cooling water bath; UV transilluminator; film cassettes and intensity screens; sonicator, Speed Vac concentrator; one large liquid nitrogen cell storage tank; 2 PC workstations for visual inspection of the data that are connected to a remote server, providing remote access to data and facilitating easy file sharing.

Shared facilities on the same floor: Nanopure water purification system, cold rooms, ultracentrifuges, autoclave and dishwashing facilities.

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: David Middle Name Last Name*: Al-Adra Suffix:

Position/Title*: ASSISTANT PROFESSOR
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: SURGERY
Division: Medicine and Public Health
Street1*: [redacted]
Street2:
City*:
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2012

Attach Biographical Sketch*: File Name: Al_Adra_K08_biosketch1039438996.pdf

Attach Current & Pending Support: File Name: Al_Adra__OS_10_14_20201039438997.pdf

Tracking Number: GRANT13237812 Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Christian Middle Name Matthew Last Name*: Capitini Suffix:

Position/Title*: Assistant Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pediatrics
Division: Medicine and Public Health
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Mentor

Degree Type: MD Degree Year: 2002

Attach Biographical Sketch*: File Name: Capitini_K08_biosketch1039337544.pdf

Attach Current & Pending Support: File Name: Capitini_OS_10_13_201039380786.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: WILLIAM Middle Name J Last Name*: BURLINGHAM Suffix: PhD

Position/Title*: PROFESSOR
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Surgery
Division: Medicine and Public Health
Street1*: [redacted]
Street2: [redacted]
City*: MADISON
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Mentor

Degree Type: PhD Degree Year: 1979

Attach Biographical Sketch*: File Name: Burlingham_K08_biosketch1039337545.pdf

Attach Current & Pending Support: File Name: burlingham_os_10_8_20201039337827.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: JOSHUA Middle Name D Last Name*: MEZRICH Suffix:

Position/Title*: ASST PROFESSOR
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Surgery - Transplantation
Division: Medicine and Public Health
Street1*: [redacted]
Street2: [redacted]
City*: MADISON
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Mentor

Degree Type: MD Degree Year: 1997

Attach Biographical Sketch*: File Name: Mezrich_K08_Biosketch1039337546.pdf

Attach Current & Pending Support: File Name: Mezrich_OS_10_8_20201039337828.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: Paul Middle Name M Last Name*: Sondel Suffix:

Position/Title*: Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pediatrics
Division: Medicine and Public Health
Street1*: [redacted]
Street2:
City*:
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Mentor

Degree Type: MD Degree Year: 1974

Attach Biographical Sketch*: File Name: Sondel_K08_biosketch1039337547.pdf

Attach Current & Pending Support: File Name: Sondel_OS_8_20_20201039337829.pdf

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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: David Al-Adra

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

POSITION TITLE: Assistant Professor of Surgery

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
University of Alberta, Edmonton, AB	BSc.	06/2002	Science-Genetics (Hon)
University of Alberta, Edmonton, AB	MD	06/2007	Medicine
University of Alberta, Edmonton, AB	PhD.	06/2012	Experimental Surgery and Immunology
University of Alberta, Edmonton, AB	FRCSC	06/2015	General Surgery Residency
University of Toronto, Toronto, ON	-	07/2017	Fellowship in Abdominal Transplantation Surgery

A. Personal Statement

As a basic scientist focusing on the mechanisms of immunological tolerance, I am attempting to induce tolerance to transplanted organs through immunomodulation. Modifying the composition of graft-resident immune cells and altering expression of cellular surface molecules to create a regulatory environment in the organ may decrease the organ's immunogenicity after transplant. Whole organ modification is possible prior to transplant using *ex vivo* normothermic machine perfusion. However, before modifying an organ, it is imperative to understand the mechanisms of tolerance present during liver transplantation. Understanding which immune cells are tolerogenic will assist in targeting these cells for modification or expansion in future research. The clinically relevant goal of these experiments is to decrease the need for induction and maintenance immunosuppression.

My prior basic science research investigated the mechanisms of immunological tolerance and how this process can be augmented by allogeneic chimerism (the presence of two or more genetically distinct cells within the same individual). The two themes of my research were: 1. Understanding the challenges in establishing chimerism in the setting of autoimmunity and creating a protocol to overcome these difficulties; and 2. Defining why the immune system will respond to certain transplants, but not others once chimerism is generated (referred to as "split tolerance"). These two themes required an in-depth understanding of peripheral tolerance and immune activation, knowledge that has prepared me well for the experiments involving regulatory dendritic cells in the current K08 proposal.

As a transplant surgeon-scientist at the University of Wisconsin-Madison (UW), I am involved in the medical and surgical care of patients who require abdominal organ transplants. I have experience in normothermic organ preservation and the technical expertise required to operate these perfusion systems. UW has a strong research history in the arena of transplantation, especially in organ preservation, and I am enthusiastic about contributing further to this field. The resources (advanced microsurgical skills) and cutting-edge technology (live-cell imaging, combination imaging/flow cytometry) available at UW make performing high-quality immunology research possible. My objective is to integrate my basic science laboratory with clinically relevant outcomes in a truly translational model of research. I intend to conduct high-quality research into the mechanisms of immunological tolerance, and will attempt to induce tolerance to transplanted organs through organ pre-treatment. I believe my surgical training and immunology background as well as the supportive research environment at UW will allow me to achieve these goals.

B. Positions and Honors

Positions and Employment

2017 – Present Assistant Professor, Department of Surgery, University of Wisconsin, Madison, WI

Honors and Awards

2020 – 2020 Surgical Society of the Alimentary Tract Career Development Award
2011 – 2012 Clinical Fellowship, Alberta Innovates Health Solutions
2011 – 2012 Incorporated Studentship for diabetes research, Cosmopolitan Foundation Canada
2012 The George R Graham Postgraduate Memorial Bursary in Surgery, University of Alberta
2014 Resident Teaching Award, Canadian Association of General Surgery

C. Contribution to Science

1. **Investigate the mechanisms of central immunological tolerance and split tolerance**
My early research revolved around creating central tolerance towards allografts by inducing allogeneic mixed hematopoietic chimerism. Specifically, I investigated the challenges in establishing mixed chimerism in the setting of autoimmunity (non-obese diabetic mouse model) and then created a protocol to overcome these difficulties. Once chimerism was established, I then further defined why the immune system will respond to certain transplants, but not others in the setting of chimerism (split tolerance). These investigations have demonstrated the resistance that Natural Killer and T cells have on chimerism induction in the non-obese diabetic mouse transplant model. In addition, we discovered some of the factors responsible for the development of split tolerance. Through the identification of these factors, we developed a successful minimalistic, non-myeloablative induction protocol that abrogates split tolerance towards islet transplants. To date, all published human mixed chimerism tolerance trials demonstrate split tolerance. Therefore, understanding the mechanisms behind this phenomenon has clinical relevance.

    a. **Al-Adra DP**, Chan WF, Anderson CC. (2011) Nonobese diabetic natural killer cells: a barrier to allogeneic chimerism that can be reduced by rapamycin. *Transplantation*. 92(9):977-84. PMID:21956197
    b. **Al-Adra DP**, Anderson CC. (2011) Mixed Chimerism and Split Tolerance: Mechanisms and Clinical Correlations. *Chimeris*m. 2(4): 89-101. PMCID: PMC3321885
    c. **Al-Adra DP**, Pawlick R, Shapiro AM, Anderson CC. (2012) Targeting Cells Causing Split Tolerance Allows Fully Allogeneic Islet Survival With Minimal Conditioning in NOD Mixed Chimeras. *American Journal of Transplantation.* 12(12):3235-3245. PMID: 22974315
    d. **Al-Adra DP**, Anderson CC. (2013) Toward minimal conditioning protocols for allogeneic chimerism in tolerance resistant recipients. *Chimerism*. 1(4):1-3. PMCID: PMC3654734

2. **Define the optimal treatment for primary liver cancer**
Primary liver cancers (hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA)) have a variety of treatment options. Optimal treatment of these cancers depends on patient, liver, and tumor factors. As new technology develops and treatment options evolve, it is important to assess which treatment modality is best suited to each patient and tumor in order to optimize survival. We investigated the role of treating unresectable CCA with radioactive embolization by performing a systematic review and pooled analysis of available literature. We demonstrated a small survival advantage in patients treated with the radioactive embolization. The results of this study changed the European Society of Medical Oncology Biliary Cancer Guidelines (cited below) to include radioactive embolization as an option for post-chemotherapy treatment of intrahepatic CCA. We then reviewed the treatment of recurrent HCC after initial therapy and showed liver transplantation is an effective therapy in these patients. The relevance of this study is that it demonstrates success of a curative option, transplantation, for the treatment of patients with recurrent HCC.

    a. **Al-Adra DP**, Gill RS, Axford SJ, Shi X, Kneteman N, Liau SS. (2015) Treatment of unresectable intrahepatic cholangiocarcinoma with yttrium-90 radioembolization: A systematic review and pooled analysis. *European Journal of Surgical Oncology*. 41(1):120-127. PMCID: PMC4316196

b. Valle JW, Borbath I, Khan SA, Huguet F, Gruenberger T, Arnold D. (2016) Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology* 27 (Supplement 5): v28–v37. PMID:27664259
c. **Al-Adra DP***, Muaddi H*, Beecroft R, Ghanekar A, Moulton C-A, Doyle A, Selzner M, Wei A, McGilvray I, Gallinger S, Grant DR, Cattral MS, Greig PD, Kachura J, Cleary SP, Sapisochin G. (2018) Liver Transplantation is Equally Effective as a Salvage Therapy for Patients with Hepatocellular Carcinoma Recurrence Following Radiofrequency Ablation or Liver Resection with Curative Intent. *Annals of Surgical Oncology*. 25(4): 991-999 PMID: 29327179.
*Authors contributed equally

3. Transplant oncology

I continue to examine questions related to my patients in terms of transplant outcomes as it relates to malignancy. This involves the selection of potential recipients with a prior diagnosis of cancer as well as treating patients who have liver cancer with transplantation. This work helps focus our lab efforts on translational aspects that can ultimately improve the health of patients.

Al-Adra DP, Hammel L, Roberts J, Woodle ES, Levine D, Mandelbrot D, Verna E, Locke J, D'Cunha J, Farr M, Sawinski D, Agarwal PK, Plichta J, Pruthi S, Farr D, Carvajal R, Walker J, Zwald F, Habermann T, Gertz M, Bierman P, Dizon DS, Langstraat C, Al-Qaoud T, Eggener S, Richgels JP, Chang GJ, Geltzeiler C, Sapisochin G, Ricciardi R, Krupnick AS, Kennedy C, Mohindra N, Foley DP, Watt KD. (2020) Pre-Existing Melanoma and Hematological Malignancies, Prognosis and Timing to Solid Organ Transplantation: A Consensus Expert Opinion Statement. *American Journal of Transplantation*. Sep 25. doi: 10.1111/ajt.16324. Online ahead of print. PMID: 32976703.

Al-Adra DP, Hammel L, Roberts J, Woodle ES, Levine D, Mandelbrot D, Verna E, Locke J, D'Cunha J, Farr M, Sawinski D, Agarwal PK, Plichta J, Pruthi S, Farr D, Carvajal R, Walker J, Zwald F, Habermann T, Gertz M, Bierman P, Dizon DS, Langstraat C, Al-Qauod T, Eggener S, Richgels JP, Chang GJ, Geltzeiler C, Sapisochin G, Ricciardi R, Krupnick AS, Kennedy C, Mohindra N, Foley DP, Watt KD. (2020) Pre-Transplant Solid Organ Malignancy and Organ Transplant Candidacy: A Consensus Expert Opinion Statement. *American Journal of Transplantation.* Sep 24. doi: 10.1111/ajt.16318. Online ahead of print. PMID: 32969590.

Complete list of published work in My Bibliography
https://www.ncbi.nlm.nih.gov/myncbi/david.al-adra.1/bibliography/public/

D. Research Support

Ongoing Research Support

[redacted] 07/01/20-06/31/22 0.72 Cal Mo

Immunomodulation of the Liver using Normothermic ex-vivo Machine Perfusion

This project aims to understand the immunological effects of NEVLP on the liver and the effects on the immune microenvironment.

Completed Research Support
None

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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: Capitini, Christian

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

POSITION TITLE: Associate 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)	END DATE MM/YYYY	FIELD OF STUDY
Drew University, Madison, NJ	BA	05/1998	Biology
University of Rochester School of Medicine and Dentistry, Rochester, NY	MD	05/2002	N/A
University of Minnesota, Minneapolis, MN	Resident	06/2005	Pediatrics
Johns Hopkins University/National Cancer Institute, Baltimore and Bethesda, MD	Fellow	06/2008	Pediatric Hematology/Oncology
National Cancer Institute, Bethesda, MD	Postdoctoral Fellow	08/2011	Immunology Section, Pediatric Oncology Branch

A. Personal Statement

My research focus has been on using mouse models of allogeneic bone marrow transplant after lethal irradiation to optimize therapies that prevent graft-versus-host-disease (GVHD). My main focus has been on understanding the biology of antigen presenting cells, such as dendritic cells and macrophages. After joining the University of Wisconsin, I received a K08 award and published how bone marrow deficient in STAT1 generated high levels of STAT3+ plasmacytoid dendritic cells that help prevent GVHD. Through this work we gained expertise in culturing and expanding dendritic cells, profiling their phenotype in vitro, and administering them as a cellular therapy in vivo. My present research focuses on the role of a new inducible macrophage, that can protect the host from GVHD and radiation injury. Many of the same assays we used to define dendritic cell subsets are still in use in our lab while we define macrophage subsets. Thus, I am in a unique position to mentor the KL2 Candidate, Dr. David Al-Adra. In addition, I have a track record of mentoring postdoctoral trainees and early stage faculty, having trained/am training 3 PhD postdoctoral fellows and 3 physician-scientists, 2 of whom are now Assistant Professors at other institutions.

B. Positions and Honors

Positions and Employment

1993 - 1993 Summer Student Researcher, Center of Molecular Medicine & Immunology, Newark, NJ
1994 - 1998 Undergraduate Research Assistant, Department of Biology, Drew University, Madison, NJ
1995 - 1995 Summer Student Researcher, Department of Microbiology, Biochemistry & Molecular Genetics, Howard Hughes Medical Institute, University of Cincinnati, Cincinnati, OH
1997 - 1997 Summer Student Researcher, Department of Pharmacology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC
1999 - 1999 Cancer Research Training Awardee, National Cancer Institute, Laboratory of Leukocyte Biology, Frederick, MD
2000 - 2000 Cancer Research Training Awardee, National Cancer Institute, Laboratory of Leukocyte Biology, Frederick, MD
2008 - 2011 Postdoctoral Fellow, National Cancer Institute, Pediatric Oncology Branch, Immunology Section, Bethesda, MD
2011 - 2020 Assistant Professor, University of Wisconsin, Department of Pediatrics, Division of Hematology, Oncology & Bone Marrow Transplant, Madison, WI

2018 - Director of Clinical Innovation, Forward BIO Institute, Madison, WI
2020 - Associate Professor with tenure, University of Wisconsin, Department of Pediatrics, Division of Hematology, Oncology & Bone Marrow Transplant, Madison, WI
2020 - Affiliate Faculty, University of Wisconsin, Department of Biomedical Engineering, Madison, WI
2020 - Co-Program Leader, Developmental Therapeutics, University of Wisconsin Carbone Cancer Center, Madison, WI

Other Experience and Professional Memberships

2015 Study section, Peer Reviewed Cancer Research Medical Program, Department of Defense (DOD)
2015 - present Section Editor, Journal for Immunotherapy of Cancer
2015 - 2016 Study section, Reconstructive Transplant Research , DOD
2016 Study section, Transplantation, Tolerance and Tumor Immunology (TTT), NIH
2016 Study section, Neuroblastoma, DOD
2016 Study section, Pediatric Brain Tumors, DOD
2016 - 2018 Study section, Neuroblastoma and Pediatric Brain Tumors, DOD
2017 Study section, Leukemia and Lymphoma, DOD
2017 - present Section Editor, Advances in Cell and Gene Therapy
2018 Study section, Neuroblastoma, DOD
2018 Study section, Reconstructive Transplant Research, DOD
2018 - 2020 Study section, Cancer Immunopathology and Immunotherapy (CII), NIH
2020 - present Study section, TTT, NIH

Honors

2011 - 2012 Scholar, Hyundai Hope on Wheels
2012 - 2015 Centennial Program Scholar for faculty from underrepresented minorities, University of Wisconsin School of Medicine and Public Health
2012 Moderator, Professional Development Session, Society for Immunotherapy of Cancer
2013 Session Chair, Gene/Cell Therapy, 3rd Annual World Drug Discovery Online Conference
2013 - 2021 Young Investigator, Pediatric Cancer Dream Team, Stand Up to Cancer/St. Baldrick's Foundation/American Association for Cancer Research
2014 Early Career Faculty Travel Award, American Association of Immunologists
2015 Faculty Abstract Awardee, Immuno-oncology Young Investigator Forum
2015 - 2016 Vilas Faculty Early Career Investigator Award, University of Wisconsin, Office of the Provost
2016 - 2018 Scholar, Hyundai Hope on Wheels
2016 - 2017 "Top 25" Teacher, UWSMPH Dept. of Pediatrics
2017 Distinguished Speaker, Combination Immunotherapy, GTCbio 9th Immunotherapeutics and Immunomonitoring Conference
2017 Moderator, Immunomonitoring and novel high throughput technologies, GTCbio 9th Immunotherapeutics and Immunomonitoring Conference
2017 Early Career Faculty Travel Award, American Association of Immunologists
2018 - 2022 Research Scholar, American Cancer Society
2018 Founding Section Editor, 4-year service award, Journal for the Immunotherapy of Cancer
2018 - 2019 Gerard B. Odell Research Award, UWSMPH Dept. of Pediatrics
2019 Outstanding New Member Science Award, Society for Pediatric Research
2019 Moderator, Hematopoietic Stem Cell Transplantation, American Association of Immunologists Annual Meeting
2019 Moderator and Organizer, 14th Annual Wisconsin Stem Cell Symposium

C. Contribution to Science

1. Separating the beneficial graft-versus-leukemia (GVL) effect from deleterious graft-versus-host-disease (GVHD) remains the “holy grail” of allogeneic hematopoietic stem cell transplantation (alloHSCT). I have demonstrated that mild, subclinical GVHD markedly impairs T cell responses to a tumor-associated antigen. Using donor bone marrow deficient in gamma interferon signaling could reverse this process. With this approach, the host could tolerate high doses of T cells without ever developing GVHD. I also demonstrated that donor antigen presenting cells play a role in extracorporeal photopheresis through an IL-10 dependent mechanism. We also found that using donor bone marrow deficient in STAT1, which is downstream of the gamma interferon receptor, prevents GVHD and leads to an expansion of anti-inflammatory, donor-derived plasmacytoid dendritic cells, enhancing immune reconstitution and the GVL effect.

    a. **Capitini CM**, Nasholm NM, Chien CD, Larabee SM, Qin H, Song YK, Klover PJ, Hennighausen L, Khan J, Fry TJ. Absence of STAT1 in donor-derived plasmacytoid dendritic cells results in increased STAT3 and attenuates murine GVHD. Blood. 2014 Sep 18;124(12):1976-86. PubMed PMID: 25079358; PubMed Central PMCID: PMC4168352.
    b. **Capitini CM**, Davis JP, Larabee SM, Herby S, Nasholm NM, Fry TJ. Extracorporeal photopheresis attenuates murine graft-versus-host disease via bone marrow-derived interleukin-10 and preserves responses to dendritic cell vaccination. Biol Blood Marrow Transplant. 2011 Jun;17(6):790-9. PubMed PMID: 21216299; PubMed Central PMCID: PMC3087832.
    c. **Capitini CM**, Herby S, Milliron M, Anver MR, Mackall CL, Fry TJ. Bone marrow deficient in IFN-{gamma} signaling selectively reverses GVHD-associated immunosuppression and enhances a tumor-specific GVT effect. Blood. 2009 May 14;113(20):5002-9. PubMed PMID: 19258593; PubMed Central PMCID: PMC2686147.

2. I have showed that GVHD impairs responses to a tumor vaccine by inhibiting antigen-specific T cell proliferation and increasing T cell apoptosis. Inhibiting perforin in GVHD-causing T cells restores vaccine responses in vivo, leading to decreased tumor growth. Using this expertise, I became involved in collaborations developing a novel humanized mouse model of chronic GVHD. Recently our lab has shown that human mesenchymal stem cell (MSC)-educated macrophages can treat xenogeneic GVHD and lethal radiation injury, and have used exosomes from MSCs to generate these macrophages in an "off the shelf" manner.

    a. Kink JA, Forsberg MH, Reshetylo S, Besharat S, Childs CJ, Pederson JD, Gendron-Fitzpatrick A, Graham M, Bates PD, Schmuck EG, Raval A, Hematti P, **Capitini CM**. Macrophages Educated with Exosomes from Primed Mesenchymal Stem Cells Treat Acute Radiation Syndrome by Promoting Hematopoietic Recovery. Biol Blood Marrow Transplant. 2019 Nov;25(11):2124-2133. PubMed PMID: 31394269; PubMed Central PMCID: PMC6861683.
    b. Bouchlaka MN, Moffitt AB, Kim J, Kink JA, Bloom DD, Love C, Dave S, Hematti P, **Capitini CM**. Human Mesenchymal Stem Cell-Educated Macrophages Are a Distinct High IL-6-Producing Subset that Confer Protection in Graft-versus-Host-Disease and Radiation Injury Models. Biol Blood Marrow Transplant. 2017 Jun;23(6):897-905. PubMed PMID: 28257800; PubMed Central PMCID: PMC5499382.
    c. Lockridge JL, Zhou Y, Becker YA, Ma S, Kenney SC, Hematti P, **Capitini CM**, Burlingham WJ, Gendron-Fitzpatrick A, Gumperz JE. Mice engrafted with human fetal thymic tissue and hematopoietic stem cells develop pathology resembling chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2013 Sep;19(9):1310-22. PubMed PMID: 23806772; PubMed Central PMCID: PMC3755109.
    d. **Capitini CM**, Nasholm NM, Duncan BB, Guimond M, Fry TJ. Graft-versus-host disease impairs vaccine responses through decreased CD4+ and CD8+ T cell proliferation and increased perforin-mediated CD8+ T cell apoptosis. J Immunol. 2013 Feb 1;190(3):1351-9. PubMed PMID: 23275602; PubMed Central PMCID: PMC3626171.

3. I have had a longstanding interest in improving usage of adoptive transfer of NK cells to improve anti-tumor effects after syngeneic and allogeneic HSCT. One of my first publications demonstrated that NK cell anti-tumor activity could be enhanced by blocking inhibitory receptors on their cell surface. As a PI, my lab recently developed the first protocol for tracking NK cells by 19F-MRI to solid tumors like neuroblastoma and melanoma as well as lymphoma. Using ex vivo expansion with IL-15 and 4-1BBL, we are presently combining NK cells with the anti-GD2 immunocytokine hu14.18-IL2 as a means of improving GVT effects against neuroblastoma after allogeneic HSCT.

   a. Damodharan SN, Walker KL, Forsberg MH, McDowell KA, Bouchlaka MN, Drier DA, Sondel PM, DeSantes KB, **Capitini CM**. Analysis of ex vivo expanded and activated clinical-grade human NK cells after cryopreservation. Cytotherapy. 2020 Aug;22(8):450-457. PubMed PMID: 32536506; PubMed Central PMCID: PMC7387178.
   b. Cho MM, Quamine AE, Olsen MR, **Capitini CM**. Programmed cell death protein 1 on natural killer cells: fact or fiction?. J Clin Invest. 2020 Jun 1;130(6):2816-2819. PubMed PMID: 32391808; PubMed Central PMCID: PMC7260016.
   c. Lieberman NAP, DeGolier K, Haberthur K, Chinn H, Moyes KW, Bouchlaka MN, Walker KL, **Capitini CM**, Crane CA. An Uncoupling of Canonical Phenotypic Markers and Functional Potency of *Ex Vivo*-Expanded Natural Killer Cells. Front Immunol. 2018;9:150. PubMed PMID: 29456538; PubMed Central PMCID: PMC5801405.
   d. Bouchlaka MN, Ludwig KD, Gordon JW, Kutz MP, Bednarz BP, Fain SB, **Capitini CM**. (19)F-MRI for monitoring human NK cells in vivo. Oncoimmunology. 2016 May;5(5):e1143996. PubMed PMID: 27467963; PubMed Central PMCID: PMC4910731.

