CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Autoreactive IgG as biomarkers of transplantation rejection

Name, degree, title, institution, and contact information including the email address of each PI

Anita S Chong, PhD, Professor, Department of Surgery, The University of Chicago, achong@bsd.uchicago.edu
Marcus R Clark, M.D., Professor, Department of Medicine, The University of Chicago, mclark@bsd.uchicago.edu

Anat R Tambur, DMD, PhD, D[ABHI], Research Professor and Director, Transplant Immunology Laboratory, Northwestern University, a-tambur@northwestern.edu
Amishi Desai, D.O., Associate Professor, Department of Medicine, Northwestern University, amishi.desai@nm.org

Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☒	**NO** ☐
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

One-paragraph lay-language summary of project

End-stage renal disease can be treated with kidney transplantation, however, despite availability of potent pharmacological immunosuppression, rejection especially when mediated by antibodies (AMR) continues to limit the longevity of transplanted kidneys. Clinical diagnosis of AMR depends on histological features in biopsies, and detection of antibodies directed at highly polymorphic human leukocyte antigens (HLA). However, >50% of AMR biopsies are not associated with detectable donor HLA-specific antibodies (DSA), suggesting that non-HLA antibodies are mediating AMR. The Clark-Chong labs reported that plasma cells accumulating in AMR biopsies produced autoantibodies that recognize nuclear autoantigens, and that autoreactive antibodies are produced during kidney allograft rejection in mice. This project will test the hypotheses that (i) antibodies recognizing non-HLA *autoantigens* are produced by patients during kidney allograft rejection; and (ii) because *autoantibodies* predominantly recognize intracellular antigens, they induce a phenotype of AMR that is distinguishable from rejection mediated by antibodies recognizing membrane-bound HLA antigens.

One-paragraph discussion of what makes this project high-risk/high-reward

The repertoire of non-HLA antigens released by damaged cells during rejection is likely to be extensive. Current testing methods using antigen arrays are limited by number of antigens on the array and high cost. **Aim 1 of this project is high-risk because we are proposing to screen for autoantibodies recognizing intracellular antigens in fixed cells, without actually identifying the autoantigens.** In Aim 2, we will test the novel hypothesis that rejection associated with autoreactive antibodies are immunologically more complex that DSA-mediated rejection. **Aim 2 is high risk because of the novelty of the hypothesis, and our proposed use of cutting-edge highly multiplexed immunofluorescence imaging to detail the immune architecture of rejecting biopsies from transplant recipients with autoantibodies or DSA. Analytical approaches to deconvolute the images and identify immunological neighborhoods in the biopsies are being developed in the Clark lab.** If this project generates data supporting the hypotheses that non-HLA autoantibodies are biomarkers and mediators of allograft injury, the project will be clinically and scientifically impactful. This study is not yet competitive for NIH funding. A CBC Catalyst Award will allow us to generate critical data, and solidify our collaboration between the UChicago research and Northwestern clinical teams.

Description of proposed research

Antibody-mediated rejection (AMR) is a major cause of late kidney allograft failure[1]. In clinical practice, up to ~50% of biopsies meeting the histological criteria for ABMR do not have detectable donor-specific antibodies (DSA), suggesting a role of non-HLA antibodies[2-7]. Non-HLA antibodies can be divided into two main groups: (i) donor-reactive antibodies that recognize polymorphic antigens differing between the donor and recipient, and (ii) autoreactive antibodies that recognize non-polymorphic *self-antigens* expressed by all donor grafts, and which may be expressed in an organ-specific manner [8-13]. Antigen-specific assays are required to detect Group (i) antibodies, whereas autoreactive antibodies recognizing non-polymorphic antigens can be detected by protein or peptide arrays **or** by a cell based assay. Protein or peptide arrays provide exquisite specificity, but they display a limited repertoire of antigens and are very expensive (~$5000/array), thus precluding large cohort studies. In contrast, cell-based assays allow for a large array of non-HLA antibodies to be tested in a single assay. Furthermore, they are relatively inexpensive thus permitting the conduct of large cohort studies, which are necessary to provide robust correlative clinical data.

We repurposed the clinical HEp-2 immunofluorescence kit (Biorad), designed to test for autoantibodies in the setting of autoimmunity, and used this assay to show that B and plasma cells infiltrating kidney grafts produced antibodies that were autoreactive[14]. Furthermore, we used this assay to show that autoantibodies are detected in the sera of mice undergoing acute or chronic kidney allograft rejection, but not in non-rejecting grafts that are genetically matched to the donor

(**Fig 1**). Importantly, autoantibodies