4. We have identified a variety of targeted therapeutic approached toward the treatment of high grade lymphomas using syngeneic and xenogeneic models. We have used small molecule inhibitors of BCL2 and PRMT5 to demonstrate critical anti-apoptotic and signaling pathways used by mantle cell and diffuse large B cell lymphoma respectively. In addition, we have helped develop a targeted radiotherapy approach for treating T cell lymphoma, and demonstrated induction of T cell memory to tumor neoantigens. We also have helped develop a novel labeling approach for analyzing CSF from patients with B cell leukemia.

   a. Yu Q, Zhong X, Chen B, Feng Y, Ma M, Diamond CA, Voeller JS, Kim M, DeSantes KB, **Capitini CM**, Patel NJ, Hoover-Regan ML, Burke MJ, Janko K, Puccetti DM, Ikonomidou C, Li L. Isobaric Labeling Strategy Utilizing 4-Plex *N*,*N*-Dimethyl Leucine (DiLeu) Tags Reveals Proteomic Changes Induced by Chemotherapy in Cerebrospinal Fluid of Children with B-Cell Acute Lymphoblastic Leukemia. J Proteome Res. 2020 Jul 2;19(7):2606-2616. PubMed PMID: 32396724; PubMed Central PMCID: PMC7334086.
   b. Zhu F, Guo H, Bates PD, Zhang S, Zhang H, Nomie KJ, Li Y, Lu L, Seibold KR, Wang F, Rumball I, Cameron H, Hoang NM, Yang DT, Xu W, Zhang L, Wang M*, **Capitini CM***, Rui L*. PRMT5 is upregulated by B-cell receptor signaling and forms a positive-feedback loop with PI3K/AKT in lymphoma cells. Leukemia. 2019 Dec;33(12):2898-2911. PubMed PMID: 31123343; PubMed Central PMCID: PMC7494157. * = co-corresponding authors
   c. Hernandez R, Walker KL, Grudzinski JJ, Aluicio-Sarduy E, Patel R, Zahm CD, Pinchuk AN, Massey CF, Bitton AN, Brown RJ, Sondel PM, Morris ZS, Engle JW, **Capitini CM**, Weichert JP. ^{90}Y-NM600 targeted radionuclide therapy induces immunologic memory in syngeneic models of T-cell Non-Hodgkin's Lymphoma. Commun Biol. 2019;2:79. PubMed PMID: 30820474; PubMed Central PMCID: PMC6391402.
   d. Li Y, Bouchlaka MN, Wolff J, Grindle KM, Lu L, Qian S, Zhong X, Pflum N, Jobin P, Kahl BS, Eickhoff JC, Wuerzberger-Davis SM, Miyamoto S, Thomas CJ, Yang DT*, **Capitini CM***, Rui L*. FBXO10 deficiency and BTK activation upregulate BCL2 expression in mantle cell lymphoma. Oncogene. 2016 Dec 1;35(48):6223-6234. PubMed PMID: 27157620; PubMed Central PMCID: PMC5102814. * = co-corresponding authors

D. Additional Information: Research Support

Ongoing Research Support

R01 CA215461, NCI/NIH Capitini, Christian (PI) 07/03/18-05/31/23
Combining hu14.18-IL2 and NK cell infusions to treat neuroblastoma
The long term objective of this proposal is to enhance the graft-versus-tumor effect against neuroblastoma.

P30 CA014520, NCI/NIH Bailey, Howard (PI) 04/01/19-03/31/21
Image-guided CAR T cell therapy for neuroblastoma
The project is an administrative supplement for UW Carbone Cancer Center's funded project, P30 CA014520. This pending project is to support research in cell-based immunotherapies of human cancer specifically to the project leader, Christian Capitini, MD. This proposal will test 3 exciting imaging technologies to study CAR T cell metabolism and track persistence in tumors non-invasively, potentially improving outcomes.
Role: Project Leader

[redacted] [redacted] (PI) 12/01/17-11/30/22
Immunogenomics to create new therapies for high risk childhood cancers
This collaborative multi-institutional consortium links genomics and immunotherapy efforts underway at 8 Pediatric Oncology Centers in order to perform preclinical and clinical collaborative work designed to move new therapies into clinical testing.
Role: Subcontract Young Investigator

[redacted] [redacted] (PI) 07/01/18-06/30/22
Ex vivo activated NK cells and immunocytokine for pediatric cancers
The goal of this project is to use murine models to develop evidence for a clinically applicable combined strategy that utilizes IC to enhance anti-tumor properties of immunologically activated, ex-vivo activated NK cells, and to track the localization of these NK cells using a novel 19F-MRI platform for neuroblastoma and osteosarcoma.

[redacted] [redacted] (PI) 05/01/20-12/31/20
Vaccine and checkpoint blockade after allogeneic BMT for neuroblastoma
This proposal will support an undergraduate, Nicholas Mohrdieck, for a Summer Fellowship in pediatric oncology research. NK cell activation via co-culture with a vaccine engineered to express CD54, CD80, CD86, and CD137L, called AgN2a 4P, will be studied to investigate NK cells' ability to induce cytotoxicity of murine neuroblastoma tumor cells in vitro and in vivo.

[redacted] [redacted] (PI) 07/01/20-06/30/22
Treating post-transplant B cell acute lymphoblastic leukemia with blinatumomab as a radiation sparing immunotherapy
This proposal will lead to the creation of innovative in vivo models for assessing gamma delta T cell activation and its role after alloHSCT, all using humanized models and leukemias.

[redacted] [redacted] (PI) 07/01/20-06/30/22
Activation of NK cells against pediatric cancers using IL-15 with TGF-beta trap
This proposal will test for the first time the potential efficacy of a "fusokine" called FIST15 with the objective of enhancing NK-mediated anti-tumor effects after alloBMT while simultaneously preventing immunosuppressive effects of the tumor by trapping TGF-beta.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

NAME: BURLINGHAM, WILLIAM J.

eRA COMMONS USER NAME (agency login): [redacted]

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Livingston College, Rutgers University, Piscataway, NJ	BS	1974	Biology
Syracuse University, Syracuse, NY	PhD	1979	Biology
Mayo Clinic, Rochester, MN	Post-Doc	1983	Immunology

A. PERSONAL STATEMENT

As a Ph.D. immunologist with a laboratory focused on basic and translational studies in transplant immunology, my goal is to develop strategies to improve transplant outcomes by inducing immunologic tolerance based on chimerism, in the great UW tradition of Ray Owen. Ray discovered mixed chimerism in red blood cells of dizygotic cattle twins in 1945, launching the fields of modern immunogenetics and transplantation. My laboratory studies two types of clinical transplants: kidney, where organs of living donors are the focus, and lung or liver, where the organs are all from deceased donors. In liver transplantation, we have found that mismatched donor HLA class I drove a Treg reponse in the host, while donor HLA-class II had a complex effect: DR(no Treg) and –DQ (weak Treg induction). Although the specific antigens driving tolerance or rejection in each organ are different, developing a favorable ratio of regulatory-to-effector-T cells both within the host and in the microenvironment of the donor organ is a common theme of my research in both. My overall goal is to use the knowledge we have gained in our studies of kidney, liver and lung transplant recipients and donors to find or create tolerance–prone donor-recipient pairs. According to our research so far, the recipe for successful tolerance involves establishment of “immune privilege” in the donor organ, combined with anergy, clonal deletion, and regulation in the host. Our focus is on adaptive and innate T cells, as well as dendritic cells(DC), which create the conditions for, and stabilize the tolerant state. The specific T cells and DC required appear to differ in each organ system, depending on the barrier and metabolic functions of the organ. My lab was the first to identify “autoimmune” Th17 responses that were critical for the chronic failure of human lung transplants, known as bronchiolitis obliterans. We also were the lab to first describe the tolerance effect of non-inherited maternal antigens (NIMA) in sibling kidney transplantation, and more recently, to recognize the importance of DC-derived exosomes in that tolerance, and living-related organ transplantation generally. Finally, our recent paper in Cell Reports (Jan. 2020) re-defines the inhibitory cytokine IL35, discovered 13 yrs ago by Dario Vignali, as a “vesikine”, i.e. a constituent of extracellular vesicles released by regulatory T and other (Bregs) cell types, and as the principle mediator of “infectious” tolerance.

B. POSITIONS AND HONORS

Positions and Employment

1983 – 1985 Project Associate, Department of Surgery, University of Wisconsin, Madison, WI
1985 – 1988 Assistant Scientist, Department of Surgery, University of Wisconsin, Madison, WI
1988 – 1990 Associate Scientist, Department of Surgery, University of Wisconsin, Madison, WI
1990 – 1993 Senior Scientist, Department of Surgery, University of Wisconsin, Madison, WI
1993 – 1999 Assistant Professor, Department of Surgery, University of Wisconsin, Madison, WI
1999 – 2005 Associate Professor, Department of Surgery, University of Wisconsin, Madison, WI
2005 – Present Professor, Department of Surgery, University of Wisconsin, Madison, WI

Honors and Awards

1994 Outstanding Service Award from the Autumn Immunology Conference, Chicago, IL
1997 ASHI Scholar Award, American Society for Histocompatibility and Immunogenetics, 23rd Annual Meeting, Atlanta, Georgia

1997 – 2002	Career Development Award, NIAID National Institutes of Health, K02-AI01452-01 "Soluble Forms of HLA-A, B and Chronic Rejection"
2004 – 2006	American Society of Transplantation "In appreciation and recognition of service and dedication," Awards and Grants Committee
2001	Excellence in Presentation: "Microchimerism and Tolerance" at Festschrift honoring Dr. Thomas E. Starzl, Pittsburgh, PA
2008	"Adaptive Tregs, Dendritic Cells and Bystander Suppression in Transplant Tolerance," The Canadian Society of Transplantation, Quebec, Canada
2008	"Inducing tolerance to transplants while maintaining tolerance to self" Int'l Society for Expt'l Microsurgery Shanghai, China
2007-2010	Member, Tumor, Transplantation, and Tolerance [TTT] study section, NIH-NIAID

C. Contribution to Science

1. My lab was the first to show that maternal microchimerism was linked to CTL anergy in a tolerant kidney transplant recipient, and the first to show a tolerogenic effect of non-inherited maternal antigens [NIMA] in renal transplants between siblings. We discovered that patients with long-standing tolerance have donor antigen-specific regulatory T cells that produce IL35 and TGFβ, and utilize CTLA-4 to suppress delayed type hypersensitivity responses via T cell-dendritic cell interactions. My group has further characterized a population of Foxp3-negative, $CD25^{int}CD4^{+}$ CTLA-4^{+}, surface $TGF\beta^{+}$ regulatory T cells specific for a HLA-B-derived donor allopeptide, in tolerant transplant recipients. We have also found using HLA class I/peptide tetramers, a tetramer-dim, CTLA-4^{+} $CD8^{+}$ T reg cell in a long term tolerant patient that recognized a human minor H antigen of the kidney donor in the context of HLA-A2. Recently, we discovered the mechanistic basis for split tolerance to non-inherited maternal antigens (NIMA) in a mouse F1 backcross model, based on microchimerism-derived exosomes, using imaging flow cytometry and TcR Tg T cell adoptive transfer approaches.

a. **Burlingham WJ**, AP Grailer, DM Heisey, FHJ Claas, D Norman, .et al. The effect of tolerance to noninherited maternal HLA antigens on the survival of renal transplants from sibling donors. *New England Journal of Medicine* 1998; 339 (23), 1657-1664.
b. Dutta P, Molitor-Dart M, Bobadilla JL, Roenneburg DA, Yan Z, Torrealba JR, **Burlingham WJ**. Microchimerism is strongly correlated with tolerance to noninherited maternal antigens in mice. *Blood.* 2009;114(17):3578-87. PMC2766676.
c. Dutta P, Dart M, Roenneburg DA, Torrealba JR, **Burlingham WJ**. Pre-transplant Immune Regulation Predicts Alllograft Tolerance. *Am J Transplant* 2011; 11(6):1296-301. PMC3110527.
d. Bracamonte-Baran W, J Florentin, Y Zhou, … **Burlingham WJ**. Modification of host dendritic cells by microchimerism-derived extracellular vesicles generates split tolerance. Proceedings of the National Academy of Sciences 2017; 114 (5), 1099-1104 PMC5293109

2. Work in my lab has identified IL17-producing T cells specific for sentinel antigens left sequestered after activation [aka 'SALSAA's] that include collagen V, vimentin & k-α1-tubulin] as a key factor in chronic rejection of clinical heart & lung transplants. These T cells require monocytes for function, develop in the thymus as T effector memory cells, and they are normally restrained by a novel class of $CD39^{+}CD4^{+}$ Treg cells, which operate independently of dendritic cells or CTLA4. Regulation may be lost over time after transplant, and is associated with fibro-obliterative disease in the transplanted lung or heart.

a. **Burlingham, W.J**., et al., IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants*. J Clin Invest*, 2007. 117(11): p. 3498-3506. PMC2040314
b. Keller MR, Haynes LD, Jankowska-Gan E, Sullivan JA, Agashe VV, Burlingham SR**, Burlingham WJ**. Epitope analysis of the collagen type V-specific T cell response in lung transplantation reveals an HLA-DRB1*15 bias in both recipient and donor. *PLOS One*. 2013 Nov 12;8(11). PMC3827168
c. Sullivan JA, Jankowska-Gan E, Shi L, Roenneburg D, Hegde S, Greenspan DS, Wilkes DS, Denlinger LC, **Burlingham WJ**. Differential Requirement for P2X7R Function in IL-17 Dependent vs. IL-17 Independent Cellular Immune Responses. *Am J Transplant.* 2014 Jul;14(7):1512-22. PMC4295495
d. Sullivan JA, Jankowska-Gan E, Hegde S, Pestrak MA, Agashe VV, Park AC, Brown ME, Kernien JF, Wilkes DS, Kaufman DB, Greenspan DS, **Burlingham** WJ.Th17 Responses to Collagen Type V, kα1-

Tubulin, and Vimentin Are Present Early in Human Development and Persist Throughout Life. .Am J Transplant. 2017 Apr;17(4):944-956. PMC5626015 doi: 10.1111/ajt.14097. Epub 2016 Dec 19.

3. In addition to my work in transplant immunology, I have been involved in collaborations regarding the role of the Treg product IL35 in cancer and autoimmunity. This has resulted in: 2 papers with Dr. Dan Greenspan on atherosclerosis and it's treatment by tolerogenesis using intranasal collagen type V, plus a paper with Dr. Doug McMeel in the UW-CCC, identifying production of IL35 by $CTLA4^{+}CD8$ T regulatory cells in prostate cancer patients as a limiting factor for immunotherapy using DNA vaccines incorporating a tumor –specific antigen. This latter work has prepared my lab's foray into analyzing "unknown" tumor antigens and PD1/PD-L1 with Dr. Ticiana Leal. Recently, we submitted a paradigm-shifting paper that was favorably reviewed, and which we are revising. It identifies IL35 as a "vesikine", i.e. not a conventional soluble cytokine, but rather one that is mobilized by association with the tetraspannin CD81 and incorporated into extracellular vesicles. When taken up by lymphocytes, IL35 components p35 and Ebi3 are free to associate at the cell surface and then bind the IL35 receptor on other cells (trans-, or 2^{0}-suppression) or on the surface of the same cell(cis- suppression), leading to exhaustion of that T or B cell. This breakthrough and its implications for transplant and tumor therapy is discussed in a recent (Sept 2020) review.

a. Dart ML, Jankowska-Gan E, Huang G, Roenneburg DA, Keller MR, Torrealba JR, Rhoads A, Kim B, Bobadilla JL, Haynes LD, Wilkes DS, **Burlingham** WJ, Greenspan DS. Interleukin-17-dependent autoimmunity to collagen type V in atherosclerosis. *Circ Res.* 2010 Oct 29;107(9):1106-16 PMC3010213
b. Park AC, Huang G, Jankowska-Gan E, Massoudi D, Kernien JF, Vignali DA, Sullivan JA, Wilkes DS, **Burlingham** WJ, Greenspan DS. Mucosal Administration of Collagen V Ameliorates the Atherosclerotic Plaque Burden by Inducing Interleukin 35-dependent Tolerance. *J Biol Chem.* 2016 Feb 12;291(7):3359-70. PMC4751380
c. Olson BM, Jankowska-Gan E, Becker JT, Vignali DA, **Burlingham** WJ, McNeel DG. Human prostate tumor antigen-specific CD8+ regulatory T cells are inhibited by CTLA-4 or IL-35 blockade. *J Immunol.* 2012 Dec 15;189(12):5590-601 PMC3735346
d. Sullivan, J.A.,Tomita, Y., Jankowska-Gan,E.,Lema, D.,Arvedson, M., Nair, A.,Bracamonte-Baran, W., Zhou, Y., Meyer K.K., Zhong, W., Sawant, D.V., Szymczak-Workman, A.L., Zhang, Q., Workman C.J.,Vignali, D.A.A., **Burlingham,** W.J. Treg-derived, IL35-coated exosomes promote infectious

   tolerance. Cell Rep, 30 (4), 1039-1051.e5 2020 Jan 28 PMID: 31995748
e. **Burlingham WJ** , Al-Adra, DA, Olson BM, McNeel DG, Sullivan,JA Infectious Tolerance as seen with 2020 vision: the role of IL-35 and Extracellular Vesicles *Frontiers in Immunology* (Aug 2020)

Complete List of Published Work in My Bibliography:
https://scholar.google.com/citations?sortby=pubdate&hl=en&user=49O6WUYAAAAJ&view_op=list_works)

D. RESEARCH SUPPORT

Ongoing Research Support

R01AI119140 (Burlingham, PI) 03/01/2016 - 02/28/2021
NIH/NIAID
Natural vs. Pathogenic Th17 responses to col Va1, Ka1tubulin and vimentin
The goal of this project is to test the hypothesis that: a) responses to certain "self" antigens common to all mammals are the result of natural Th17 cells that escape clonal deletion and arise during ontogeny under strict Treg control. The challenge in the transplant setting is that addition of a "new" HLA-DR by way of a DR-mismatched lung or heart graft, results in additional col Va1, Ka1tubulin and vimentin peptide reactivities which the recipient may or may not be able to control, leading to long term survival of some, or chronic rejection of other, DR-mismatched lung transplants.

U01AI102456 (Kaufman, PI) 07/01/2012-06/30/2022
NIH/NIAID
Tomotherapy and Hematopoietic Cells for Tolerance to Kidney Transplants
This project aims to test the hypothesis that tolerance to MHC mismatched living related kidney transplant

can be effectively and safely achieved by establishing a stable immune mixed chimeric state in non-human primates using a novel non-myeloablative, helical tomotherapy-based total lymphoid irradiation (TLI) conditioning regimen followed by Mozobil + G-CSF mobilized donor hematopoietic cell infusions.
Role: Co-I

R01AI110617 (Fernandez, PI) 04/01/2015-03/31/2020
NIH/NIAID
The Role of Complement Inhibition in Expanded Criteria Kidney Transplantation
The overarching goal of this proposal is to investigate the pattern of complement activation in expanded criteria brain death donors and to test the efficacy of human (C1INH) in preventing renal damage using a novel, highly translational kidney transplantation/DGF model in non-human primates (NHP).
Role: Co-I

1U01HL134764-01 Zhang (contact PI), Kamp (Co-PI) 9/1/2016-8/31/2023
NIH/NHLBI

"Integrated Cellular and Tissue Engineering for Ischemic Heart Disease"
The long-term goal of this project is to generate a functional human cardiac tissue patch using derivatives of human pluripotent stem cells for repair of ischemic myocardium overcoming immunological and arrhythmia barriers.
Role: Subproject Co-PI
$[redacted] annual funding from this grant, now in its 3rd year

[redacted] 07/01/05-present
This core will provide investigators with assay systems to evaluate the immunogenicity of ES-derived tissue transplants. Services will be provided in immunology and pathology.
Role: Core Leader

Completed Research Support

R01 AI066219-10 (Burlingham, PI) 06/15/2006-11/30/2016
NIH/NIAID
Maternal Microchimerism and Neonatal Tolerance
Specific Aims: 1) We will determine the influence of maternal exposure upon the development and phenotype of maternal antigen specific T regulatory and T effector cells in an F1 backcross breeding model that results in transplant tolerance to maternal antigens; 2) We will examine the strain differences in mouse F1 back-cross breeding models that exhibit either tolerance or rejection of heart allografts carrying the non-inherited maternal antigens; and 3) We will investigate the peculiar resistance of the long-term surviving, maternal antigen + heart allograft to chronic rejection by testing the hypothesis that maternal microchimerism induces T regulatory cells that can suppress autoimmunity to cardiac myosin in susceptible mouse strains.

1P01 AI084853-01 09/15/2010-08/31/2015
NIH/NIAID
TH17 Autoimmunity to Type V Collagen in Heart and Lung Transplant
The goal of this project is to test the hypothesis that collagen V plays a central role in chronic rejection of heart and lung transplants, by: 1) presentation of immunodominant peptides derived from the α1 chain of col(V) to Ag-specific CD4 T cells; and 2) activation in the pro-inflammatory matrix of the transplanted organ where alloreactivity and abundant IL6 allows escape from normal T-regulatory control; and differentiation of the col (V) -specific T cells into TH17 effectors.
Role: Project Leader

[redacted] 05/01/2003-04/30/2015
The objectives of this study are to identify patients who are functionally tolerant following kidney transplantation as well as relevant groups for comparison, to enter demographic and clinical data from these patients into an electronic database thereby creating a registry of tolerant kidney transplant recipients in order to facilitate

subsequent mechanistic studies and to use cutting edge research technologies to define surrogate markers of tolerance induction in humans.
Role: Principal Investigator

[redacted] 12/01/2010-11/30/2015
[redacted]
The central focus of the proposed cooperative work programme is to produce distinct populations of haematopoietic regulatory cells and comparatively test their safety and efficacy in minimizing pharmacological immunosuppression in solid organ transplants.
Role: Project Leader

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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: Joshua D. Mezrich

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

POSITION TITLE: Associate Professor of Surgery

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
Princeton University, Princeton, NJ	BA	1989-1993	Slavic Languages and Literature
Cornell University Medical College, New York, NY	MD	1993-1997	Medicine
University of Chicago Hospitals and Clinics, Chicago, IL		1997-2005	Surgery
Massachusetts General Hospital, Boston, MA		1999-2002	Transplant Immunology
University of Wisconsin Hospital and Clinics, Madison, WI		2005-2007	Transplant Surgery

A. Personal Statement

I have been running a laboratory at the University of Wisconsin since I came on faculty in 2007. Our lab focuses on the role of the Aryl Hydrocarbon Receptor (AHR) in immune response, particularly its role in the differentiation of T cells and activation of DCs. We see the AHR as a thermostat that responds to signals in the environment and communicates with the cells of the immune system. AHR ligands are made endogenously, but also are products of the microbiome, found in the diet, and found in pollution. We have multiple projects looking at dietary supplementation as a potential to treat autoimmunity, transplant rejection, and hospital-acquired infection. I have significant background working with T-cell differentiation and regulatory cell generation both *in vitro* and in a large animal model. My initial work was conducted at the Transplantation Biology Research Center in Boston, where I investigated regulatory tolerance in combined heart-kidney transplantation in miniature swine. Over the last 10 years I have worked extensively with T cells *in vitro* and *in vivo* in mouse models of autoimmunity, airway disease, and transplantation. I work extensively with Chris Bradfield PhD (an expert in the AHR) and Jamie Schauer PhD, (an expert in atmospheric exposures, and the director of air chemistry at the Wisconsin State Laboratory of Hygiene).
I have benefited greatly from wonderful mentorship since I came here, and have learned a lot about what makes a good mentor for trainees at different points in their careers, from undergraduates, to medical students, to residents and fellows, to graduate students to junior faculty. One of the responsibilities I take most seriously and get the most gratification from is my role as a mentor. This is highlighted by my involvement in leading a T32 and T35, and my role as a mentor to graduate students both in my own lab and on various committees around campus. I have mentored a number of junior faculty, and have particular insight into surgeons trying to balance a clinical practice and basic science research. It is this commitment and background that gives me confidence I can serve as a useful mentor on Dr. Al-Adra's K08 application.

B. Positions and Honors

Positions

1994 Research Assistant in the Laboratory of Dr. Jerome Posner, Memorial Sloan Kettering Cancer Center, New York, NY

1994-1997 Tissue Procurement Leader Team Leader, The New York Firefighter's Skin Bank, New York Hospital – Cornell Medical Center
1997-2005 Resident in General Surgery/Research Fellow, University of Chicago Hospitals and Clinics, Chicago, IL
1999-2002 Post-Doctoral Research Fellowship in the Laboratory of Dr. David H. Sachs, Transplantation Biology Research Center, Massachusetts General Hospital
2005 Administrative Chief Resident, University of Chicago Hospitals and Clinics, Chicago, IL
2005-2007 Clinical Instructor of Surgery/Transplant Fellow, University of Wisconsin School of Medicine and Public Health, Madison, WI
2007-2015 Assistant Professor of Surgery, Division of Transplantation, University of Wisconsin School of Medicine and Public Health, Madison, WI
2015-present Associate Professor with tenure, Division of Transplantation, University of Wisconsin School of Medicine and Public Health, Madison, WI

Honors

1989 National Merit Finalist
1989 Garden State Scholarship
2000-2002 T35 Training Grant – 2-year grant to fund research and salary; Massachusetts General Hospital
2002 American Transplant Congress Domestic Young Investigator Award; Massachusetts General Hospital
2005 Administrative Chief Resident; University of Chicago Hospitals and Clinics Department of Surgery

C. Contributions to Science

1. My early publications focused on transplant immunology, and particularly tolerance induction in a large animal model of organ transplantation. I studied a combined heart and kidney transplant model in miniature swine, trying to determine how kidney transplants were able to provide protection, and even tolerance, to combined heart transplants. I discovered that the kidney transplant led to the generation of peripheral regulatory T cells that were dependent on the presence of the thymus, and that loss of these cells would lead to rejection of the heart transplants.

a. **Mezrich, JD**, Yamada K, Lee RS, Mawulawde K, Benjamin LC, Schwarze ML, Maloney ME, Amoah HC, Houser SL, Sachs DH, Madsen JC. Induction of tolerance to heart transplants by simultaneous cotransplantation of donor kidneys may depend on a radiation-sensitive renal cell population. Transplantation 2003; 76(4):625-31. PMID:12973099
b. **Mezrich JD**, Kesselheim JA, Johnston DR, Yamada K, Sachs DH, Madsen JC. The role of regulatory cells in miniature swine rendered tolerant to cardiac allografts by donor kidney cotransplantation. Am J Transplant 2003; 3(9):1107-15. PMID:1219090
c. **Mezrich JD**, Benjamin LC, Sachs JA, Houser SL, Vagefi PA, Sachs DH, Madsen JC, Yamada K. Role of the thymus and kidney graft in the maintenance of tolerance to heart grafts in miniature swine. Transplantation 2005; 79(12):1663-73.PMID:15973167

2. The AHR, originally described as the receptor for TCDD, was identified to have an important role in the generation of regulatory T cells (Tregs) from naïve T cells. It has long been known that the AHR has important endogenous ligands, although identification of clinically relevant endogenous ligands had been difficult. I combined my previous experience with Tregs with my current understanding that tryptophan metabolites can be ligands of the AHR, to identify that IDO, the enzyme responsible for dendritic cell-generation of Tregs, generates kynurenine that binds to the AHR on T cells to generate Tregs. This finding has been cited numerous times and has led to many high profile publications and findings, including a mechanism whereby tumors prevent their own destruction by generating IDO and kynurenine.

a. **Mezrich JD**, Fechner JH, Zhang X, Johnson BP, Burlingham JW, Bradfield CA. An interaction between kynurenine and the Aryl Hydrocarbon Receptor can generate regulatory T cells. J Immunol 2010 Sep 15; 185(6):3190-8. PMCID: PMC2952546

3. Recently we have focused on the role of the AHR as a sensor to environmental signals at organs at the interface with the outside world. We tested the hypothesis that the AHR is activated by components of inhaled pollution, and in response to these, including polycyclic aromatic hydrocarbons, enhances

effector Th17 differentiation of T cells, that over time can lead to autoimmunity and chronic transplant rejection. This is an important concept that may be a central mechanism to pollution-induced autoimmunity and a new theory as a cause for chronic rejection in lung transplants or potentially other organs. We have further examined the importance of the AHR in the gut, and how it responds to ligands found in the diet or the microbiome. In addition, we did describe an example where ligands of the AHR can be used for prevention or treatment of disease.

a. O'Driscoll CA, Owens LA, Gallo ME, Hoffmann EJ, Afrazi A, Han M, Fechner JH, Schauer JJ, Bradfield CA, **Mezrich JD**. Differential Effects of Diesel Exhaust Particles on T Cell Differentiation and Autoimmune Disease. Part Fibre Toxicol. 2018 Aug 24:24;15(1):35 PMCID:PMC6109291
b. O'Driscoll CA, Owens LA, Hoffmann EJ, Gallo ME, Afrazi A, Han M, Fechner JH, Schauer JJ, Bradfield CA, **Mezrich JD**. Ambient Urban Dust Particulate Matter Reduces Pathologic T-Cells in the CNS and Severity of EAE. Environ Res 2019 Jan;168:178-192 PMCID:PMC6263800
c. O'Driscoll CA, Gallo ME, Hoffmann EJ, Fechner JH, Schauer JJ, Bradfield CA, **Mezrich JD**. Polycyclic aromatic hydrocarbons (PAHS) present in ambient urban dust drive proinflammatory T cell and dendritic cell responses via the aryl hydrocarbon receptor (AHR) in vitro. PLoS One 2018 Dec 21;13(12) PMCID:PMC6303068
d. O'Driscoll CA, Gallo ME, Fechner JH, Schauer JJ, **Mezrich JD**. Real-world PM extracts differentially enhance Th17 differentiation and activate the aryl hydrocarbon receptor (AHR).*Toxicology* 2019 Feb 15;414:14-26 PMCID:PMC7065493.

4. Over the past few years we have also been exploring the potential role for the AHR as a target for immunomodulation, and the possibility of applying this strategy to models of organ transplantation. This represents a new paradigm for transplant, where we can utilize a natural receptor that already serves as a thermostat to the immune system that maintains the balance of effector and regulatory responses to both exogenous and endogenous signals, to increase regulation in the setting of a transplanted graft.

a. Pauly SK, Fechner JH, Zhang X, Torrealba J, Bradfield CA, **Mezrich JD**. The Aryl Hydrocarbon Receptor Influences Transplant Outcomes in Response to Environmental Signals. Toxicol Environ Chem. 2012;94(6):1175-1187. PMC3445427
b. **Mezrich JD**, Nguyen LP, Kennedy G, Nukaya M, Fechner JH, Zhang X, Xing Y, Bradfield CA. SU5416, a VEGF receptor inhibitor and ligand of the AHR, represents a new alternative for Immunomodulation. Plos One. 2012;7(9). PMCID: PMC3432581
c. Julliard W, De Wolfe TJ, Fechner JH, Safdar N, Agni R, **Mezrich JD.** Amelioration of Clostridium difficile Infection in Mice by Dietary Supplementation With Indole-3-carbinol. *Ann Surg*. 2016 Jun 8. PMCID: PMC5743052
d. Julliard W, Fechner JH, Owens L, O'Driscoll CA, Zhou L, Sullivan JA, Frydrych L, Mueller A, **Mezrich JD**. Modeling the Effect of the Aryl Hydrocarbon Receptor on Transplant Immunity. Transplant Direct. 2017 Apr 25;3(5):e157. PMCID: PMC5441988

5. In addition, I continue to examine questions related to my patients, in terms of transplant outcomes, organ selection, immune response, and novel strategies for immunosuppression. This work helps focus our lab efforts on translational aspects that can ultimately improve the health of patients.

a. Scalea JR, Redfield RR, Arpali E, Leverson GE, Bennett RJ, Anderson ME, Kaufman DB, Fernandez LA, D'Alessandro AM, Foley DP, **Mezrich JD**. Does DCD Donor Time-to-Death Affect Recipient Outcomes? Implications of Time-to-Death at a High-Volume Center in the United States. Am J Transplant. 2017 Jan;17(1): 191-200. PMID: 27375072
b. Scalea JR, Redfield RR, Rizzari MD, Bennett R, Anderson ME, Anderson JE, Kaufman DB, Sollinger HW, Fernandez LA, D'Alessandro AM, **Mezrich J**. When Do DCD Donors Die?: Outcomes and Implications of DCD at a High-volume, Single-center OPO in the United States. Ann Surg. 2016 Feb;262(2): 211-6. PMID:26181480
c. **Mezrich JD**, Pirsch JD, Fernandez LA, Foley DP, Bellingham JM, Odorico JS, Leverson GE, Munoz-Del-Rio A, Sollinger HW, Kaufman DB, D'Alessandro AM. Differential outcomes of expanded-criteria donor renal allografts according to recipient age. Clin J Am Soc Nephrol. 2012 Jul; 7(7): 1163-71.PMCID: PMC3386667
d. Jankowska-Gan E, Sheka A, Sollinger HW, Pirsch JD, Hofmann RM, Haynes LD, Armbrust MJ, **Mezrich JD**, Burlingham WJ. Pretransplant Immune regulation predicts allograft outcome:

bidirectional regulation correlates with excellent renal transplant function in living-related donor-recipient pairs. Transplantation. 2012 Feb 15;93(3): 283-90. PMCID: [redacted]

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/joshua.mezrich.1/bibliography/47509071/public/?sort=date&direction=ascending

D. Research Support

Active

[redacted] 07/01/2019-06/30/2023

In this proposal we investigate the role that inhaled pollution plays in aggravation of autoimmune disease, using a mouse model for experimental autoimmune encephalomyelitis (EAE). We focus on the components of particulate matter that alter T cell and DC cell response, and examine the role of the AHR in this effect. We will further screen source samples that are specific to the military, including burn pit samples, and will examine the capacity for dietary interventions to mitigate pollution-induced aggravation of disease.

T32AI125231 Mezrich (Co-Director) 07/01/2016-06/30/2021
NIH/NIAID
UW Transplant Research Training Program
The overall goal of the "UW Transplant Research Training Program" is to provide postdoctoral trainees who are strongly motivated toward a career in transplant related research, with a two-year, comprehensive, hypothesis-based research experience.

NIH T35DK062709 Mezrich (Director) 05/01/2016-04/30/2019
NIH/NIDDK
Surgery Summer Research Experience for Medical Students
The program provides medical students with a focused, 8-12 week, mentored research and training experience that guides students towards a career pathway which integrates biomedical research, with a secondary goal of encouraging students to in the field of academic surgery.

Completed

1R01ES023842 (Mezrich, PI) 12/1/2014-11/30/2019
NIH/NIEHS
A Novel Mechanism for Environmentally Induced Airway Disease
Goal: In this project we extend our own experience with the mechanisms of Bronchiolitis Obliterans Syndrome after lung transplant to explore whether the Th17-driven lung inflammation seen in this syndrome is similar to the pathogenesis of other EIADs. By documenting which components of inhaled pollution aggravate airway pathology and through what specific mechanism in which cells, we will be able to propose avoidance strategies and novel targets for future pharmaceutical treatment of patients suffering from these illnesses.

1R21ES025304 Mezrich (PI) 04/01/2015-03/31/2017
NIH/NIEHS
Pollution Aggravates Autoimmunity Through the Aryl Hydrocarbon Receptor
In this proposal we extend our own experience with inhaled particulate matter (PM) and its effects on the immune system, particularly its ability to activate the aryl hydrocarbon receptor (AHR) and aggravate Th17 differentiation. We will apply this to a model of Experimental Autoimmune Encephalomyelitis (EAE) in mice.

[redacted] Mezrich (PI) 06/01/2009-05/31/2013

Specific Aims: 1) To establish an in vitro model of TCDD-mediated thymic involution and use this model to determine the important effector molecules that induce TCDD-mediated thymic involution; 2) Determine if the AHR plays a role in the natural course of thymic involution or its ability to repopulate after involution; and

3) Determine the degree to which modulation of the AHR can lead to thymic-dependent immunosuppression, partially attributable to generation of Tregs. This can have an effect on organ rejection and development of tolerance.

[redacted] Mezrich (PI) 07/01/2010-06/30/2013 [redacted]

The hypothesis for the project is that the AHR is integral to the crosstalk between DCs and T cells leading to T cell differentiation. The indoleamine 2,3-dioxygenase (IDO) pathway leads to the tryptophan breakdown product kynurenine, which acts directly through the AHR and leads to Regulatory T cell generation.

[redacted] Mezrich (PI) 07/01/2009-06/30/2011 [redacted]

Our experimental design is to 1) refine our current model using a variety of mouse strains and AHR ligands and to characterize the alterations in the immune system that lead to immunosuppression following TCDD-treatment of skin allograft recipient mice, 2) determine the extent to which the thymic involution, and alterations in T-cell trafficking and differentiation play in promoting allograft survival and 3) use in vitro models to determine the specific molecular changes that occur following immunosuppression mediated by activation of the AHR.

[redacted] Mezrich (PI) 07/01/2012-06/30/2014 [redacted]

Goals: This proposal is based on the hypothesis that exposure to polycyclic aromatic hydrocarbons (PAHs) present in the environment has a direct effect on T-cell differentiation in an aryl hydrocarbon (Ah) receptor-dependent manner, that aggravates reactive airway disease and asthma.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH (9-23-20)

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: Sondel, Paul M.

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

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
University of Wisconsin, Madison, WI	BS w/honors	9/1968-8/71	Genetics
University of Wisconsin, Madison, WI	PhD	9/71-8/72, 6/74-8/75	Immuno-Genetics
Harvard Medical School, Boston, MA	MD (Magna Cum Laude)	9/72-5/74, 9/75-6/77	Medicine-Immunology
Farber Cancer Ctr. (Harvard), Boston, MA	lab post-doc (50%)	9/75-6/77	Tumor Immunology

A. PERSONAL STATEMENT

Research: Since joining the lab of Fritz Bach in Dec. 1969 as a 2nd year undergrad, my training and career have been focused on using the immune system as cancer treatment. In 1980 I became faculty at UW-Madison and have led a research team that has pursued: a) basic cancer immunology; b) early clinical immunotherapy trials; and c) the translational-preclinical research needed to connect basic science to clinical testing. This work has been supported through many sources, including continuous NCI support, with me as PI/PD since 1982 (including R01, R35 and multi-investigator grants). From 1990-2016, I led our UW Division of Pediatric Hematology, Oncology and Bone Marrow Transplantation, and am now Research Director. I have served nationally on many cancer immunology advisory committees and have a steady record of service to the NCI, through study sections, advisory boards, and review panels. I have previously held other administrative leadership positions at UW (i.e., Vice Chair of Pediatrics; Asso. Director of the UWCCC; and leader of the UWCCC Immunology-Immunotherapy Program). Over the past few years we have seen real clinical benefit from the immunotherapy approaches my research team, and many others, are pursuing. I am convinced that far greater application of immunotherapy principles can be integrated into (or replace components of) the “standard” for most forms of cancer. The goal is more effective therapy with less long- and short-term toxicity. Furthermore, I believe the widespread application of effective immunotherapy to worldwide cancer treatment will be best served by strategies that utilize off-the-shelf agents. My efforts are focused on these important research and translational goals.

We have identified a novel combination therapy (“*In Situ* Vaccination”) given directly to a large existing tumor that enables it to function as a potent vaccine, providing long-lasting cure of mice from large tumors that were previously non-curable with other immunotherapy approaches. Following local radiotherapy (RT), we directly inject a tumor-reactive immunocytokine [a tumor-reactive monoclonal antibody (mAb) linked to IL2, a potent immune cell activator]. Most mice bearing a single large B78 melanoma show tumor elimination with long lasting protective immunity with this approach (Morris et al, Cancer Research, 76:3929, 2016). In mice with 2 tumors, delivering RT to both the 1st + 2nd tumors, or adding checkpoint-blockade therapy using an anti-CTLA-4 mAb, enables injection of immunocytokine to the 1st tumor to eradicate it as well as the 2nd tumor in most mice. (Morris et al, Cancer Immunology Research,6: 825-834, 2018). We’ve shown results are similar in mice bearing pancreatic cancer or neuroblastoma. Clinical translation of these preclinical concepts have recently begun, in clinical trials of these concepts via protocols that are open here at UW, under INDs that I hold; one for a trial of molecular targeted radiotherapy in combination with anti-GD2 mAb + anti-PD-1 in refractory/relapsed neuroblastoma, and the other for a trial of local RT plus intratumoral anti-GD2 immunocytokine together with ipilimumab and nivolumab for advanced melanoma. We hypothesize that *In Situ* Vaccination will also be effective in other tumors that appear resistant to checkpoint-blockade. Our ongoing preclinical work is evaluating the potency of *in situ* vaccination in distinct mouse models, characterizing the

immune suppression from multiple tumors, determining how best to achieve meaningful-lasting tumor eradication, and identifying the mechanisms underlying these important clinically translatable findings. Our goal is to transform these approaches into evidence-based clinical testing and ultimately, clinical efficacy.

In addition to my own effort on research, during the 40 years that I have been a member of the UW-Madison faculty, I have had over 70 graduate students and postdoctoral fellows train within my research laboratory. The majority are in academic positions as independent researchers. I have served on 49 separate mentoring/promotion committees for UW Assistant Professors (27 in The Dept. of Pediatrics, and 22 in other departments). I have served as research grant mentor for graduate students, residents, fellows and faculty that have received NIH training/career-development awards. I am eager to serve as a member of Dr. Al-Adra's mentoring committee and as a research/career mentor for him.

B. POSITIONS

7/77-6/78 Intern, Dept. of Pediatrics, Univ. of Minnesota Hospital, Minneapolis
7/78-6/80 Resident, Dept. of Pediatrics, Univ. of Wisconsin Hospitals, Madison
1980-87 Asst. and Assoc. Professor, Depts. of Pediatrics, Human Oncology and Genetics, UW-Madison
1987- Professor, Depts. of Pediatrics, Human Oncology, and Genetics, UW-Madison
1990-2016 Head, Div. Pediatric Hematology/Oncology, Dept. of Pediatrics, UW-Madison
1990-08 Leader, UWCCC, Program/Working Group in Immunology and Immunotherapy
2002- Reed and Carolee Walker Professor of Childhood Cancer Research, UW-Madison
2006-09 Associate Director Translational Research, UW Carbone Cancer Center (UWCCC)
2006-08 Vice Chair for Research, UW Department of Pediatrics
2009- Co-leader, UWCCC working group on Immunotherapy
2016- Director of Research, Div. Pediatric Hematology/Oncology, Dept. of Pediatrics, UW-Madison

Honors and National Committees (Selected)

1981-84 G.A. and J.L. Hartford Foundation Fellow
1981-86 Scholar of the Leukemia Society of America
1987-91 NIH Experimental Immunology Study Section
1988-02 Children's Cancer Study Group - New Agents Vice Chairman - Director of Immunotherapy
1988- American Society of Clinical Investigation
1991- Editorial Boards (selected-past and present), [J.N.C.I., Blood, J. Immunotherapy, Clinical Cancer Research (95-99 Sr Ed), Blood and Marrow Transpl. Cancer Imm. Immunotherapy (and others)]
1991-95 American Cancer Soc. - Immunology Review Committee (Vice Chair - 1993-1994, Chair 1994-95)
1993-96 NCI Division of Cancer Treatment - Member, Board of Scientific Counselors
1996- University of Wisconsin Rusch Professorship (Converted to Walker Professorship in 2002)
1997-01 NCI Cancer Centers Review Committee
2002-04 NCI Biologic Resource Branch Oversight Committee
2004-09 NCI Board of Scientific Counselors
2007 UW Hilldale Award in Biology and Rusch Award in Translational Research
2008-11 Chairman, St. Jude Children's Research Hospital (Memphis TN), Scientific Advisory Board
2013- NCI- New Experimental Therapeutics, Special Emphasis Panel
2014 UW Dean's Medical Research Mentorship Award
2015-22 NCI-R35 Outstanding Investigator Award Recipient
2017 Smalley Annual Lectureship Award; Society for Immunotherapy of Cancer

C. CONTRIBUTIONS TO SCIENCE

1. ***The biology of graft-versus-leukemia reactions, demonstrating the importance of alloantigens;*** Possibly the first effective application of antitumor immunotherapy was the graft vs. leukemia (GVL) effect. The overriding hypothesis in the 70's involved immunity to leukemia specific antigens. Our lab demonstrated that minor histocompatibility antigens (**a**), likely distributed on leukemia and hematopoietic cells (**b**), were targets for this GVL effect. We also pursued how leukemia can escape from GVL (**c**), and worked collaboratively to demonstrate the potency of GVL (**d**).
   a. **Sondel PM**, Hank JA, Wendel T, Flynn B, Bozdech MJ. HLA identical leukemia cells and T cell growth factor activate cytotoxic T cell recognition of minor locus histocompatibility antigens in vitro. J Clin Invest 71:1779-86, 1983. [PMID: 6223050, PMCID: PMC370383].
   b. Sosman JA, Oettel K, Smith SD, Hank JA, Fisch P and **Sondel PM.** Specific recognition of human leukemic cells by allogeneic T cells: II. Evidence for HLA-D restricted determinants on leukemic cells that

are crossreactive with determinants present on unrelated nonleukemic cells. Blood 75:2005-2016, 1990. [PMID: 1692492].

c. **Sondel PM**, Hank JA, Molenda J, Blank J, Borcherding W, Longo W, Trigg ME, Hong R and Bozdech MJ. Relapse of host leukemic lymphoblasts following engraftment by an HLA-mismatched marrow transplant: Mechanisms of escape from the "graft versus leukemia" effect. Experimental Hematology 13:782-790. 1985. [PMID: 2931298].

d. Horowitz MM, Gale RP, **Sondel PM**, Goldman JM, Kersey J, Kolb H-J, Rimm AA, Ringden O, Rozman C, Speck B, Truitt RL, Zwaan FE and Bortin MM. Graft-versus-leukemia reactions after bone marrow transplantation. BLOOD 75:555-562, 1990. [PMID: 2297567].

2. ***Activation of anti-tumor immune destruction with Interleukin-2 (IL2).*** Our team was the first US center to be testing clinical trials of IL2, outside of the NCI. We demonstrated that lymphokine activated killer cells (LAK) could be generated in vivo (without requiring ex-vivo activation and expansion) (**a**), and that a moderate dose regimen, not inducing ICU-level toxicity, could generate meaningful antitumor effects (**b**). We demonstrated which subpopulations of NK cells responded to IL2 in patients (**c**), and clarified the roles of IL2R β and γ_c chains in regulating the response (**d**).

a. Hank JA, Kohler PC, Weil-Hillman G, Rosenthal N, Moore KH, Storer B, Minkoff D, Bradshaw J, Bechhofer R and **Sondel PM**. In vivo induction of the lymphokine-activated killer phenomenon: Interleukin 2-dependent human non-major histocompatibility complex-restricted cytotoxicity generated in vivo during administration of human recombinant Interleukin 2. Cancer Research 48:1965-1971, 1988. [PMID: 3258180].

b. Sosman JA, Kohler PC, Hank JA, Moore KH, Bechhofer R, Storer B, **Sondel PM**. Repetitive weekly cycles of recombinant human IL-2: Responses of renal carcinoma with acceptable toxicity. J Nat Cancer Inst 80:60-3, 1988. [PMID: 3257526].

c. Weil-Hillman G, Voss SD, Fisch P, Schell K, Hank JA, Sosman JA, Sugamura K and **Sondel PM.** Natural killer cells activated by Interleukin-2 treatment in vivo respond to Interleukin-2 primarily through the p75 receptor and maintain the p55 (TAC) negative phenotype. Cancer Res 50:2683-2691, 1990. [PMID: 1691679].

d. Voss SD, Robb RJ, Weil-Hillman G, Hank JA, Sugamura K, Tsudo M and **Sondel PM.** Increased expression of the Interleukin 2 (IL-2) receptor beta chain (p70) on $CD56^+$ natural killer cells after in vivo IL-2 therapy: p70 expression does not alone predict the level of intermediate affinity IL-2 binding. J Exp Med 172:1101-1114, 1990. [PMID: 1698909].

3. ***Combining IL2 with tumor reactive monoclonal antibodies (mAbs) to facilitate tumor killing by leukocytes [Antibody Dependent Cell-mediated Cytotoxicity (ADCC)].*** We found that NK cells from patients showed potent augmentation of their ADCC capability after in vivo IL2 administration (**a**). We thus added tumor-reactive mAb to this IL2 regimen, and demonstrated that we created in vivo conditions that led to ADCC, as measured ex vivo with patient samples (**b**). We led Phase I/II trials of this combination in adults with melanoma and children with neuroblastoma (**c**). This culminated in a large Phase III study that demonstrated the clinical efficacy of this approach (**d**), which was FDA approved in 2015.

a. Hank JA, Robinson RR, Surfus J, Mueller BM, Reisfeld RA, Cheung N-K, **Sondel PM**. Augmentation of ADCC following in vivo therapy with recombinant IL-2. Cancer Res 50:5234-9, 1990. [PMID: 2386933].

b. Hank J, Surfus J, Gan J, Chew T-L, Hong R, Tans K, Reisfeld R, Seeger R, Reynolds CP, Bauer M, Wiersma S, Hammond D and **Sondel PM.** Treatment of neuroblastoma patients with antiganglioside GD_2 antibody plus Interleukin-2 induces antibody dependent cellular cytotoxicity against neuroblastoma detected in vitro. J Immunother 15:29-37, 1994. [PMID: 8110728].

c. Gilman AL, Ozkaynak F, Matthay K, Krailo M, Yu A, Gan J, Sternberg A, Hank J, Seeger R, Reaman G, **Sondel P.** Phase I Study of ch14.18 with Granulocyte-Macrophage Colony-Stimulating Factor and Interleukin-2 in Children with Neuroblastoma After Autologous Bone Marrow Transplantion or Stem Cell Rescue: A Report from the Children's Oncology Group. J. Clin. Oncol. 27:85-91, 2009. [PMID: 19047298, PMCID: PMC2645092].

d. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman S, Chen H, Smith M, Anderson B, Villablanca J, Matthay KK, Shimada H, Grupp SA, Seeger R, Reynolds CP, Buxton A, Reisfeld RA, Gillies SD, Cohn SL, Maris JM, **Sondel PM.** Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. New England J. Med. 363:1324-1334, 2010. [PMID: 20879881, PMCID: PMC3086629].

4. ***The mechanisms of augmented tumor destruction using tumor-reactive antibodies directly linked to IL2.*** In collaboration with Steve Gillies and Ralph Reisfeld, we have pursued the lab and clinical anti-tumor

activity of immunocytokines, in which IL2 is linked to tumor reactive mAbs. These immunocytokines show superior antitumor effects over the combination of mAb and IL2 as separate molecules. In mice this is very potent, particularly against "non-bulky" disease. Our Phase II study of this showed potent antitumor effects in patients with non-bulky disease (**a**). In our mouse models, we are able to have a potent impact on bulky disease by giving the immunocytokine as an intra-tumor injection following a low (immunomodulating) dose of local radiotherapy; enabling the immunotherapy to convert the existing tumor into an "*in situ vaccine*" (**b,c**). This approach has been extended to effective eradication of very "cold" tumors, with low tumor mutation burden (such as MYC-driven neuroblastoma) when additional danger signaling (via CpG) and dendritic cell activation (via agonist anti-CD40 mAb) are added (**d**).

a. Shusterman S, London WB, Gillies SD, et al. Hank JA, Voss S, Seeger RC, Reynolds CP, Kimball J, Albertini MA, Wagner B, Gan J, Eickhoff J, DeSantes KD, Cohn SL, Hecht T, Gadbaw B, Reisfeld RA, Maris JM, **Sondel PM**. Anti-tumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J. Clin. Oncology, 28(33):4969-4975; 2010. [PMID: 20921469, PMCID: PMC3020698].
b. Morris ZS, Guy EI, Werner LR, Carlson PM, Heinze CM, Kler JS, Busche SM, Jaquash AA, Sriramaneni RN, Carmichael LL, Loibner H, Gillies SD, Korman AJ, Erbe AK, Hank JA, Rakhmilevich AL, Harari PM, **Sondel PM**. Tumor-specific inhibition of in situ vaccination by distant untreated tumor sites. Cancer Immunology Research, 6:825-834, 2018.
c. Morris ZS, Guy EI, Werner LR, Carlson PM, Heinze CM, Kler JS, Busche SM, Jaquash AA, Sriramaneni RN, Carmichael LL, Loibner H, Gillies SD, Korman AJ, Erbe AK, Hank JA, Rakhmilevich AL, Harari PM, **Sondel PM**. Tumor-specific inhibition of in situ vaccination by distant untreated tumor sites. Cancer Immunology Research, 6:825-834, 2018.
d. Voeller J, Erbe A, Slowinski J, Rasmussen K, Carlson PM, Hoefges A, VandenHeuvel S Stuckwisch A, Wang X, Gillies SD, Patel RB, Farrell A, Rokita JL, Maris JM, Hank JA, Morris ZS, Rakhmilevich AL, **Sondel PM**. Combined Innate and Adaptive Immunotherapy Overcomes Resistance of Immunologically Cold Syngeneic Murine Neuroblastoma to Checkpoint Inhibition. Journal for Immunotherapy of Cancer, Published ahead of print on-line Dec. 2019.

5. ***The role of KIR and KIR-Ligand in controlling NK-mediated ADCC responses in the clinical setting.*** Our murine model demonstrated tumors escape from immunocytokine by upregulating MHC class-I, suggesting that Ly49 (KIR-like) receptors regulate the antitumor ADCC response (**a**). Our clinical immunocytokine data were consistent with this in relapsed neuroblastoma, where KIR genotype was associated with response (**b**). We alaso demonstrated that KIR geneotype appeared to prognosticate which newly diagnosed neuroblastoma patients would respond to dinutuximab (anti-GD2 mAb) + IL2 + GM-CSF (**c**), and have most recently demonstrated KIR genotype is associated with benefit from rituximab immunotherapy for follicular lymphoma (**d**).

a. Neal ZC, Imboden M, Rakhmilevich AL, Kim KM, Hank JA, Surfus J, Dixon JR, Lode HN, Reisfeld, RA, Gillies SD and **Sondel PM**. NXS2 murine neuroblastomas express increased levels of MHC class I antigens upon recurrence following NK-dependent immunotherapy. Cancer Immunology and Immunotherapy, 53:41-52, 2004. [PMID: 14504825].
b. Delgado DC, Hank JA, Kolesar J, Lorentzen D, Gan J, Seo S, Kim KM, Shusterman S, Gillies SD, Reisfeld RA, Yang R, Gadbaw B, DeSantes KD, London WB, Seeger RC, Maris J, and **Sondel PM**. Genotypes of NK Cell KIR Receptors, Their Ligands, and Fcγ Receptors in the Response of Neuroblastoma Patients to Hu14.18-IL2 Immunotherapy. Cancer Research, 70:9554-9661, 2010. [PMID: 20935224, PMCID: PMC299644].
c. Erbe AK, Wang W, Carmichael L, Kim KM, Mendonça EA, Song Y, Hess D, Reville PK, London WB, Naranjo A, Hank JA, Diccianni MB, Reisfeld RA, Gillies SD, Matthay KK, Cohn SL, Hogarty MD, Maris JM, Park JR, Ozkaynak MF, Gilman AL, Yu AL, **Sondel PM**. Neuroblastoma Patients' KIR and KIR-ligand Genotypes Influence Clinical Outcome for Dinutuximab-based Immunotherapy: A Report from the Children's Oncology Group. Clinical Cancer Research, 24: 189-196, 2017. [PMID:28972044]
d. Erbe AK, Wang W, Carmichael L, Hoefges A, Grzywacz B, Reville P, Ranheim EA, Hank JA, Kim KM, Seo S, Mendonca EA, Song Y, Kenkre VP, Hong F, Gascoyne RD, Paietta E, Horning SJ, Miller JS, Kahl BS, **Sondel PM**. Follicular lymphoma patients with KIR2DL2 and KIR3DL1 and their ligands (HLA-C1 and HLA-Bw4) show improved outcome when receiving rituximab. Journal for ImmunoTherapy of Cancer, 7(1):70, 2019. [PMID: 30871628; PMCID: PMC6419437].

Listing of 329 manuscripts in PubMed: http://www.ncbi.nlm.nih.gov/pubmed/?term=Sondel+P
Listing of 103 manuscripts in Pub Med Central: http://www.ncbi.nlm.nih.gov/pmc/?term=sondel+p
Listing of 410 manuscripts in myNCBI: http://bit.ly/2f7bXmY

D. RESEARCH SUPPORT (current, partial list);

R35 CA197078 (Sondel, PI) 08/01/15-07/31/22
NIH/NCI, Outstanding Investigator Award
Enhancing Antibody-directed Innate Immunity to Improve Cancer Outcome
This multi-focused program includes basic, preclinical and clinical testing, in order to better enable tumor-reactive mAbs and their genetic derivatives to facilitate recognition, killing and immune memory against clinical cancer.
Role: PI

NCI-Clinical Immunotherapy Trials Network (Cheever, PI) 04/01/11-04/31/21
National Consortium of 27 centers doing collaborative clinical trials of early immunotherapy.
Role: Local UW Co-PI (with D. McNeel MD PhD)
NCI-Pediatric Clinical Immunotherapy Trials Network (Cheever; Mackall, MPIs) 04/01/11-04/31/21
National Consortium of 10 pediatric oncology centers doing collaborative clinical trials of early immunotherapy.
Role: Local UW PI (with K. DeSantes)

[redacted] 12/01/17-06/30/21
Immunogenomics to Create Therapeutics for High-Risk Childhood Cancers (Pediatric Cancer Dream Team)
This consortium links genomics and immunotherapy efforts underway at 7 Pediatric Oncology Centers in order to move new therapies into clinical testing.
Role: Local PI and Executive Committee Member

U54 CA232568 (Maris, Mackall PD/MPI) 07/01/18-06/30/23
NIH/NCI-Discovery and Development of Optimal Immunotherapeutic Strategies for Childhood Cancers
Project 3: Discovery & development of pediatric cancer antigenic targets recognized by adaptive immune responses
Role: Sondel- MPI for Project 3 (of 3 projects) (Multiple PI role, shared with John Maris)

[redacted] 07/01/18-06/30/22
Ex vivo activated NK cells and immunocytokine for pediatric cancers
This project will provide preclinical evidence for a platform for incorporating recently developed immune-therapies (immunocytokine and natural killer cells) for neuroblastoma and osteosarcoma. Role: Collaborator

U01 CA232494 (Morris, Weichert PD/MPI) 09/18/18-08/31/23
NIH/NCI
Immunomodulation of the Tumor Microenvironment with Molecular Targeted Radiotherapy to Facilitate an Adaptive Anti-Tumor Immune Response to Combined Modality Immunotherapies
The goal of this proposal is testing and developing synergy between checkpoint blockade, intratumoral mAb + IL2 and molecular targeted radiation therapy in cold murine cancers, and begin translation in a canine model.
Role: Sondel (Co-Investigator)

[redacted] 02/01/19-12/31/21
UW Innovations in Malignancy Personalized Advanced Cell Therapies (UW-IMPACT)
Role: Co-Investigator

OTHER SUPPORT

AL-ADRA, DAVID PETER

ACTIVE

[redacted] 07/01/20-06/31/22 0.72 Cal Mo
$[redacted] TC
Immunomodulation of the Liver using Normothermic ex-vivo Machine Perfusion

This project aims to understand the immunological effects of NEVLP with and without cytokine therapy on the liver and then use NEVLP to modify the immune microenvironment.

PENDING

1K08AI155816-01 (Al-Adra) 09/01/20-08/31/25 9.00 Cal Mo
NIH $[redacted] TC
Targeting Donor Regulatory Dendritic Cells During Normothermic Ex-Vivo Liver Perfusion to Overcome Rejection after Liver Transplant

This project aims to understand the mechanisms of the Dentritic Cells within the liver and enhance their function after transplant

[redacted] 07/01/21-06/30/23 9.00 Cal Mo
$[redacted] TC
Targeting Donor Regulatory Dendritic Cells During Normothermic Ex Vivo Liver Perfusion to Overcome Rejection after Liver Transplant

This project aims to understand the mechanisms of the Dentritic Cells within the liver and enhance their function after transplant

OVERLAP

Note: We understand that the PI would not be eligible to receive both the NIH K08 and ICTR KL2. In the event of being awarded both grants, we will relinquish the KL2 award.

OTHER SUPPORT

Capitini, Christian

ACTIVE

R01 CA215461 (Capitini, PI) 07/01/18–05/31/23 3.0 calendar
NIH/NCI $[redacted] – total project award
Combining hu14.18-IL2 and NK cell infusions to treat neuroblastoma
The goal of this project is to use murine alloHSCT models to develop evidence for a clinically applicable combined strategy that utilizes the immunocytokine hu14.18-IL2 to enhance the GVT effect of immunologically activated, ex-vivo activated NK cells, and track the localization of these NK cells using a novel ^{19}F-MRI platform.

P30 CA014520-45S6 (Bailey, PI) 04/01/19–03/31/21* 1.2 calendar
NIH/NCI $[redacted] total project costs *in NCE
Administrative Supplements for P30 Cancer Centers Support Grants (CCSG): Image-guided CAR T cell therapy for neuroblastoma
The goals of this project are to test the following aims: (1) Optimize multiscale imaging (OMI and ^{13}C-MRI) to correlate GD2 CAR-T cell metabolic function with memory formation and persistence in vivo; and (2) Utilize ^{19}F imaging as a means of tracking the location and number of infused GD2 CAR T cells in vivo.
Role: Project Leader

[redacted] 12/01/17–11/30/21 1.2 calendar
$[redacted] – total project award to UW
Immunogenomics to Create New Therapies for High-Risk Childhood Cancers
This collaborative multi-institutional consortium links genomics and immunotherapy efforts underway at 9 Pediatric Oncology Centers in order to perform preclinical and clinical collaborative work designed to move new therapies into clinical testing.
Role: Subcontract Young Investigator

[redacted] 07/01/18–06/30/22 1.2 calendar
$[redacted] – total project award
Ex vivo activated NK cells and immunocytokine for pediatric cancers
The goal of this proposal is to provide preclinical evidence for a potential platform for incorporating recently developed immunotherapies, namely immunocytokine and natural killer cells, for neuroblastoma and osteosarcoma.

[redacted] 05/01/20 – 12/31/20 0 calendar
$[redacted] total project costs
Vaccine and checkpoint blockade after allogeneic BMT for neuroblastoma
This proposal will support an undergraduate, Nicholas Mohrdieck, for a Summer/Fall Fellowship in pediatric oncology research. NK cell activation via co-culture with a vaccine engineered to express CD54, CD80, CD86, and CD137L, called AgN2a 4P, will be studied to investigate NK cells' ability to induce cytotoxicity of murine neuroblastoma tumor cells in vitro and in vivo.
Role: Mentor

UL1 TR002373 (Brasier, PI) *11/01/20 – 10/31/21 0 calendar
NIH-NCATS/UW-ICTR $[redacted] (total project costs)
Translational Pilot Grant
Uncovering Predictive Cellular Biomarkers of GVHD Following HSCT
This proposal is a pilot award from the University of Wisconsin Institute of Clinical and Translational Research (ICTR). The primary aim of this pilot application is to test this hypothesis by investigating whether circulating T cells show similar indicators of activation in human HSCT patients who go on to develop aGVHD.
Role: Pilot Grant Co-PI (w/J. Gumperz), mentor to Dr. Nicholas Hess

[redacted] 07/01/17–06/30/21 0.6 calendar
$[redacted] – total project award
Activation of NK cells against pediatric cancers using IL-15 with TGF-beta trap

The goal of this research is to use alloBMT of pediatric sarcomas, such as rhabdomyosarcoma and osteosarcoma, with post-transplant administration of FIST15 to activate donor-derived NK cells and reduce serum TGFβ.

[redacted] 07/01/17–06/30/21 0.6 calendar
$[redacted] – total project award
Treating post-transplant B cell acute lymphoblastic leukemia with blinatumomab as a radiation sparing immunotherapy
The goal of the project is to determine if gamma/delta T cells are able to generate a graft-versus-leukemia effect in the presence of blinatumomab and eliminate the need for TBI as a form of alloHSCT conditioning.

[redacted] 12/01/14–xx/xx/xx 0.12 calendar
~$[redacted]/patient
Long Term Follow-Up of Patients Exposed to Lentiviral-Based CD19 directed CART Cell Therapy
The purpose of this study is to monitor all patients exposed to CD19 directed CAR T-cells (CD19 CART) for 15 years following last CD19 CART (e.g., CTL019) infusion to assess the risk of delayed adverse events (AEs), monitor for replication competent lentivirus (RCL) and assess long-term efficacy, including vector persistence.

[redacted] 02/22/15–xx/xx/xx 0.12 calendar
~$[redacted]/patient
Phase II Study of Redirected Autologous T cells Engineered to Contain anti-CD19 Attached to TCR and 4-1BB Signaling Domains in Patients with Chemotherapy Resistant or Refractory Acute Lymphoblastic Leukemia
To evaluate the efficacy of CTL019 therapy as measured by overall remission rate (ORR), which includes Complete Remission (CR) and CR with incomplete blood count recovery (CRi) as determined by independent review committee (IRC) assessment.

[redacted] 04/10/19 – xx/xx/xx
~$[redacted]/patient
A Phase II, single arm, multicenter open label trial to determine the safety and efficacy of tisagenlecleucel in pediatric patients with relapsed or refractory mature B-cell non-Hodgkin lymphoma (NHL) (BIANCA)
The purpose of the study is to assess the efficacy and safety of tisagenlecleucel in children and adolescents with r/r B-cell NHL.

[redacted] 08/05/19 – xx/xx/xx
No expenses posted yet
A Phase II Trial of Tisagenlecleucel in First-Line High-Risk (HR) Pediatric and Young Adult Patients with B-cell Acute Lymphoblastic Leukemia (B-ALL) who are Minimal Residual Disease (MRD) Positive at the End of Consolidation (EOC) Therapy. AALL1721
To evaluate the efficacy of tisagenlecleucel therapy as measured by the 5-year disease-free survival (DFS) by investigator assessment.

PENDING

MSN243683, R01 (Evans, PI) 03/31/21-02/28/26 0.6 calendar
NIH $[redacted] total project costs
Harnessing adaptive NK cell transfer to deplete viral reservoirs
The proposal leverages the combined expertise of our team to investigate: 1) the contribution of viral peptides to NK cell recognition of virus-infected cells through activating receptors; 2) the extent to which adoptive transfer of *ex vivo* expanded NK cells in combination with latency reversal can deplete viral reservoirs; and 3) the potential for NK cell transfer together with an Env-specific antibody to enhance the depletion of viral reservoirs.
Role: Co-Investigator

MSN245981, R01 (Saha, Capitini, Karumbaiah, MPIs) 07/01/21-06/30/26 1.2 calendar
NIH $[redacted] total project costs
A Synthetic Biology Framework to Engineer Potent CAR T Immunotherapies

This proposal will identify how unique cell surface properties of tumors influence T cells, uncover novel gene regulatory networks associated with T cell differentiation and exhaustion, generate lead microenvironment programmed CAR T products, and test their potency in vivo.
Role: Multiple PI

OVERLAP

There is no budgetary, commitment, or scientific overlap with the work proposed.

OTHER SUPPORT

BURLINGHAM, WILLIAM J., PHD

ACTIVE

1R01AI119140-01A1 (Sullivan, PI) 03/01/16 - 02/28/21 2.40 calendar
NIH/NIAID $[redacted]
Natural vs. Pathogenic Th17 responses to col Va1, Ka1tubulin and vimentin
In this project, we propose to test the hypothesis that Col V, kα1tubulin, and vimentin-reactive T cells are natural type 17 TcRαβ memory T cells.

2U01AI102456-06 (Kaufman, PI) 08/01/17 - 07/31/22 0.90 calendar
NIH/NIAID $[redacted]
Tomotherapy and Hematopoietic Stem Cells for Tolerance to Kidney Transplants
The goal of this project is to develop a tolerance induction protocol for major histocompatibility complex (MHC) disparate kidney transplants in rhesus macaques.
Role: Co-Investigator

2R01DC012773-06 (Thibeault, PI) 05/15/18 - 04/30/23 0.12 calendar
NIH/NIDCD $[redacted]
Mechanisms of innate immune-microbial interactions in vocal fold inflammation
The goal of this project is to further our knowledge of host-mediated immune defenses and inflammatory- based tissue alterations in the vocal fold mucosa when the mucosa are exposed to defined bacteria species.
Role: Key Person

1U01HL134764 (Zhang J, PI / Kamp, Sub PI) 09/15/16-05/31/23 0.60 calendar NIH/NHLBI $[redacted]
Integrated Cellular and Tissue Engineering for Ischemic Heart Disease
The goal of this consortium is to develop clinical size human myocardial tissue equivalents (“patches”) fabricated from pluripotent stem cells with engineered immunoprivilege. Also, to overcome critical barriers to generating large, fully functional human cardiac tissues that can be integrated safely into the native myocardium to provide a powerful new approach for treatment of advanced ischemic heart disease.
Role: Co-Investigator

R21AI147157-01 (Sullivan, PI) 09/01/19-08/31/21 1.20 calendar
NIH $[redacted]
Exosome-mediated Tolerance in Combined Kidney and Stem Cell Transplantation
Our goal: to discover the role of Treg- and DC-derived exosomes in a clinical tolerance trial.

5R01DC010777-08 (Welham, PI) 08/01/16-07/31/21 0.24 calendar
NIH/NIDCD $[redacted]
Regeneration of Multi-Layered Vocal Fold Mucosa
The goal of the proposed research is to further develop and refine a tissue engineering approach to create replacement vocal fold tissues for patients with severely damaged vocal folds. Such patients have significant voice difficulties which often contribute to reduced quality of life.
Role: Co-I

1 R03 AI146688-01A1 (Sullivan, PI) 01/01/20-12/30/22 0.24 calendar
NIH $[redacted]
Detection of IL35+ Exosomes as a Marker for Peripheral Tolerance
The successful completion of the outlined specific aims in this proposal will fill a significant gap in our understanding of the cytokine IL35, and will ideally lead to future applications that further our understanding of the therapeutic implications of IL35 for humans.
Role: Co-Investigator

PENDING

None

OVERLAP

None

OTHER SUPPORT

MEZRICH, JOSHUA
UW Madison Appointment 0.5 FTE

ACTIVE

1R01ES023842 (Mezrich, PI) 12/01/14-11/30/19 3.96 cal mo
NIH/NIEHS $[redacted]
A Novel Mechanism for Environmentally Induced Airway Disease

The goal of this project is to extend our own experience with the mechanisms of Bronchiolitis Obliterans Syndrome after lung transplant to explore whether the Th17-driven lung inflammation seen in this syndrome is similar to the pathogenesis of other EIADs.

T32AI125231 (Kaufman, PI) 07/01/16-06/30/21 1.20 cal mo
NIH/NIAID $[redacted]
UW Transplant Research Training Program

The goal of the “UW Transplant Research Training Program” is to provide postdoctoral trainees who are strongly motivated toward a career in transplant related research, with a two-year, comprehensive, hypothesis-based research experience.
Role: Associate Program Director

NIH T35DK062709-14 (Mezrich, PI) 05/01/19-04/30/24 0.60 cal mo
NIH/NIDDK $[redacted]
Surgery Summer Research Experience for Medical Students

The program provides medical students with a focused, 8-12 week, mentored research and training experience that guides students towards a career pathway which integrates biomedical research, with a secondary goal of encouraging students to in the field of academic surgery.

PENDING

[redacted] 07/01/20-06/30/21 1.20 Cal Mo
$[redacted]
The Role of Pollution and Sex Differences in Severity of COVID-19 Infection

In this proposal we will utilize a well-defined hamster model of COVID-19 infection using the same virus infecting humans, looking at the role of sex and exposure to inhaled pollution prior to infection.

OVERLAP

None

MEZRICH, JOSHUA
[redacted]

ACTIVE

[redacted] 07/01/19-06/30/23 2.40 cal mo
$[redacted]
Differential Effects of Particulate Matter on Autoimmunity

The goal of this project is to analyze which parts of pollution alter the immune system to make autoimmunity worse, and to explore if military-specific pollutions do this.

PENDING

[redacted] 10/01/20-09/30/22 2.40 Cal Mo
$[redacted]
COVID19: Understanding Infectivity Based on Risks, Comorbidities, and Pollution Exposure

In this proposal we will first obtain organs and tissues from deceased organ donors from high- and low-risk groups. We will examine cells from every organ in culture, and then inoculate them with SARS-CoV-2 virus. We will then expose these cells to pollution, including diesel, cigarette smoke, and burn pit extract. Then we will study a well-defined hamster model of COVID-19 infection using the virus, looking at the role of sex and exposure to inhaled pollution prior to infection.

OVERLAP

There is no scientific overlap or budgetary overlap between the VA and UW appointments. There is no scientific or budgetary overlap between active and pending VA grants.

ADDITIONAL NOTES:

Dr. Mezrich has a dual appointment with both the University of Wisconsin (UW) and the William S. Middleton [redacted] in Madison, WI (VA). As part of an agreement, Dr. Mezrich has been granted a 5/8ths position at the [redacted]. The effort for [redacted] grant comes from the new appointment. This arrangement has bee defined in a formal [redacted]. The number of person months in this application represents UW effort on the proposed project in relation to professional effort for the UW appointment only.

OTHER SUPPORT

SONDEL, PAUL M.

ACTIVE

R35 CA197078 (Sondel) 08/01/15 – 07/31/22 6.0 calendar
NIH/NCI $[redacted] (total award)
Enhancing Antibody-directed Innate Immunity to Improve Cancer Outcome
This multi-focused program includes basic, preclinical and clinical testing, in order to better enable tumor-reactive mAbs to facilitate recognition, killing and immune memory against clinical cancer.
Role: PI

[redacted] 12/01/17 – 11/30/21 1.2 calendar
$[redacted] (total subaward costs)
Immunogenomics to Create New Therapeutics for High Risk Childhood Cancers
This collaborative multi-institutional consortium proposes to link genomics and immunotherapy efforts underway at 7 Pediatric Oncology Centers [Children's Hospital of Philadelphia, NCI, Baylor, University of Washington, University of British Columbia, University of Toronto, and University of WI] in order to perform preclinical and clinical collaborative work designed to move new therapies into clinical testing, renewed by St. Baldrick's for 4 more years.
Role: Local PI and Executive Committee Member

UL1 TR002373 (Brasier) 09/21/07 – 06/30/22 **1.08 calendar
NIH/NCRR $[redacted] direct to UW (none to our lab)
Institutional Clinical and Translational Science Award
The goal of the UW Institute for Clinical and Translational Research is to create an environment that facilitates the transformation of research at the University into a continuum extending from investigation through discovery to translation into practice, linking basic research to practical improvements in human health.
Role: Chair, Scientific Review Committee

[redacted] 07/01/17 – ongoing 0.12 calendar
$[redacted]
Determining the Influence of KIR/KIR-ligand Genotypes in the Outcome of High-Risk Neuroblastoma Patients Following Anti-GD2 Based Immunotherapy. This project is analyzing genotyping data from our lab for associations with outcome in COG clinical trials.
Role: PI

R01 CA205101 (Skala) 08/01/16 – 07/31/21 *0.24 calendar
NIH & Morgridge Institute for Research $[redacted] (total costs for UW subaward)
Quantitative in vivo optical imaging of tumor heterogeneity
The goal of this proposal is to develop and validate tools to quantify the metabolic heterogeneity of tumors in living animals over a time-course of treatment.
Role: Co-Investigator

U01 CA233102 (Morris, Weichert, PD/MPI) 09/01/18 – 08/31/23 *0.36 calendar
NIH/NCI $[redacted] (total project costs)
Immunomodulation of the Tumor Microenvironment with Molecular Targeted Radiotherapy to Facilitate an Adaptive Anti-Tumor Immune Response to Combined Modality Immunotherapies
The goal of this proposal is testing and developing synergy between molecular targeted radiation therapy and various combined immunotherapies in 3 separate syngeneic models of adult cancers.
Role: Co-investigator

U54 CA232568 (Maris, Mackall PD/MPI) 07/01/18 – 06/30/23 1.2 calendar
NIH/NCI $[redacted] ($[redacted] for UW subaward)
Discovery and Development of Optimal Immunotherapeutic Strategies for Childhood Cancers

Project 3: Discovery and development of pediatric cancer antigenic targets recognized by adaptive immune responses
The goal of this proposal is testing and developing synergy for combined immunotherapy approaches to induce effective tumor eradication via induction of “in situ vaccines” and characterize the antigenic targets that are involved. The focus is on immunologically cold tumors, with initial work on TH-MYCN driven neuroblastoma, and then validation work in other analogous murine models of cold pediatric solid-tumor malignancies.
Role: MPI for Project 3 (of 3 projects) (Multiple PI role, shared with John Maris)

[redacted] 02/01/19-12/31/21 *0.36 calendar
$[redacted] (total project costs)
UW Innovations in Malignancy Personalized Advanced Cell Therapies (UW-IMPACT)
The goal of this grant is to investigate the function of GIFT4 (a fusion cytokine consisting of GM-CSF + IL4) treated B cells as a means to enhance separate strategies for inducing augmented adaptive immunity in tumor-bearing mice.
Role: Co-Investigator (leader for project #2)

[redacted] 07/01/18-06/30/22 *0.24 calendar
$[redacted] (total project costs)
Ex vivo activated NK cells and immunocytokine for pediatric cancers
This project will provide preclinical evidence for a platform for incorporating recently developed immune-therapies (immunocytokine and natural killer cells) for neuroblastoma and osteosarcoma. Role: Collaborator

[redacted] 01/21/19 – 01/21/21 0.12 calendar
$[redacted] (total project costs)
SNIPER Multispecific Antibodies
The goal of this project is to develop novel bispecific antibodies that can provide potential therapeutic advantage by requiring co-recognition of 2 distinct cell surface antigens simultaneously. These can potentially be used to more selectively target and inactivate certain immune cells for immunomodulatory purposes, or for delivering immune mediated or other destructive “payloads” to tumor cells with far less delivery to normal tissues.

PENDING

P01 CA250972 (Weichert J and Morris Z,. MPIs) 07/01/20 – 06/30/25 1.2 calendar
NIH/NCI $[redacted] (total costs requested)
Molecular targeted radionuclide therapy for tumor immunomodulation and enhancing immunotherapy response
Project 3: Combining targeted radionuclide therapy with a localized in situ vaccine to overcome immune suppression in the tumor microenvironment and augment T cell responses.
Goal: Targeted radionuclide therapies are a type of cancer treatment that can be injected into a patient’s vein and, after circulating through the patient’s body, these will preferentially accumulate in tumors and selectively deliver radiation to these locations. Radiation can damage tumors in a way makes them more susceptible to being killed by a patient’s own immune cells. Here, we will evaluate what effects targeted radionuclide therapies have on immune recognition of tumor cells and we will test whether these agents may enhance response to diverse forms of cancer immunotherapy – all in an effort to develop non-toxic, curative treatment approaches for patients with metastatic cancers of any type.
Role: Project leader for Project 3.

[redacted] 10/01/20 – 09/30/22** 0.6 calendar
$[redacted] (total costs to UW)
Combinatorial targeting of oncogene-driven childhood cancer
The goal of this project is to identify approaches capable of using local and systemic therapy to cure distant tumours in mice bearing large, chemotherapy resistant, measurable tumors in multiple sites, in the face of disseminated microscopic disease. This is the setting that best simulates that of high-risk and relapsed pediatric neuroblastoma.
** *This grant has been awarded; start date is pending.*

OVERLAP
There is no scientific overlap on any of the above grants.

*If the pending P01 grant is funded, Dr. Sondel plans to decrease his effort from the following four grants to accommodate the additional 1.2 calendar months on the P01:
R01 CA205101 (Skala), from 0.24 to 0.12 calendar months; U01 CA233102 (Morris/Weichert), from 0.36 to 0.12 calendar months; [redacted] from 0.36 to 0.12 calendar months; and [redacted] from 0.24 to 0.12 calendar months.

**We have received notification that the pending grant from [redacted], (Anderson, PI) will be funded, but start date is pending and is anticipated to be October 1, 2020 and last for 2 years. To accommodate this additional 0.6 calendar months of effort on [redacted], Dr. Sondel plans to decrease his effort on UL1 TR002373 (Brasier, PI) from 1.08 calendar months to 0.48 calendar months.

OTHER DISCLOSURES
Dr. Sondel is not part of any foreign talent program or foreign visiting professorship program.

As listed in **Pending** support, Paul Sondel is a co-investigator on an international grant, [redacted] that has recently been funded jointly by [redacted] and [redacted], that is being led by [redacted] and [redacted]. The collaborative funding by the [redacted] and [redacted] foundations required that labs from both the USA and [redacted] be collaborating on this grant, that is funded by USA and [redacted] funds. The total grant is for $[redacted] over 2 years, with $[redacted] of support over 2 years to [redacted] lab at UW-Madison (reflecting 0.6 calendar months effort for [redacted]).

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		David		Al-Adra		PD/PI	[redacted]	9			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2021 **End Date*:** 08-31-2022 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition Remission	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification1039439100.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		David		Al-Adra		PD/PI	[redacted]	9			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2022 **End Date*:** 08-31-2023 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition Remission	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	183,266.00	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification* File Name: Budget_Justification1039439100.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		David		Al-Adra		PD/PI	[redacted]	9			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2023 **End Date*:** 08-31-2024 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition Remission	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification1039439100.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		David		Al-Adra		PD/PI	[redacted]	9			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2024 **End Date*:** 08-31-2025 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition Remission	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification* File Name: Budget_Justification1039439100.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		David		Al-Adra		PD/PI	[redacted]	9			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-20-203 . Received Date: 2020-11-04T09:42:09.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 09-01-2025 **End Date*:** 08-31-2026 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition Remission	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification1039439100.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

David Al-Adra, MD, PhD, Principal Investigator (75% effort, 9.00 calendar months) Dr. Al-Adra is an Assistant Professor in the Department of Surgery at the University of Wisconsin- Madison. He is a hepatobiliary and transplant surgeon who specializes in the mechanisms of immunological tolerance. He studies methods to decrease rejection of transplanted organs and decrease cancer recurrence rates after transplant through organ pre-treatment. Dr. Al-Adra will be responsible for overseeing the project, designing experiments, conducting experiments, and analyzing data.

Fringe Benefits:
The professional fringe benefit rate for Dr. Al-Adra is budgeted at 33.6% for the duration of the grant.

Mentors (no salary requested):

Christian Capitini, PhD (Primary Research Mentor) is an Assistant Professor in the Department of Pediatrics at the University of Wisconsin- Madison. His research focuses on using allogenic hematopoietic stem cell transplant to cure pediatric cancers. His past research support encompasses twenty awards, including an NIH K08. Currently, he holds ten active awards, including a NIH R01. Dr. Capitini will serve as primary mentor to the Principal Investigator (PI) and guide the overall direction of the PI's research and career development through weekly meetings.

William Burlingham, PhD (Co-Mentor) is a Professor in the Department of Surgery at the University of Wisconsin-Madison. Dr. Burlingham's research program focuses on acquired immunologic tolerance. His lab hopes to gain insight into graft acceptance by studying transplant recipients who have survived after stopping immunosuppressive drugs. He has an extensive history of independent funding and mentoring success. He has been the principal investigator on over twenty R01 and R21 grants and is currently a PI of an R01 and an R21, as well as a Co-I on a U01 grant. Dr. Burlingham and the PI will meet weekly to discuss the immunological aspects of the projects contained in this proposal.

Paul Sondel, MD, PhD (Co-Mentor) is a Professor in the Department of Pediatrics at the University of Wisconsin- Madison. Dr. Sondel's research focuses on tumor immunology. He pursues basic, preclinical and clinical mechanisms to induce *in vivo* activated innate immune effector cells to provide anti-tumor benefit. Dr. Sondel has had continuous NCI support since 1982, which includes several R01s and U01s, and he is currently the project leader on a U54 and PI of an R35. Dr. Sondel and the PI will meet monthly to discuss the immunological aspects of the projects contained in this proposal.

Josh Mezrich, MD (Co-Mentor) is an Associate professor in the Department of Surgery at the University of Wisconsin-Madison. Dr. Mezrich's research focuses on transplant tolerance and how environmental exposures alter the immune system. Dr. Mezrich has a successful basic science research program. He is the recipient of a K award followed by an R01, and has just secured a VA Merit grant. Dr. Mezrich will help the PI develop technical expertise using monthly formal meetings.

Other Research Personnel:

Feridoon Najmabadi, Researcher (Y1-Y5: 15% effort, 1.80 calendar months). Dr. Najmabadi holds a PhD in biology and is an expert in cell culture and the analysis of biological samples using cellular and molecular biology techniques. He also has vast experience with immuno-histochemistry, which will be invaluable for the proposed project. Dr. Najmabadi will be responsible for experimental design, conduct of experiments, and data analysis.

Fringe Benefits:
The professional fringe benefit rate for Dr. Najmabadi is budgeted at 33.6% for the duration of the grant.

Supplies/Other Expenses

Cell Isolation/Culture Consumables (Y1: $[redacted]

- Cell isolation consumables (glass slides, cell strainers, tubes)
- Cell culture consumables (culture dishes, media, media additives)

Pre-operative/Operative Cost & Disposables (Y1: $[redacted])

- Operative disposables, suture, bandages
- Pre-operative costs (Analgesia, Isoflurane)
- Operative costs (liver cannulation, NEVLP set-up)

Perfusion Additives & Consumables (Y1: [redacted]

- Perfusion consumables (tubing, sterilization, buffers)
- Perfusion additives (IL-2, IL-10, TGF-β)

Histology Service Fees (Y1: $[redacted]

The UW Histology core facility will be instrumental in characterizing the cellular phenotypes of immune cells present in the liver.

Flow Cytometry Services Fees (Y1: $[redacted]

Usage time in the UW Flow Cytometry core facility will be required (cost of $65/hour). We anticipate ~100 hours of time to complete Aims 1 and 2, for a cost of approximately $[redacted] The reagents and anti-bodies required to characterize the cellular phenotypes using flow cytometry will cost an additional $[redacted]

Nanosight Imaging Microscope (Y2-Y5: $[redacted])

This state-of-the-art imaging modality will be used for the characterization of exosomes. We anticipate ~100 hours of time to complete Aims 1 and 2, for a cost of approximately $[redacted]. The reagents and anti-bodies required to characterize the exosomes will cost an additional $[redacted].

Animal Expenses including purchasing, shipping, housing per diems (Y: [redacted]

Animal purchase will occur on a quarterly basis. Animals will be purchased from a commercial vendor at an estimated cost of $[redacted] per animal. A total of 400 rats will be purchased to perform the necessary experiments ($[redacted] over 5 years). Housing per diems at UW-Madison are $[redacted]/cage/day; with an estimated 3500 cage-days = $[redacted]

Travel (Y1-Y5: $[redacted])

Requesting funds for travel costs associated with attending the American Transplant Congress Conference, which occurs annually. Estimated costs for a 3-day conference are based on Flight (~$[redacted]), Registration fees ($[redacted]), Meals/Hotel $ (~$[redacted] per night).

Indirect Cost Rate: 8% per RFA

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	
Total Number Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	
1. Domestic	
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	
Section K, Total Costs and Fee (I + J)	[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	Al_Adra_rev_Introduction_final1039438874.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate_Section1039337538.pdf
Research Plan Section	
3. Specific Aims	Al_Adra_Aims1039438879.pdf
4. Research Strategy*	Research_Strategy1039438881.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	Responsible_conduct_of_research1039337540.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Mentor_Plan_with_Letters_10_6_20201039337542.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	OS_Docs_Combined1039439002.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	Institutional_Environment1039337543.pdf
11. Institutional Commitment to Candidate's Research Career Development	Chair_Letter_Al_Adra_K081039337818.pdf
12. Description of Candidate's Contribution to Program Goals	
Other Research Plan Section	
13. Vertebrate Animals	Vertebrate_Animals1039337822.pdf
14. Select Agent Research	Research_Using_Select_Agents1039337821.pdf
15. Consortium/Contractual Arrangements	
16. Resource Sharing	Resource_Sharing_Plan1039337819.pdf
17. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Biol1039337820.pdf
Appendix	
18. Appendix	

Funding Opportunity Number: PA-20-203. Received Date: 2020-11-04T09:42:09.000-05:00

PHS 398 Career Development Award Supplemental Form

Citizenship*:

19. U.S. Citizen or Non-Citizen National?* ● Yes ❍ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: ❑

Introduction to Resubmission: My sincere appreciation to the Committee for the comprehensive review of my application and for the suggestions and concerns identified in the 7/26/20 summary statement. This resubmission directly responds to all weaknesses, which I believe substantially improves the application. It includes a revised career development plan and a more focused and mechanistic experimental strategy complete with new preliminary data. This brief introduction page highlights the major conceptual and strategic revisions I have made in this revised application based on the review:

1. **Career development and gaps in training:** Reviewer 1 felt the proposed coursework could be designed to better address my gaps in training and Reviewer 3 felt the career development plan could be more succinct. I have completely revised the career development plan to make this section concise and have identified additional coursework that can accurately fulfill my knowledge gaps in transplant immunology. I have recently completed The American Association of Immunologists Advanced Course in immunology and plan to attend additional advanced immunology courses that focus on regulatory dendritic cell (DC) biology.
2. **Microsurgical training**: Reviewer 1 commented that the time to master small animal microsurgery may be better spent on other research initiatives. I have now collaborated with a team of experienced microsurgeons (Drs. Poore and Zeng – see letter of support) to optimize the rat liver transplant model that is crucial to the conduction of experiments to evaluate the liver-resident DCs (see new figure 5).
3. **Preliminary data**: The lack of preliminary data suggested by Reviewer 1 is an excellent point. In this resubmission, we have generated new data demonstrating we have increased the number of experiments conducted in a reproducible fashion that has allowed us to increase rigor. These data are complete with the representative gating strategies used to characterize liver-resident DCs (see figure 4). In addition, I have made progress towards my research collaboration and team science goals by presenting these research findings at the University of Wisconsin's Surgery Research Summit 2020 and aim to submit a full manuscript in the winter of 2020.
4. **Specificity for DCs:** All reviewers commented on the "innovative strategy" with "intriguing preliminary data" to decrease liver rejection after transplant by focusing liver-resident DCs. However, Reviewers 1 and 3 suggested that the approaches to evaluate liver-resident DCs are not specific to this cell population. I agree with this assessment and have added additional experiments that will allow the investigation off-target cellular effects. Specifically, in addition to characterizing liver-resident DCs, we will also characterize T cells, B cells, and macrophages present in the liver using newly designed flow cytometry panels. We have also expanded the number of cytokines we are evaluating during NEVLP to include ones that are not associated with DCregs, to determine which other cells are being affected by our interventions (see aim 2.1).
5. **Mechanistic experiments:** Reviewer 3 suggested a significant revision of aim 1 to successfully determine the regulatory effect DCregs have on liver transplant. We now propose a more rigorous assessment of the role liver-resident DCs play in liver transplantation (see Aim 1). The experimental strategy includes new sub-aims with expansion of DCs both *in vivo* (new preliminary data in figure 7) and *ex vivo*, both in a transplantation model. We also now propose DC depletion experiments in a transplant model, as suggested by Reviewer 1. In addition, we have specified the precise methods being used to deplete the major regulatory aspects of DCregs (PD-L1 and extracellular vesicles) to determine the contribution of these regulatory qualities to transplant tolerance.
6. **Immunological endpoints:** We agree with Reviewer 3's comment that using clinical "rejection" of a liver transplant as an endpoint may miss the temporal kinetics of the immune response. Therefore, to increase sensitivity of detecting immunological responses, we have added early predetermined time points to understand what is occurring in the graft soon after transplant and how these immunological changes may relate to long-term transplant outcomes (see aims 1.1 and 2.2). Furthermore, we have included a more extensive analysis of cytokines and have included standardized criteria that are being evaluated by histopathology.
7. **Extracellular Vesicle characterization:** Reviewer 3 commented on the lack of preliminary data related to the composition and function of the extracellular vesicles being analyzed. We have now included new preliminary data in figure 9, which outlines our experience isolating and quantifying the extracellular vesicles being produced by the liver. Their function will be quantified in mixed cellular proliferation assays (Aim 2.1).

We remain dedicated to answering the questions posed in this proposal. As such, my laboratory is making progress on both aims as we are eager to continue moving this project work forward, to address both mechanistic and translational questions that we believe are key to ultimate clinical application. As testament to this, we have had an abstract published in the *American Journal of Transplantation,* and my research was selected for a Surgical Society of the Alimentary Tract award. Thank you again for the constructive comments and suggestions.

CANDIDATE STATEMENT

The University of Wisconsin (UW)-Madison Department of Surgery excels in research and training (ranked 8th in NIH funding for Departments of Surgery), and is an ideal environment in which to achieve my career goals as a surgeon-scientist. Thus, I was delighted to join the department in 2017 as a tenure-track Assistant Professor. My primary career objective is to pursue and answer original research questions and become a recognized authority in the field of Transplantation Immunology, to improve outcomes of patients after transplantation. To this end, my K08 Mentored Clinical Science Research Career Development proposal addresses areas where I have identified gaps in my education and training (outlined below). I have assembled an outstanding and committed mentorship team that will guide and enhance my development in core competencies, including communication, biostatistics, and ethical research design. I aspire to improve the outcomes of patients after solid organ transplantation and have dedicated my career to performing basic and translational research to investigate the mechanism of immunological rejection of the liver and develop strategies to decrease this rejection. Through the K08 mentored process, I will develop a high-impact research program that will ultimately translate into an independent research career devoted to advancing transplant patient care.

Candidate Background

I established my research trajectory early in my undergraduate training at the University of Alberta, where I was first challenged in a basic science lab to ask original research questions, work in a team, and critically analyze data. I was fortunate to continue my research pursuits while in medical school at the same institution (**Table 1A**), and upon graduation I was recognized with an "MD with Special Training in Research." During my General Surgery residency, I completed a basic science PhD in Experimental Surgery with the ultimate aim of starting a basic science laboratory after completion of my clinical training. My doctoral studies fueled my enthusiasm for and devotion to Transplantation Immunology (**Table 1B**). I was awarded a prestigious Clinical Fellowship from Alberta Innovates Health Solutions to study a subset of donor-derived antigen presenting cells (APCs) after bone marrow transplant. During my solid organ transplant fellowship at the University of Toronto, I engaged in clinical science, helped develop original clinical questions, collected and analyzed data, and presented and published these findings. In addition, I was part of the study team that researched the first North American clinical application of normothermic machine liver preservation (NEVLP) technology (**Table 1C**). Presently, as an early stage faculty member, I am interested in combining my background in APCs with the study of regulatory dendritic cells (DCreg), and applying knowledge of APC biology with NEVLP to enhance liver transplant outcomes by decreasing acute rejection (**Table 1D**).

Table 1. Basic Science and Clinical Research Experience

	Year(s)	Training / Experience	Description	Achievements
A	2005	University of Alberta (K Bagnall), Medical student research	Human X-ray measurement and calculations to investigate the effects of vertebral rotation on scoliosis development	1 first author publication
B	2009-2012	University of Alberta (C Anderson, AMJ Shapiro), PhD research	Mouse studies to evaluate the role of APCs on the development of central tolerance	4 first author publications; 2 basic science awards; several regional / national podium presentations
C	2015-2017	University of Toronto (P Greig, M Selzner), Transplant surgery fellowship	Clinical research evaluating outcomes after various solid organ transplants	8 publications, including 2 first author or co-first author
D	2017-present	University of Wisconsin (Primary mentor Dr. Christian Capitini), PI	Basic research investigating the ability of DCregs to be expanded *ex vivo* to decrease acute rejection of a liver transplant	Developed rat NEVLP model; received *Hope Meets Gratitude Research Grant*; *Surgical Society of the Alimentary Tract Career Development Award*

Despite my rich research experience, I have identified specific gaps in my education and training that could be addressed through a formal mentored research plan, with instrumental support from the K08 Award and an exceptional mentorship team.

Candidate Career Goals and Objectives

I will hold my appointment as Assistant Professor of Surgery for the duration of the K08 Award. My expectation, and that of my Department Chair (Dr. [redacted] and Division Chief (Dr. [redacted]), is that I will focus on developing my research laboratory over the first 5-7 years of my appointment.

Objectives. Scientifically, the objective of this K08 Career Development Award is to investigate the ability of *ex vivo* expanded donor-derived regulatory dendritic cells (DCregs) to decrease acute rejection in a rat model of Normothermic *Ex-Vivo* Liver Perfusion (NEVLP), using a novel model of liver transplantation. My professional objective is to dramatically improve my communication and teamwork skills, solidify my immunologic knowledge base, and develop the skills required to ethically design methods to expand DCregs *ex vivo* using NEVLP technology. These skills will allow me to establish the foundation for an independent research program.

Goals. My short-term goals are to develop the skills necessary to advance my scientific and professional career. This effort will build a solid foundation for my transition to an independent investigator and a leader in the field of Transplantation Immunology, where I can eventually apply my discoveries to patients who have received a liver transplant. Upon completion of the K08 Award, I believe I will have accrued sufficient mentorship and training to secure and maintain independent NIH funding for the duration of my career. I will also develop strong collaborations with investigators both within and outside of UW-Madison through networking at basic science meetings, and develop multi-PI studies that have the potential to enhance the clinical impact of basic science findings.

My long-term goal is to lead an independently funded basic science research program in Transplant Immunology that investigates the mechanisms behind liver transplant rejection, and leverage these mechanistic translational T0 studies into therapeutic translational T1 studies to decrease rejection rates and improve liver transplant outcomes. In addition, although my current K08 proposal focuses on liver transplants, findings from the proposed experiments can be translated to other solid organ transplants, such as kidney and lung. Furthermore, these data will provide the basis for presentations at scientific meetings and publications in high-impact journals, and will facilitate my ultimate goal of applying for an NIH R01 grant. A future R01 application could include subsequent translational T2 investigations with aims to (1) Determine the effects of DCreg modification during NEVLP in order to “cloak” the liver from the recipient immune system by upregulation and over-expression of the co-inhibitory molecule programmed death ligand-1; and (2) Investigate the effects of increasing the number and life span of graft-resident passenger lymphocytes, specifically APCs, on the development of chimerism and central tolerance towards the transplanted liver.

My short and long-term goals complement the diverse array of research performed in the Division of Transplantation and significantly strengthen the research portfolio in the Department of Surgery. My laboratory will also serve as an excellent opportunity for mentoring surgical residents and medical students interested in basic science research. Over time, I anticipate expanding my research team to include several graduate students, surgical residents, medical students, and undergraduates with the aim of inspiring the next generation of surgeon-scientists and fostering their careers.

Candidate Career Development and Training Plan

I have worked closely with my expert mentor team to develop the proposed plan, which addresses specific gaps in my training and development through a combination of *research activities related to my project* (development of technical skills and collaborations), *educational activities* (courses, workshops, and conferences), and *mentorship* from an exceptional team. These activities will allow me to establish my research independence, focusing on organ modification prior to transplant to decrease immunological rejection of the liver. The main components of this plan are outlined below, and in **Table 2**.

1. Develop Academic Expertise in the Fields of Transplant Immunology, Dendritic Cell Biology, and Biostatistics

Transplantation Immunology. Transplantation Immunology is a major focus of my research; therefore, I will invest significant time to address knowledge gaps in immunology related to transplantation. *First,* I will attend the Advanced Course in Basic and Clinical Immunology offered through the Federation of Clinical Immunology Societies. During this focused, intensive course (March 1-3, 2021), world-renowned immunologists present recent advances in the biology of the immune system and cover current topics in immunology and laboratory techniques. *Second,* I will attend the annual Great Lakes Transplant Immunology Forum, a regional meeting held yearly in the fall, where cutting-edge transplant immunology is discussed, and important research collaborations are made. *Third,* I will attend and present at the weekly UW Immunology Journal Club led by Dr. Burlingham (Mondays, 9AM), which focuses on current immunologic research and critically analyzes the interpretation of experimental results. *Last*, I will routinely review advanced components of the transplant immune system related to dendritic cells, mechanisms of transplant rejection, and check-point inhibitors with Drs. Burlingham, Sondel, and Mezrich.

Dendritic Cell Biology. To strengthen my knowledge base with respect to DC biology, I have recently taken the Advanced Immunology Course offered by the American Association of Immunologists. This weeklong course contained sections on fundamental principles governing the interaction between DCs, T cells, and B cells. In addition, the Advanced Course in Basic and Clinical Immunology (mentioned above) includes educational sessions on DCs and the cytokines they produce. These didactic courses will be supplemented by directed discussions with Dr. Capitini regarding specific subsets of DCs and how these subsets relate to immunity and tolerance.

Biostatistics. To advance my knowledge in experimental data examination and improve my ability to critically analyze data, I will enroll in training in experimental design and statistical methodology by enrolling in one course per semester for the first two years of the award period. At UW, the biostatistics program includes BMI 541 (Introduction to Biostatistics; Fall 2021 Tuesdays 4:00 - 5:15), and BMI 542 (Introduction to Clinical Trials I; Spring 2022 Thursdays 3:30-4:30).

2. Develop Research Vision and Focus Through Regular Meetings my with Mentorship Team. I will meet weekly (after Monday afternoon lab meting) with my primary mentor, Christian Capitini, MD, Associate Professor of Pediatrics, for directed discussions on career development and transition to independence (e.g., research design, collaborations, basic science meetings, networking, effective mentoring, and acquisition of extramural (independent) funding). In addition, I will frequently meet with Josh Mezrich, MD, Associate Professor of Surgery, to discuss navigating a surgeon-scientist career, including balancing clinical work while focusing on research pursuits. An accomplished author and public speaker, Dr. Mezrich will also help me refine my skills in communication and grant/manuscript writing. William Burlingham, PhD, Professor of Surgery, will provide expertise in transplant immunology, input on research design, and education on various experimental techniques necessary for my proposed investigations, especially related to extracellular vesicles. I will continue to attend his Friday morning laboratory meetings on a weekly basis. Paul Sondel, MD, PhD, Professor of Pediatrics, will provide an immunology education component; we will meet monthly for discussions in the highly specialized area of checkpoint inhibitors. I will also leverage the extensive and successful careers of Drs. Sondel and Burlingham to gain valuable insight into effective laboratory staff recruitment, training, and mentorship. To ensure appropriate progress, I will present my training plan and achievements to my mentor team (Drs. Capitini, Mezrich, Burlingham, and Sondel) during semi-annual, two-way, critical feedback meetings.

3. Develop Laboratory Training and Experience Leading an Independent Research Group
Investigative Techniques. I will receive advanced training from Drs. Capitini, Burlingham, and Mezrich to address gaps in investigative techniques and research design that will be important for my future success. This training includes isolating immune cells from livers, isolating and characterizing exosomes from liver during *ex vivo* perfusion, and evaluating immune cell activation and cytokine production. Becoming proficient with these research methods will allow comprehensive examination of dendritic cells in the rat liver.

Research Development. I will meet with Dr. Capitini weekly to discuss experiments, results, and relevant next steps in my proposed research. My proximity to the offices of Drs. Burlingham and Mezrich allows me to speak with them frequently to discuss proposed experiments and receive advice on relevant investigative techniques.

In the process of completing the aims of my proposal, my mentor team and I will discuss and review manuscript submission, with the goal of 4 publications during the career development award.

***Research Progression*.** Presenting and publishing my work will be important for establishing my research program. Data from investigation of the modifying liver-resident dendritic cells will form the basis for presentations at scientific meetings, where I can network and develop collaborations with investigators from other institutions. I will submit research abstracts (average two per year) and present at the annual meetings of the American Transplant Congress (July), and the Great Lakes Transplant Immunology Forum (fall), leveraging departmental professional support up to $[redacted]/year. The collaborations formed with investigators at these meetings will complement the collaborative efforts that I will cultivate at UW. I plan to work with scientists in other specialties on multi-PI grants that may enhance the clinical impact of our basic science findings.

***Research Administration*.** To develop and manage a successful research team, I will train to be an ethical and responsible principle investigator. In years 3, 4 and 5 of the Career Development Award, I will engage in the customized mentor training curricula at the UW Center for the Improvement of Mentored Experiences in Research. This curriculum will help me gain laboratory management and operational skills in maintaining effective communication, cultivating ethical behavior, and fostering independence of laboratory members. I will hold discussions with, and gain valuable insight from Drs. Burlingham and Sondel, who have managed successful laboratories for decades.

4. Professional development

Career Development and Leadership Skills. I will attend the UW Institute for Clinical and Translational Research (ICTR) career development seminars and complete a customized mentee training curriculum through the UW-Madison Center for the Improvement of Mentored Experiences in Research. The UW ICTR seminars are highly pertinent for early career scientists (e.g., research methods, bioinformatics, core service technologies, leadership) and will complement my monthly career development discussions with Dr. Capitini. Furthermore, I will hold monthly discussions with Drs. Mezrich and Sondel, focused on mechanisms to build a successful clinician-scientist career. To expand my leadership skills, I will take the 3-day American College of Surgeons (ACS) Surgeons as Leaders course in Year 2 of the award. In addition, I will attend professional development programs offered through UW, including the daylong Leadership and Management Development Conference, which focuses on building communication skills, giving/receiving constructive feedback, and fostering inclusivity to enhance teamwork and team science.

Responsible Conduct in Research. I will enroll in Pharmacy 800 (Research Ethics, Scientific Integrity and the Responsible Conduct of Research, Fall 2021 Mondays 4:40 – 6:10), which is approved by the Office of Research Integrity and meets the National Institutes of Health (NIH) requirements for the responsible conduct of research. This course consists of face-to-face faculty instruction in principles and concepts of research ethics through presentations and discussion of case studies. Further, I will discuss the Responsible Conduct in Research training with my mentors and advisors during the formal semi-annual meetings. My primary mentor, Dr. Capitini, will ensure that I continue to update my knowledge of responsible conduct of research at our monthly directed learning sessions; he will be available at any time to discuss ethical issues that may arise.

Candidate Benchmarks for Successful Completion. To achieve my ultimate goal of developing an independent research laboratory, by the end of this K08 award, I will generate a competitive R01 application. In Year 4, I will participate in a K to R grant writing workshop offered through UW-ICTR to obtain critical feedback on development of a research project grant. In Years 4-5, I will leverage the experiences and expertise of all my mentors to prepare and submit an R01 grant. I will also actively seek out opportunities to present my work, as speaking engagements represent progression to becoming an expert in the field of Transplantation Immunology. The R01 submission will be the largest benchmark for successful completion of my research, training, and professional goals, and will also be important for my promotion along the tenure track.

Summary: My K08 plan provides the research strategy, advanced education, training, and mentorship required to cultivate an independent basic and translational science research program. It will provide a platform to secure R01-level funding to further investigate the ability of liver-resident immune cells to be modified in order to decrease organ rejection after liver transplant. This work will position me to be a leader in the field of

Transplantation Immunology and advance therapeutic strategies aimed at increasing the survival of patients with liver failure.

Table 2. Summary Timeline of K08 Research and Training Activities (assumed start date Sept 2021)

	Year	Year	Year	Year	Year
Research	**1**	**2**	**3**	**4**	**5**
Research Plan: Specific Aim 1					
Characterize exosomes (optimize technique w/ Capitini and Burlingham labs)					
Multiparameter ELISA and flow cytometry	*In progress*				
Research Plan: Specific Aim 2					
Compare DCregs in treated NEVLP livers with untreated livers	*In progress*				
Rat liver transplantation					
Training					
Responsible Conduct in Research course	Fall				
American College of Surgeons: Surgeons as Leaders course		Apr			
Advanced Course in Basic and Clinical Immunology	Mar				
Biostatistics Research Courses: BMI 541 and BMI 542	Fall	Spring			
UW Immunology Journal Club					
Dept. Surgery Research-in-Progress seminars					
UW Center for the Improvement of Mentored Experiences in Research: customized training					
Grant Writing					
Wisconsin Partnership Program New Investigator grant		Sep			
K to R grant writing workshop					
NIH R01 grant submission (key benchmark)				Winter 2025	
Publications					
Manuscripts		1st	2	3 & 4	
Conferences					
UW Leadership & Management Development Conference			Nov	Nov	
Great Lakes Transplant Immunology Forum	Fall	Fall	Fall	Fall	Fall
American Transplant Congress	July	July	July	July	July

SPECIFIC AIMS

Liver transplantation is the only treatment option for patients with end-stage liver disease, with over 8,000 liver transplants performed yearly in the US[1]. Unfortunately, immunological rejection is common, and despite the long-held belief that rejection episodes do not affect the liver graft, evidence now shows that such episodes are indeed detrimental to the transplanted organ[4,5]. Development of innovative strategies to decrease rejection of the transplanted liver remains a major, unmet need. Current strategies to decrease organ rejection utilize dangerous, lifelong anti-rejection drugs that suppress the immune system and heighten the risk of life-threatening infections. In contrast, our innovative strategy will entail modifying the immune microenvironment at the time of transplantation to enhance immune regulation and suppress rejection.

Previous attempts to decrease organ rejection have demonstrated the importance of regulatory dendritic cells (DCregs) for prolonging transplant survival, especially DCregs that come from the organ donor[6-8]. The ability of DCregs to prolong organ transplants may be due to their expression of co-inhibitory molecules, such as PD-L1, and through the production of extracellular vesicles (EVs) that contain molecules and cytokines that can influence the immune microenvironment of the transplanted organ[9-11]. However, due to the difficulty of obtaining the number of DCregs required for therapy, the impact of donor-derived DCregs on deceased donor liver transplant outcomes remains uncertain. Recent advances in normothermic *ex vivo* liver perfusion (NEVLP)[12-14] have led to a paradigm shift in the way livers are stored prior to transplantation; we now have the exceptional ability to modify cell subsets in their native microenvironment within the graft prior to transplantation[15,16]. Consequently, our lab is intently focused on using NEVLP to increase the number of DCregs and improve their function, to allow for reduced graft rejection and improved graft survival.

The overall objective of this proposal is to expand and enhance the regulatory function of DCregs using NEVLP in a rat model of liver transplantation. Successful completion of the proposed aims will provide a substantive foundation to achieve the clinically relevant long-term goal of decreasing acute rejection rates and minimizing the need for anti-rejection drugs. Based on prior studies demonstrating the pivotal role of dendritic cells in the prolongation of transplant survival, our central hypothesis is that expansion of liver-resident DCregs during NEVLP will promote a regulatory environment for the organ after transplant through their expression of co-inhibitory proteins and production of regulatory EVs. We will test our hypothesis through two specific aims:

Aim 1. Determine the dominant regulatory mechanism of liver-resident DCregs. The mechanisms by which liver-resident DCregs affect graft rejection have not been well characterized. Based on prior data regarding DCreg inhibitory function[9-11], our working hypothesis is that liver-resident DCregs regulate the rejection response towards the transplanted liver through their expression of co-inhibitory proteins and production of regulatory EVs. To test this hypothesis, we will use a model of rat orthotopic liver transplantation from LEW to BN rats, to determine if expanding liver-resident DCregs *in vivo* or during NEVLP can decrease rejection after transplant. We will then use a model of rat liver transplant that *does not reject* and assess whether tolerance in this model can be broken by the selective depletion of DCregs. We will further assess whether tolerance in this model can be broken through the addition of GW4869 to inhibit EV production, or the addition of anti-PD-L1 antibody to block PD-L1 expressing cells. We expect a greater immune response towards livers that are lacking DCregs or unable to produce EVs or express PD-L1.

Aim 2. Measure the impact of expanded liver-resident DCregs generated by combination cytokine therapy during NEVLP on liver graft rejection *in vitro* and *in vivo*. Our preliminary data show that anti-inflammatory cytokines can increase DCreg frequency during NEVLP. The working hypothesis is that efficiently expanded liver-resident DCregs during NEVLP can promote a regulatory environment for the organ after transplant. To test this hypothesis, rat livers will be treated with the anti-inflammatory cytokines IL-10 and TGFβ during NEVLP[20-22]. The frequency and phenotype, and regulatory function of the DCregs will be determined using multiparameter flow cytometry and mixed cellular reactions, respectively. We will then compare NEVLP livers treated with and without anti-inflammatory cytokines in a rat liver transplant model and expect the addition of anti-inflammatory cytokines during NEVLP will induce the formation of DCregs in the liver and decrease immune responses after transplant.

My career goals are to become a surgeon-scientist with a clinical practice in transplant surgery and an independent research laboratory focused on strategies to decrease immunological rejection of organ transplants. Through the proposed K08 educational activities, mentorship, and research-focused training, I will dramatically improve my knowledge and skills, so that I may successfully evaluate discoveries in the laboratory and translate them into therapies for patients in my own practice. Upon completion of this project, I will have developed unique expertise in DCregs and their mechanisms of regulating transplant rejection, which is relevant to develop strategies to decrease acute rejection and improve patient survival. This expertise is critical for generating a R01 proposal that will further test these mechanisms in a large animal transplant model.

RESEARCH STRATEGY

A. SIGNIFICANCE

Overall Scientific Premise. Liver transplantation is the only treatment option for patients with end-stage liver disease. While more than 8,000 liver transplants are performed yearly in the US, over 13,000 patients languish on the waiting list, and 2,000 patients die each year without receiving a life-saving transplant[1]. Ultimately, the solution to this organ shortage will involve both increasing the number of available livers and preventing liver disease, which will require many years to accomplish. At the same time, the 5-year survival rate of liver grafts after transplant remains constant at 75%[24]. Rejection episodes, seen in up to 25% of liver transplant recipients[4,5], were commonly thought to have no bearing on long-term graft survival, and no strategies to prevent rejection have been developed. However, recent studies have demonstrated that acute rejection is not only common, but also has deleterious effects (4.4 times decreased graft survival and 3.9 times decreased patient survival)[4,5]. Efforts to simply treat rejection episodes with high doses of steroids, a therapy that is not always successful, or increase the amounts of immunosuppression after transplant, have been shown to increase infectious complications and morbidity and reduce the survival of liver recipients[25]. Furthermore, despite advances in immunosuppressive therapy, the rates of chronic rejection[26] and late-graft loss remain unchanged[27].

One approach to the problem of graft rejection is to attenuate the recipient's immune response toward the transplanted liver. Regulatory dendritic cells (DCregs), a subset of immune cells within the liver, are important for the maintenance of immune homeostasis in the liver[28]. Given their primary role of preventing an immune response to harmless self-antigens[29], these liver-resident cells also have the potential to attenuate the recipient's immune response after liver transplantation and decrease rejection. Recent studies in small animals have exhibited the importance of recipient-[30,31] and donor-derived[7] DCregs on prolonging heart and kidney transplant survival, respectively. DCreg therapy has also been shown to be advantageous in non-human primates, where the combination of donor-derived DCregs with the immunosuppressive agents CTLA4-Ig and rapamycin prolonged kidney graft survival[8]. To limit a liver recipient's immune response toward a transplanted organ and decrease rejection, there is a critical need to develop strategies to effectively leverage the regulatory function of DCregs.

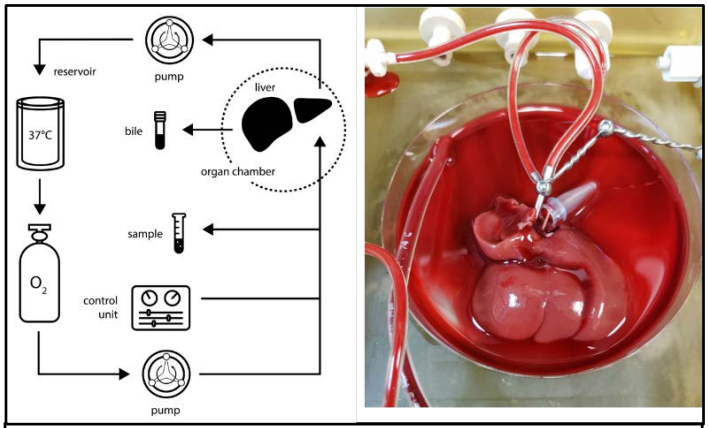

Figure 1. Normothermic Perfusion. Schematic of perfusion system (left). A rat liver undergoing NEVLP in the Al-Adra laboratory (right).

The recent development of a novel organ preservation approach known as normothermic *ex vivo* liver perfusion (NEVLP) provides an ideal platform to perform therapeutic interventions in livers before transplantation (**Fig. 1**). During NEVLP, the donor liver is housed at physiologic temperature while being perfused with nutrients and oxygen[32]. In this environment, the donor organ resumes physiologic activity, and can be effectively treated with therapeutic agents[33]. In this proposal, we will first characterize the contribution of liver-resident DCregs towards tolerance in rat liver transplantation, then determine the dominant regulatory mechanisms of liver-resident DCregs (Aim 1). Next, we will measure the impact of specific therapies to expand liver-resident DCregs to decrease immunological rejection after organ transplant *in vitro* and *in vivo* (Aim 2).

Rigor of the prior research. In prior studies utilizing DCreg therapy[6,10,30,31], DCregs were isolated, cultured, and administered to the recipient *prior* to the solid organ transplant. These precisely conducted experiments demonstrated the powerful effects of DCreg therapy; however, these approaches are only compatible with living donation, such that the *in vitro* expansion of donor or recipient DCregs can occur in co-ordination with the scheduled donation. Therefore, these protocols are not compatible with deceased donation (where scheduling donation is not possible), which is much more common in liver transplantation (95.6% of liver transplants)[1]. The lack of relevance in these prior studies towards deceased donation serves as the key support for the proposed project, where DCreg therapy can be used for all forms of liver transplantation.

Scientific Premise for Aim 1. Liver-resident DCregs (CD4+, CD11b/c^{+}, CD45^{+}, CD103^{+}, MHC II^{lo}, CD40^{lo}, CD80^{lo}, CD86^{lo}, PD-L1^{+}) are important for the maintenance of immune homeostasis in the liver[28] and play a crucial role in the development of immune tolerance toward harmless self-antigens and ingested peptides[2,34]. These cells have therapeutic potential to decrease the recipient's immune response to a transplanted organ

(**Fig. 2**)[11,35]. Recent evidence demonstrates that DCregs exert their immunosuppressive effects by communicating with host dendritic cells (DCs) at the immune synapse via extracellular vesicles (EVs)[7]. Specifically related to liver, in a mouse liver transplantation model, donor-derived EVs were responsible for "cross-dressing" recipient DCs and inducing tolerance[36].

Our research group has found that EV acquisition by host DCs can alter their phenotype and lead to increased expression of programmed death ligand 1 (PD-L1)[37]. Furthermore, we have recently shown that exosomes from primed mesenchymal stem cells can create anti-inflammatory macrophages[38]. Although our studies demonstrate EV effects in models of maternal membrane acquisition, and acute radiation syndrome, they show the regulatory effects of EVs produced from tolerogenic cells.

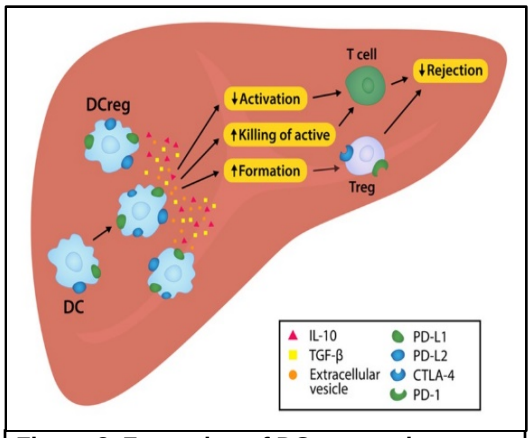

Figure 2. Formation of DCregs to decrease liver rejection. DCregs are characterized by upregulation of the co-inhibitory proteins (programmed death ligand (PD-L)1 and PD-L2, and down regulation of co-stimulatory proteins (not shown). Through direct cellular interaction and production of cytokines (transforming growth factor β (TGFβ) and interleukin (IL)-10) and EVs, DCregs can cause antigen-specific suppression of T cell responses[2,3], and can induce T cell anergy[17], apoptosis[18], and the differentiation of regulatory T cells (Tregs)[19]. In turn, Tregs can further suppress T cell, NK cell, macrophage, and dendritic cell responses through direct cellular or indirect cytokine mediated interactions[23] that can decrease rejection.

Our previous data and that of others support the use of DCregs in liver transplantation to decrease rejection and suggest that DCregs are a relevant target with significant potential for therapeutic intervention[39,40], but alternative strategies are needed to extend DCreg therapy beyond the living donor setting. One such approach is NEVLP, where donor-derived DCregs can be manipulated within a living organ itself, prior to transplantation.

While previous studies have established the importance of DCregs in decreasing immune rejection and the importance of EVs, and PD-L1, their overall contribution in liver transplantation tolerance is unknown. In addition, the dominant regulatory mechanisms of liver-resident DCregs have not been investigated. The proposed study could yield valuable insight into the mechanisms that drive the inhibitory properties of liver-resident donor-derived DCregs. Our working hypothesis is that liver-resident DCregs regulate the rejection response towards the transplanted liver through their expression of co-inhibitory proteins and production of regulatory EVs.

Scientific Premise for Aim 2. Donor-derived antigen presenting cells (APC) and their EV products play a prominent role in allograft rejection, as they are responsible for priming recipient T cells[41]. Yet certain APC subsets, such as DCregs, can actually decrease T cell responses and allograft rejection[10]. However, DCregs can transform into mature APCs and add to immune system activation if they experience cellular stress, which begins during procurement, where donor organs are cooled for storage and transport, and culminates when the organ is transplanted and undergoes ischemia-reperfusion injury[42,43]. Therefore, DCregs must be maintained in an unstimulated, regulatory state to optimize this therapy.

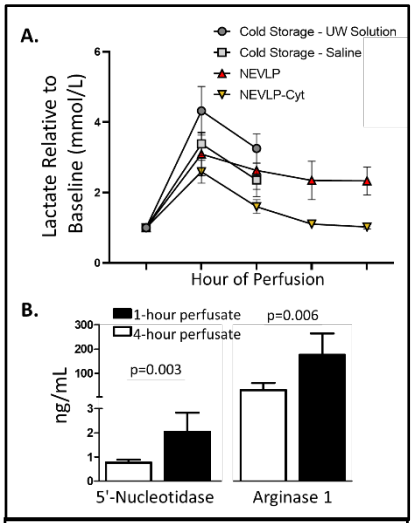

Figure 3. Liver function and damage during perfusion. **A.** During NEVLP, the liver is functioning as demonstrated by lactate clearance relative to the starting lactate in the perfusate (n=3-5 per group) See aim 1 for experimental details. **B.** Liver-specific enzymes released by hepatocytes into the perfusate are markers of liver damage. (n=7)

NEVLP may be an efficient platform to maintain DCregs in an unstimulated state during organ storage. Since NEVLP maintains the liver in a physiological state and avoids the stress of cold storage, organ injury and inflammation are decreased after transplant[32], which may prevent DCreg transformation into mature APCs. Indeed, this occurs with another type of regulatory cell: *Regulatory T cells* (*Tregs)* isolated from donor organs after normothermic perfusion, are functional and capable of inhibiting recipient immune responses and prolonging allograft survival[44]. Yet, since NEVLP operates in a closed perfusion circuit, any inflammatory mediators that are released from the organ can accumulate[45,46] and potentially activate liver-resident DCregs to become pro-inflammatory. Our data is in accordance with these prior studies, as we have found that even though the liver is metabolically active and functioning during NEVLP, as seen by lactate clearance (**Fig. 3A**) and bile production (not shown), liver damage is also occurring evidenced by the release of hepatic enzymes (**Fig. 3B**). We

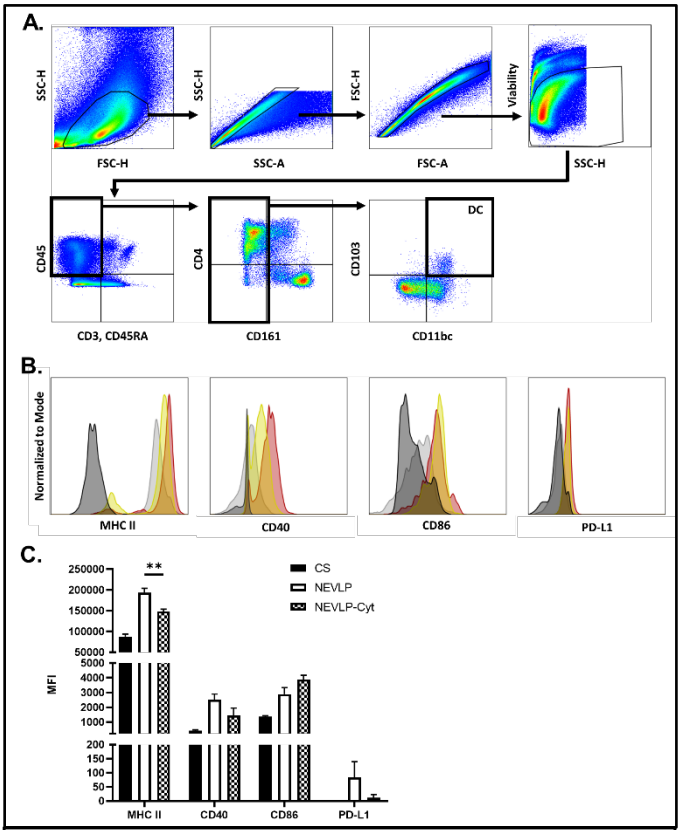

Figure 4. Cell surface phenotype characterization of liver-resident DCregs. A. Representative flow cytometry gating strategy for DCs ($CD4^+$, $11b/c^+$, $CD45^+$, $CD103^+$). Top panels select for viable single cells of the appropriate size, bottom panels exclude proteins not linked to rat DCs. **B.** Representative histograms of cell surface expression of co-inhibitory and co-stimulatory proteins to quantify DCregs. (Light Gray = Cold Storage, Red = NEVLP, Gold = NEVLP-Cytokines, Dark Grey = Fluorescence minus one. **C.** Median fluorescent intensity of DCreg markers in livers undergoing cold storage (n=3), NEVLP (n=5), and NEVLP-Cytokines (n=5). **p=0.004

conducted these standardized NEVLP experiments on randomly selected, 10-12-week-old, male and female rats to obtain robust, unbiased results. If DCregs can be maintained and expanded during NEVLP, these cells would be present in the liver itself at the time of organ transplant, allowing this therapy to be successfully used in all types of donors (including deceased donation). Prior studies have shown that anti-inflammatory cytokines (IL-10 and TGFβ) hold major promise for stimulating DCreg formation *in vitro*[20-22], and our own results confirm this. Furthermore, DCregs generated with IL-10 demonstrate a stable phenotype even under pro-inflammatory conditions[20]. Anti-inflammatory cytokines may be especially effective during NEVLP, considering that the unique liver microenvironment plays a role in the generation and maintenance of DCregs *in vivo*[28]. Thus, we attempted to counteract any pro-inflammatory signals produced as a result of liver damage during NEVLP by adding the anti-inflammatory cytokines IL-10 and TGFβ to maintain DCregs in an unstimulated, regulatory state. Our preliminary data show that the addition of IL-10 and TGFβ to the NEVLP circuit shows decreasing expression of MHC class II (p=0.004) and trends towards decreasing the co-stimulatory protein CD40 (p=0.11) on the surface of the DCregs (**Fig. 4**). However, another co-stimulatory protein, CD86, and PD-L1 expression are similar between NEVLP and NEVLP with anti-inflammatory cytokines. Even if the frequency of DCregs can be increased, their regulatory function and the mechanisms of regulation require investigation. Our working hypothesis is that efficiently expanded liver-resident DCregs during NEVLP can promote a regulatory environment for the organ after transplant.

Significance of the expected research contribution. In Aim 1, we will identify the dominant mechanisms by which liver-resident DCregs induce immunological regulation, and in Aim 2, we will establish the therapeutic benefit of anti-inflammatory cytokines on DCregs in rat livers undergoing NEVLP. The ability to decrease rejection of a transplanted liver could have an immediate effect on management of the recipients of these organs. This project is significant because for the first time, immune cells within the liver itself will be conditioned to decrease liver rejection by the recipient, prior to transplantation. Utilization of clinically available NEVLP technology and reagents could lead to immediate clinical application. The development of strategies to decrease rejection in an NEVLP liver model also holds potential for generalization of these strategies and application to other organs where normothermic perfusion is available, namely lung and kidney transplantation. This study of the immunological mechanisms by which DCregs promote organ acceptance will advance the field of transplant immunology and may be applicable to fields such as tumor immunotherapy.

B. INNOVATION

The proposed project is characterized by two major innovations: **1)** The therapeutic strategies represent novel approaches to alter the phenotype of liver-resident DCregs. No previous therapies have been developed to target DCregs within the organ prior to transplantation in order to decrease acute rejection rates after deceased donor liver transplantation. **2)** The method by which these therapies will be delivered represents a novel use of NEVLP. NEVLP holds great promise as a platform for delivering therapeutic interventions *ex vivo* because liver grafts retain physiologic activity. The concept of *ex vivo* cellular modification may provide an advantage over existing methodologies of *in vitro* DCreg expansion, as these cells will be expanded within the liver microenvironment. Expanding DCregs during NEVLP will also allow the use of this cellular therapy in the deceased donor setting, a scenario that is not possible with existing DCreg protocols. We anticipate that these

interventions will not only decrease the immune response of the recipient and decrease rejection of the liver, but also serve as proof-of-concept to optimize other solid organs prior to transplantation.

C. APPROACH

Overall Hypothesis. Expansion of liver-resident DCregs during NEVLP will promote a regulatory environment for the organ after transplant through the expression of co-inhibitory proteins and production of regulatory EVs.

Experimental model rigor and reproducibility. The rat experimental system will be used because all antibodies and cytokine detection supplies are commercially available (see Authentication, key biological resources), and it is an established, reproducible liver transplant model. We will use Lewis (LEW; $RT1^l$) and Brown Norway (BN; $RT1^n$) rats, which are completely allogeneic. We can detect cells of each strain using anti-RT1 antibodies. Based on the donor-recipient combination, BN to LEW *will not* generate liver rejection but LEW to BN *will* develop liver rejection; therefore, these strains will allow us to investigate properties of DCregs without other confounding biological variables[47,48].

Consideration of Relevant Biological Variables. Gender will be randomly assigned for both donor and recipient. Female and male BN rats will be used in these studies as transplant donors, and female and male LEW rats will be used as transplant recipients. Rats will be approximately 10-12 weeks of age at the time of transplantation, as this age provides an ideal size of liver and ability to tolerate liver transplantation.

Aim 1 – Determine the dominant regulatory mechanisms of liver-resident DCregs.

Objective and Rationale: Our objective is to determine the mechanisms of regulation by liver-resident DCregs. Our working hypothesis is that the primary mechanism by which liver-resident DCregs regulate the immune response is through their expression of co-inhibitory proteins and production of regulatory EVs. To test this hypothesis, we will use a model of rat orthotopic liver transplantation from LEW to BN, to determine if expanding liver-resident DCregs can delay or decrease rejection. We will then use a model of rat liver transplant that *does not reject* (BN into LEW) and assess whether tolerance in this model can be broken by the selective depletion of DCregs. The rationale for completing this aim is to understand how the regulatory function of liver-resident DCregs influences transplant outcomes and offer targets for therapeutic intervention.

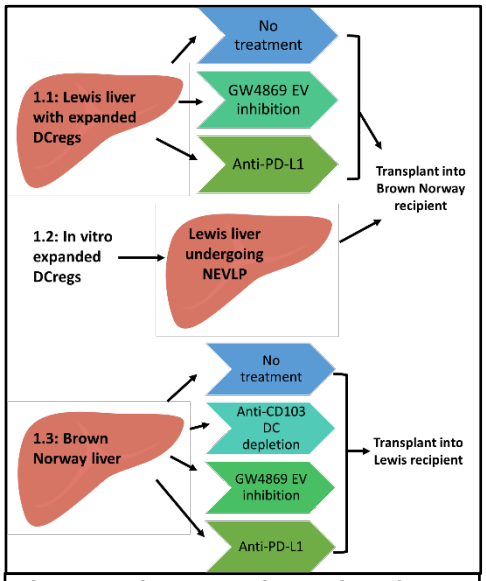

Figure 5. Aim 1 Experimental Design.

Aim 1.1: Determine the ability of DCreg expansion to decrease liver rejection. Liver transplantation from LEW to BN begin showing signs of acute liver rejection by Day 7 and are mostly rejected by Day 40[47]. We will determine if an increase in liver-resident DCregs can decrease the rejection seen in this model (**Figs. 5 and 6;** Dr. Samuel Poore; see letter of support). We will expand the number of DCregs in the LEW liver *in vivo* through a 10-day course of Fms-like tyrosine kinase 3-ligand (Flt3-L) injection and use these livers as donors. We have shown that Flt3-L injection can increase the number of DCregs (**Fig. 7**). After transplant, recipient rats will have peripheral blood collected weekly to assess for total bilirubin and aspartate transaminase liver enzyme release as markers of liver rejection. Blood will also be analyzed to determine the phenotypes of circulating lymphocytes such as T cells ($CD4^+$, $CD8^+$, and Treg), B cells, natural killer cells, macrophages and DCs by multiparameter flow cytometry. The distinction between donor and recipient cells will be made using antibodies towards RT1l (LEW) and RT1n (BN). In addition, characterization of EV cell of origin, size distribution, and concentration by Nanosight imaging will be conducted on the weekly samples. Nine recipients in each group (see power calculation below) will be used for long-term survival observations and will be euthanized upon clinical or laboratory evidence of rejection. Other recipients in each group will be euthanized at the pre-determined time points of 5, 10, and 20 days, to identify early rejection events post-transplantation. At the experimental endpoint, a portion of liver will be fixed and stained for assessment of the rejection activity index according to the Banff scoring schema by an independent histopathologist (Dr. Yongjun Liu; see letter of support). The remaining liver will be enzymatically digested with collagenase into a single cell suspension. The supernatant of this

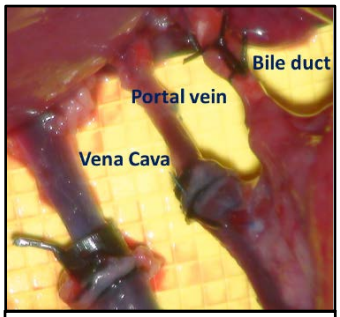

Figure 5. Successful rat liver transplant. Demonstration of the major vessel anastomosis required for rat liver transplantation.

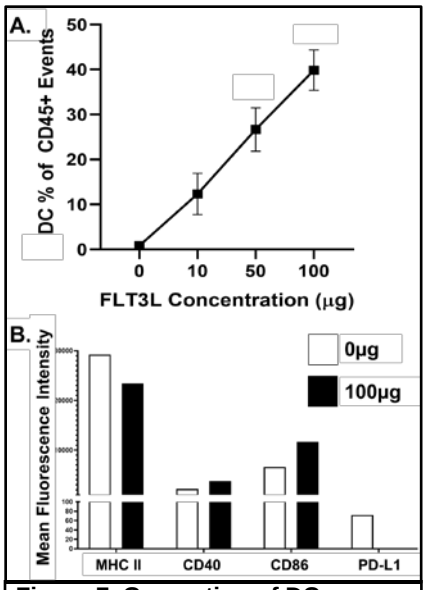

Figure 7. Generation of DCregs through Flt3-L. Lewis rats were injected for 10 days with Flt3-L. **A.** Dose-response curve to optimize DCreg frequency in the liver. **B.** MHC and co-stimulatory markers on DCs from control and Flt3-L injected animals demonstrating DCs remain in an unstimulated state. Gating strategy for DCregs was identical to that shown in Figure 4.

cell suspension will be used to measure markers of liver damage and inflammation (liver-type arginase 1, aspartate transaminase 1, α-glutathianone S-transferase, sorbitol dehydrogenase, and 5'-Nucleotidase) by a multiplex assay using Luminex technology. The cell suspension will then be used to quantify the lymphocytes present in the liver in each experimental group, with special attention focused on their inflammatory and regulatory phenotypes using multiparameter flow cytometry.

To determine the mechanisms of DCreg immune inhibition, wildtype and Flt3-L expanded DCreg rats will be treated with the neutral sphingomyelinase inhibitor, GW4869, to inhibit EV production by DCregs[49] one day prior to liver procurement and transplantation (**Fig. 5**). In a separate group, wildtype and Flt3-L expanded DCreg rats will be treated with an anti-PD-L1 antibody to block PD-L1$^+$ DCregs. Using the pre-determined time points of 5, 10 and 20 days, these experiments will allow the evaluation of the effects of EV production and PD-L1 expression on liver rejection.

Aim 1.2: Determine the ability of NEVLP to deliver DCregs directly to the liver to prevent rejection. In a separate group of experiments, liver-resident DCregs isolated from LEW rats that have undergone Flt3-L injection, will be further expanded *in vitro* for five days. These cultured DCregs will be labeled with Qtracker 705 (Invitrogen) and infused into wildtype LEW livers undergoing NEVLP. The number and distribution of the labeled DCregs in the liver will be determined by fluorescence microscopy to detect the tracking dye. After NEVLP, liver transplantation will be performed into BN recipients with outcome and rejection episodes monitored as above. We expect the infusion of DCregs into livers during NEVLP to decrease the immune response after transplantation.

Aim 1.3: Determine the role of DCreg depletion in liver transplant tolerance. In another cohort of animals, rat liver transplantation will be performed with BN rats as the donor and LEW rats as the recipients, a strain combination that *does not* reject the liver. We will measure the frequency and absolute number of liver-resident regulatory cells (DCregs and Tregs) in each rat strain to determine if strain variation in regulatory cell numbers account for differences in transplant rejection. Next, to assess the contribution of DCregs to the tolerance seen in this model, we will selectively deplete DCregs in the donor prior to liver transplantation using anti-CD103 (OX-62). If the DCreg depletion breaks tolerance, regulatory mechanisms will be investigated by treating donor animals with GW4869, to inhibit EV production, and/or anti-PD-L1 antibody to block PD-L1$^+$ DCregs, one day prior to liver procurement and transplantation. Liver transplant outcomes will be assessed as above. We expect the depletion of DCregs will lead to the rejection of these liver grafts.

Power Calculation and Statistical Analysis. Sample size calculations were performed assuming a difference in means between groups of 20% with a standard deviation of 15%. With an alpha value set at 0.05 and a study powered at 80%, we calculate that 9 animals would be needed per group. Multiplex Luminex assays will be performed in triplicate. For continuous variables, differences between groups will be analyzed by paired t-test if normally distributed or rank-sum test if non-normally distributed. Categorical variables will be analyzed by Fischer's exact test. A p-value <0.05 will be considered significant in reporting data.

Expected Results. We expect that increasing the number of liver-resident DCregs will prolong rat liver transplantation survival in the LEW to BN model. We anticipate that this increase in DCregs can be accomplished with both *in vitro* and *ex vivo* approaches. Furthermore, in these experiments, we will be able to deplete two major regulatory functions of DCregs, allowing us to determine the contributions of EV secretion and direct PD-L1 interactions on liver rejection. We also predict liver-resident DCregs are a major contributor to tolerance seen in the BN to LEW liver transplantation model. By selectively depleting these cells, we expect to break tolerance in this model and examine the contribution of regulatory EV production and PD-L1 expression.

Anticipated Pitfalls and Alternative Approaches. **1.** The tolerance in the BN to LEW model may not be solely dependent on DCregs. If this is the case, we will deplete passenger lymphocytes (macrophages, CD4$^+$ T cells, CD8$^+$ T cells, Tregs and B cells) within the donor liver in a systematic fashion to determine which cell(s) are required for tolerance in this model. If alternative cells are found, they will be targeted for expansion in the LEW to BN rejection model. **2.** DCregs may not be able to decrease rejection in the LEW to BN liver transplant

model. We will then look at expansion of Tregs, a regulatory cell type that is relevant in many transplantation tolerance models. We will analyze the frequency and phenotype of Tregs present in the donor liver and expand the frequency of these cells *in vivo* through the injection of anti-CD28 (clone JJ316)[50]. We will then assess the ability of expanded Tregs to decrease rejection after liver transplantation.

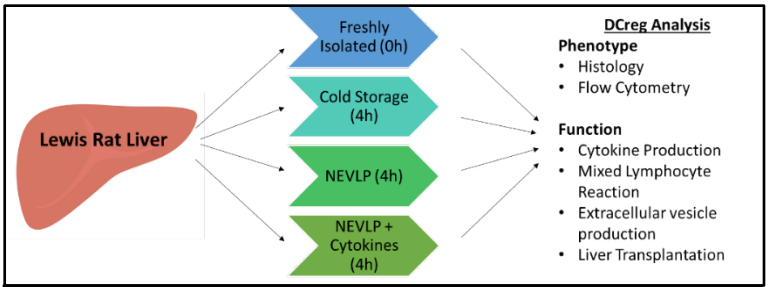

Figure 8. Aim 2.1 Experimental Design.

Aim 2 – Measure the impact of expanded liver-resident DCregs generated by combination cytokine therapy and NEVLP on liver graft rejection *in vitro* and *in vivo*.

Objective and Rationale: Increasing the number of functional DCregs in the liver may lead to decreased rejection rates and improved outcomes after liver transplantation. Therefore, our objective is to increase the number of DCregs in the rat liver using NEVLP combined with anti-inflammatory cytokine therapy. Our working hypothesis is that efficiently expanded liver-resident DCregs during NEVLP can promote a regulatory environment for the organ after transplant. To test this hypothesis, we will compare the number and function of DCregs in two experimental conditions: 1) NEVLP, and 2) NEVLP with anti-inflammatory cytokines. The rationale for completing this aim is that findings will allow us to determine the effects that therapeutic intervention during NEVLP has on liver-resident DCregs and ability to decrease rejection after transplantation.

Aim 2.1: Expansion of DCregs during NEVLP. Procured LEW rat livers will be placed on our NEVLP apparatus (as in Aim 1), with or without the addition of IL-10 and TGFβ cytokines (**Fig. 8**). A dosage of 20 ng/mL for both cytokines will be added to the perfusate, based on our protocols to expand DCregs *in vitro*[21,22]. Freshly isolated and cold stored livers will serve as controls. The perfusate will be sampled every hour to measure electrolytes, lactate, and markers of liver damage (as in aim 1.1; **Fig. 3**) and cytokine release (IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17A, IL-18, TNF-α, VEGF) by multiplex assays using Luminex technology. Hourly samples of the perfusate will also be used to assess the cell of origin, size distribution, and concentration of EVs by Nanosight imaging, as done previously[51] (**Fig. 9**). The function of these EVs will be assessed by their ability to supress responses in mixed cellular reactions. After 4-hours of perfusion, a portion of liver will then be fixed and stained for examination of liver architecture by an independent histopathologist (Dr. Yongjun Liu) using the Suzuki criteria (**Fig. 10**). The remaining liver will be enzymatically digested with collagenase into a single cell suspension. Using multiparameter flow cytometry, we will quantify the DCregs present in the liver in each experimental group (**Fig. 4**) and assess their functionality by measuring cytokine production using multiplex Luminex assay and mixed lymphocyte culture with allogenic BN responder cells (**Fig. 11**). We expect the DCregs isolated from NEVLP+cytokine treated livers to be higher in frequency when compared with NEVLP alone.

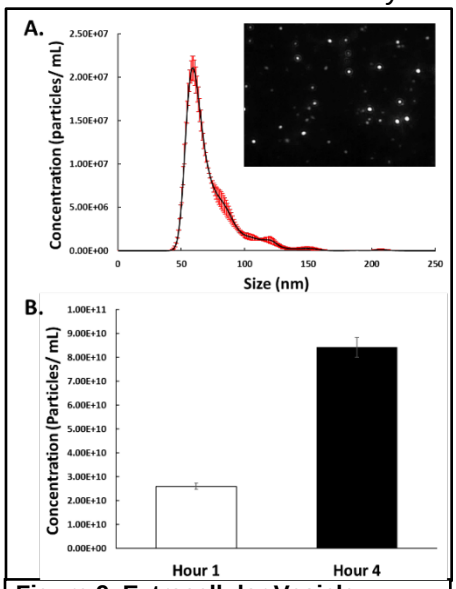

Figure 9. Extracellular Vesicle Characterization by Nanosight. During NEVLP, perfusate is collected and EVs are enriched by centrifugation and qEV column purification. **A.** Representative particle size distribution of hour 4 perfusate. **Insert** – Raw Nanosight image of EVs. **B.** Comparison of EV concentration during NEVLP. After 4 hours of perfusion, the average concentration of EVs is 3.8 times higher than hour 1. (n=7)

Aim 2.2: Rat Liver Transplant Model. Experimental perfused (with and without anti-inflammatory cytokines) and control non-perfused LEW rat livers will be transplanted into BN rat recipients. Recipients in each group will be used for long-term survival observations and will be euthanized upon clinical or laboratory evidence of rejection. Peripheral blood collection with cytokine and cellular analysis will be performed as in Aim 1. Other recipients in each group will be euthanized at the pre-determined time points of day 5, 10, and 20, to identify early rejection episodes. At the experimental endpoint, a portion of liver will be fixed and stained for assessment of the rejection activity index according to the Banff scoring schema by an independent histopathologist.

Power calculation and statistical analysis. Markers of liver damage and cytokines produced while undergoing NEVLP will be compared between all experimental groups. We will use an analysis of variance (ANOVA) performed at a significance level of 0.05. For power considerations, we focused on the comparison of 5'-nucleotidase measured at 4 hrs. between

the NEVLP and NEVLP + cytokines groups. Based on pilot data (**Fig. 3**), we estimate the mean 5'-nucleotidase value in the NEVLP group to be 2.05 ng/ml with a standard deviation of 0.73 ng/ml. We anticipate the mean of the NEVLP + cytokines group to be 50% smaller, or 1.025 ng/ml. Using a two-tailed, two-sample t-test at significance level 0.05, we would have at least 80% power for finding these differences as significant with 8 animals per group.

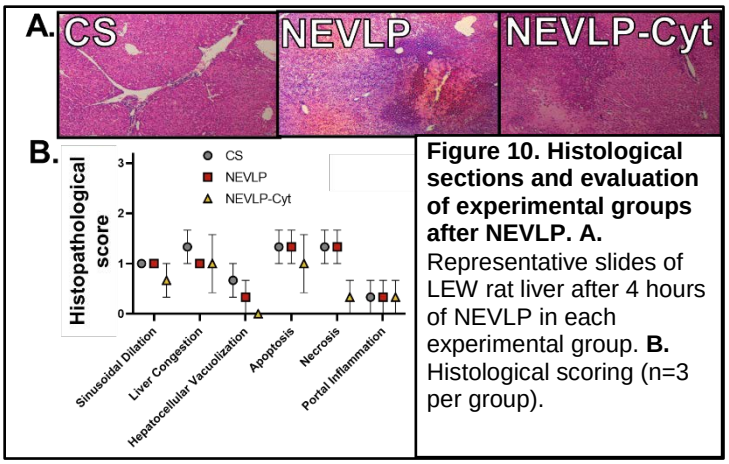

Figure 10. Histological sections and evaluation of experimental groups after NEVLP. A. Representative slides of LEW rat liver after 4 hours of NEVLP in each experimental group. **B.** Histological scoring (n=3 per group).

Expected Results. We predict the NEVLP+cytokine group will have similar histological findings to the NEVLP alone group, as we are not targeting pathways that affect ischemia-reperfusion pathways. However, we anticipate that there will be differences in DCreg numbers and function between the NEVLP and the NEVLP+cytokine groups. Using flow cytometry, we expect the DCregs isolated from NEVLP+cytokine treated livers to be higher in frequency and more resistant to activation when compared with NEVLP alone. In addition, using multiplex Luminex assays, we expect to find higher concentrations of anti-inflammatory cytokines produced by the liver in both the perfusate and the total liver after cellular isolation in the NEVLP+cytokine treated group. Similarly, we predict the livers treated with NEVLP+cytokines to produce EVs with the most suppressive function in mixed lymphocyte culture. Last, in our liver transplant model, we expect to find the least liver inflammation, immune cell infiltrate, and hepatocyte necrosis in the livers treated with NEVLP+cytokines when compared with any of the other experimental groups.

Anticipated Pitfalls and Alternative Approaches. **1.** The anti-inflammatory cytokine intervention during NEVLP is not specific for DCreg development. To investigate off-target cellular effects, we will quantify the numbers of lymphocytes including T cells ($CD4^+$, $CD8^+$, and Treg), B cells, and macrophages present in the liver after NEVLP, using newly designed flow cytometry panels. In addition, by evaluating a large number of cytokines released during NEVLP that are not associated with DCregs, we may be able to determine which cells are being affected by the intervention. **2.** Inability to expand the number of DCregs with anti-inflammatory cytokines: The cytokines used for differentiation will be initially dosed according to *in vitro* concentrations (20ng/mL); however, increased concentrations may be required given the relatively short exposure time during perfusion. Dose ranging studies will be performed to optimize cytokine effect during NEVLP. These dosing experiments will be conducted with rat livers while undergoing NEVLP and the anti-inflammatory cytokine effects measured by surface marker characterization of DCregs by flow cytometry. **3.** The amount of time the liver can be perfused during NEVLP may not be long enough to detect differences in the phenotype of immune cells present in the liver between experimental groups. Although CD8 T cells can complete a cell cycle in 2 hours[52], this cell division is antigen-dependent and may not be applicable to DCs. Therefore, the feasibility of running NEVLP with different cellular perfusate solutions to allow NEVLP for longer periods of time, will be investigated. However, the methods described above will be able to detect markers of cellular stress and inflammation on DCs since these proteins are upregulated quickly[53].

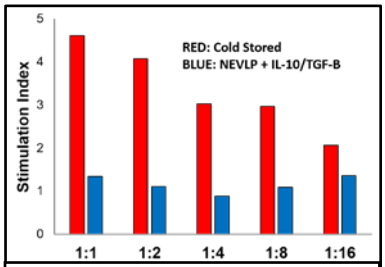

Figure 11. Mixed Lymphocyte Reaction. LEW rat liver-resident DCs were isolated after cold storage or NEVLP + anti-inflammatory cytokines and mixed with BN responder cells in various ratios. Cell proliferation was measured in a representative sample using flow cytometry with a proliferation dye.

Conclusion and Future Directions. Successful completion of the proposed work will establish a regulatory environment within the transplanted liver by increasing the number and function of resident DCregs to decrease rejection after transplant. These studies will generate mechanistic insight into how DCregs prevent liver rejection, such as through EV generation or PD-L1 expression. The resulting data will allow me to submit an R01 proposal by the end of Year 5 of the award, as the first step toward achieving my research career independence. The objective of the R01 will be to establish the safety and efficacy of these therapeutic strategies in large animal models and to improve the precision of therapeutic delivery to modify distinct cells within the liver. We will aim to upregulate the co-inhibitory molecule PD-L1 on DCregs to increase the regulatory capacity of these cells and decrease graft rejection. These approaches for organ immunomodulation during normothermic machine perfusion can be used in deceased liver donation, and potentially translated to other transplant types, such as kidney and lung.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

Format and Duration of Instruction
The University of Wisconsin (UW)-Madison strives to foster the highest scholarly and ethical standards among its students, faculty, and staff. As such, I will enroll in **Responsible Conduct of Research** courses approved by the Office of Research Integrity and accepted by the National Institutes of Health (NIH). The first course will be taken in the fall of 2021 and is offered through the UW-Madison. This course, Pharmacy 800 (Research Ethics, Scientific Integrity and the Responsible Conduct of Research), consists of 2.0 hours each week (Monday evenings) of face-to-face faculty instruction in principles and concepts of research ethics, and small group discussion of case studies (14 weeks, total of 28 hours). There is an additional 2-3 hours of assigned reading each week. Junior faculty and federally funded fellows often complete this course, as it covers NIH recommended topics.

Subject Matter
Formal instruction includes the following topics:

1. Animal welfare and safe laboratory practices
2. Collaborative science
3. Conflict of interest and commitment
4. Data acquisition, management, sharing and ownership
5. Protection of human subjects
6. Mentor/mentee responsibilities and relationships
7. Responsible authorship and publication and peer review
8. Peer review
9. Research misconduct
10. Societal and environmental impacts of scientific research

Course #/Name	Faculty	Credits/Frequency/Format/Duration	Subject Matter
Pharmacy 800: Research Ethics, Scientific Integrity and the Responsible Conduct of Research	Steven M. Swanson	2 credits, 2hrs/wk x 14 weeks, Formal course with instruction, review and discussion (Year 1)	All above topics
Introduction to the Principles and Practice of Clinical Research (NIH webinar)	Numerous	Non-credit, yearly, on-line series throughout the entire award period	Ethical, legal, and regulatory issues in clinical human subjects research Role of Institutional Review Boards Regulatory requirements Human subjects protection Good Clinical Practice

Consistent with UW-Madison policy, I have also completed and maintain current certification in the following courses related to the responsible conduct of research:

- Health Insurance Portability and Accountability Act (HIPAA) UW Privacy Training: 1/2/20
- Creating a Respectful and Welcoming Learning Environment: 3/30/19
- Conflict of Interest Training: 11/5/18

I will maintain these certifications throughout the duration of the award.

Faculty Participation
To complement the face-to-face instruction and real time discussion, I will discuss the Responsible Conduct in Research training with my mentors and advisors during the formal semi-annual meetings. My primary mentor, Dr. Christian Capitini, will ensure that I continue to update my knowledge in the responsible conduct of research and research ethics, and we will discuss many of the principles at our monthly directed learning sessions.

Frequency of Instruction
This training plan recognizes the need for ongoing and recurring training in the responsible conduct of research. Details regarding frequency are outlined in the table above.

INSTITUTIONAL ENVIRONMENT

The **University of Wisconsin-Madison (UW)** has long been recognized for excellence in education and research. UW offers one of the largest graduate programs in the United States (US) with 162 master's degree programs and 125 doctoral degree programs. According to the National Science Foundation 2018 rankings, UW-Madison placed 8th in the nation for research and development expenditures. The university is a research powerhouse, with more than $[redacted] billion in annual expenditures for research, about half of which comes from federal awards. UW-Madison consistently ranks in the top 10 amidst public institutions in the nation for federal funding and research expenditures. Twenty Nobel Prizes and 38 Pulitzer Prizes have been awarded to UW faculty or alumni. The **UW School of Medicine and Public Health (UWSMPH)** consistently ranks among *U.S. News & World Report's* best medical schools for research, and has the largest research commitment of any school or college on the UW-Madison campus, receiving more than $[redacted] million in extramural research support in 2017-18. Medical school facilities such as the Wisconsin Institutes for Medical Research (WIMR) ensure that the UW will remain at the forefront of basic, clinical, translational and public health research. Over 30 research centers and institutes are connected to the UWSMPH, including notable federally funded centers such as the UW Institute for Clinical and Translational Research (U54 Clinical and Translational Science Award [CTSA]). The abundance of resources and investigators involved in collaborative research make UW a rich environment in which to conduct this research.

The **UW Department of Surgery (UWDOS)** has over 150 full-time faculty members and its portfolio of research funding exceeds $[redacted] million annually, with approximately two-thirds of this support coming from the National Institutes of Health. In 2018, the department ranked 8th in NIH funding in Departments of Surgery across the United States. The Department of Surgery has over 20,000 square feet of research space within the UW hospital complex. The department employs 7 full-time biostatisticians and programmers who consult with faculty on protocol design and planning, data collection, data analysis and interpretation, publication and presentation of data, file manipulation and maintenance, as well as grant and manuscript review. The UWDOS Accounting Office staffs 5 full-time specialists who provide post-award grant financial services; the Research Office affords assistance in grant preparation and fulfilling requirements for assurances and certifications. Nine full-time information technologists (IT) maintain the extensive IT network.

The **UW Institute for Clinical and Translational Research (ICTR)**, funded by an NIH CTSA, further strengthens the institutional culture for research training. ICTR's educational offerings target scientific writing, mentoring, clinical research essentials, qualitative research, dissemination and implementation, and transition to independent investigation. Dr Al-Adra has participated in these ICTR programs and will continue to be involved throughout the award period.

Dixon Kaufman, MD, PhD, joined the UWDOS in 2011 as the Ray D. Owen Professor and Chair, and is the Director of the Transplantation Service Line. Dr. Kaufman is a world-renowned surgeon-scientist with extensive experience managing highly productive research laboratories and providing administrative leadership and mentorship. Throughout his career, Dr. Kaufman has demonstrated special dedication to producing future generations of academic surgeons. Under Dr Kaufman's leadership, 3 Division of Transplantation faculty have held K-awards (**Robert Redfield, David Foley and Josh Mezrich**), demonstrating how his appointment has enhanced the department's focus on developing the next generation of surgical leaders who will not only provide excellent clinical care, but also produce important scientific innovations.

In addition to the applicant's mentorship team, **Drs. John Odorico and Luis Fernandez** are NIH-funded investigators in the Division of Transplantation who will support and collaborate with Dr. Al-Adra. Their labs are purposefully located adjacent to Dr. Al-Adra's lab. This close proximity will facilitate sharing of resources and equipment to enhance the research proposed in this application.

The UW, UWDOS, and the Division of Transplantation are committed to supporting Dr. Al-Adra's success. Dr. Al-Adra will be given protected time, which limits his clinical practice, teaching, and administrative duties so that he can devote 75% of his effort to the development of his research program. In addition to protected time, Dr. Al-Adra has been provided with $[redacted] in start-up funds over the first three years of his appointment to support his research program. With these funds, he has hired a full time Assistant Researcher to help conduct experiments and advance his research.

In summary, Dr. Al-Adra will have all the physical and personnel resources to complete and support the research and career development proposed in this grant and to write future applications as he transitions to becoming an independent investigator.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	❍ Yes ● No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

VERTEBRATE ANIMALS

The UW veterinary care program is maintained in AALAC-accredited institutional facilities under supervision of full-time board-certified veterinarians. All veterinary care is established, approved, and monitored by Lab Animal Resources staff of the University of Wisconsin through the Research Animal Resources Committee. Several full-time veterinarians are available to provide care and consultation of animals used in the study. The animals will be housed at the animal care facility of the Wisconsin Institutes for Medical Research. This facility is in compliance with AALAC and NIH guidelines.

1*. Description of Procedures.*
Donor hepatectomy. All surgeries will be performed under cone mask anesthesia with continuous 5% isoflurane for induction, and 2–3% isoflurane during the procedure, with 2 L/min oxygen flow. The abdomen will be opened by a midline and transverse incision. A stent fashioned from a 24 gauge angiocatheter will be inserted into the common bile duct and secured. The proper hepatic artery and gastrosplenic and duodeno-pancreatic branches of the portal vein will be isolated and divided. Heparin (1 IU/g bodyweight) will be injected through the infrahepatic vena cava with a 30 gauge needle. Five minutes later, the portal vein will be cannulated with a perfusion cannula and the liver flushed with 40 mL of cold University of Wisconsin preservation solution. The liver then will be explanted, weighed, and stored on ice until subsequent use on the normothermic machine or transplanted.

Rat orthotopic liver transplantation. After induction of general anesthesia with inhaled anesthetic as above, a midline laparotomy will be performed. The native liver will be mobilized from its attachments. In the porta hepatis, the common bile duct, portal vein, and hepatic artery will be ligated and divided close to the native liver to preserve length. The infrahepatic and suprahepatic vena cava will be clamped and divided close to the liver, and the native hepatectomy will be completed. The donor liver then will be brought to the operative field for implantation. First, the suprahepatic IVC anastomosis will be hand sewn with 8-0 prolene suture. Following that, the infrahepatic IVC and portal vein anastomoses will be completed by inserting the vascular cuffs into the recipient vessels and securing with ties. Reperfusion of the transplanted liver then will occur by removing the vascular clamps and restoring blood flow to the graft. Finally, the bile duct anastomosis will be performed by placing a 24 gauge stent between the donor and recipient bile duct and securing with ties. No hepatic arterial anastomosis will be performed in this model. The abdomen will be closed with sutures and the animal allowed to recover on a heated pad.

Vascular cuffs made from polyethylene tubing will be placed in the infrahepatic vena cava (3mm outer diameter) and portal vein (2.08 mm outer diameter) to facilitate anastomosis in the recipient.

2. *Justification*:
Animal Species: *Rat* (*Rattus norvegicus)*. Rat liver transplantation is a well-established and reproducible pre-clinical model for investigation of liver transplant biology and testing novel therapies to improve graft function. The rat strain choices have been selected because non-arterialized orthotopic liver transplantation from Lewis (RT1l) to Brown Norway (BN; RT1n) rats, without immunosuppression, begin showing signs of acute T cell- and antibody-mediated rejection by Day 7 and are mostly rejected by Day 100. The rat is an ideal model for the proposed work due to similarity with human biology, low cost, and the experience our laboratory has developed in this field, especially in the area of normothermic liver perfusion. The strategies proposed to modify liver cells during normothermic perfusion have already been demonstrated *in vitro*, but are not developed enough to justify experiments in larger pre-clinical models (porcine, non-human primate) or human transplantation. Use of animals is necessary as computational analysis, at this time, cannot replicate the all biological variables involved in the liver microenvironment and cellular response to transplantation.

Consideration of Relevant Biological Variables: Gender will be randomly assigned for both donor and recipient. Female and male Lewis rats will be used in these studies as transplant donors and female and male Brown Norway rats will be used as transplant recipients. Rats will be approximately 10-12 weeks of age at the time of transplantation, as this age provides an ideal size of liver and ability to tolerate liver transplantation.

Total number of animals: Our power calculations indicate that 9 animals will be require for each experimental group in aim 1, and 9 animals for each experimental group in aim 2. Therefore, we will require 350 animals to

complete these experiments. Assuming a 10% technical failure rate in the NEVLP and liver transplantation studies, a total of 400 animals will be required.

3. *Procedures to minimize pain and discomfort:* Transplantation surgeries will be performed under inhalational isoflurane anesthesia in adult rats in the Wisconsin Institutes for Medical Research animal care surgical suites. Pre- and post-transplantation surgery, animals will be given buprenorphine as analgesic. Meloxicam may be given 24 hours post-surgery if needed. Animals will be inspected daily for signs of discomfort or distress. If overt disability or illness is observed, animals will be euthanized.

4. *Euthanasia*: Animals will be euthanized by CO2 inhalation, as is the approved method of the American Veterinary Medical Association Panel on Euthanasia.

RESEARCH USING SELECT AGENTS

This proposal does not use Select Agents for research.

RESOURCE SHARING PLAN

The results and accomplishments of the activities within this application will be made available to the research community and to the public at large. Sharing of data generated by this project with the community of scientists interested in transplant immunology is an essential part of the scientific process and necessary to avoid unnecessary duplication of research. In addition, we will welcome collaboration with others studying similar scientific questions in this rapidly moving discipline.

Our sharing plan includes the following:

Presentations at national scientific meetings. From the proposal, it is expected that approximately 3-4 presentations at national meetings will be given. The primary meeting targeted by our research group is the American Transplant Congress, the largest annual transplant meeting in the United States. This meeting highlights the latest developments in pre-clinical and clinical transplant related research. As outlined in the training plan, I will plan to attend and present our work at this meeting annually.

Intramural forums. As a prominent research institution, the University of Wisconsin has many opportunities on campus for researchers to share their ongoing work. I am an active participant in the Department of Surgery Research in Progress Seminars series, which is a weekly presentation given by PIs and collaborators working in the Department of Surgery. I will continue to present at this forum twice a year. In addition, the Department of Surgery has an annual Research Day where postdoctoral fellows and faculty are given the opportunity to present both oral and poster presentations. Our group will submit abstracts to this local meeting yearly.

Publication in peer reviewed journals. Most data generated by this proposal will be reported in the form of peer-reviewed journal articles. The PI plans to generate 2-3 manuscripts from the data generated by the proposal. Final accepted manuscripts from data acquired under this proposal will be deposited into PubMed Central in compliance with the NIH Public Access Policy. To aid in result dissemination, options for open access of publications will be chosen.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES

To ensure highest quality science, only biological and chemical resources from authentic suppliers will be used in the proposed research. These suppliers validate and guarantee their products for scientific use. The suppliers listed below have been used for the purchase of these key biological and chemical resources.

Animals: Inbred Brown Norway and Lewis rats will be purchased from the [redacted].

Authentication: Once in our lab, the rat major histocompatibility phenotype (RT1) will be confirmed by tail vein bleed and flow cytometry with appropriate antibodies. To control for variation in microbiome, rats within the same experiment will be co-housed. To control for variation in environment, rats will be housed in standardized facilities and supplied with identical day/night lighting, food, and water supply.

Cell culture media: All cell culture media will be purchased mainly from the following suppliers:
[redacted] - Cat# [redacted])
[redacted] (Corning - Cat# [redacted])
[redacted] - Cat# [redacted])

Authentication: Media will be tested for pH balance and sterility prior to use, using in lab pH meters and culture plates.

Enzymes:
[redacted] Cat# [redacted])

Authentication: Collagenase activity can vary between lots. Therefore, collagenase will be tested on a per-lot basis by using a Collagenase Activity Test ([redacted], Cat# [redacted]) to quantify enzymatic activity.

Chemicals:
[redacted] - Cat# [redacted]
[redacted] - Cat# [redacted])
[redacted] – Cat# [redacted])
[redacted] - Cat# [redacted])
[redacted] – Cat# [redacted])

Authentication: The chemicals listed above are guaranteed endotoxin-free, pure, and sterile from their suppliers. The chemicals listed are added to culture media and will be tested for sterility upon mixing with the liquid media. Through collaboration with the [redacted] at the [redacted], we have access to a High performance Liquid Chromatography machine where the media supplements will be tested for purity.

Antibodies:
Various antibodies against cell surface proteins for immune cell detection with flow cytometry will be purchased from commercial vendors who can provide documentation of the antibody's purity and structure.

Authentication: Antibodies will be tested for lot variability and fluorescent intensity by performing titration studies on spleen and lymph node cells using flow cytometry.

Cytokines:
[redacted] - Cat# [redacted])
[redacted] - Cat# [redacted])

Authentication: [redacted] will be purchased from commercial vendors who can provide documentation of the cytokines purity and activity. [redacted] activities will be measured by ability to stimulate or inhibit cell growth in proliferation assays.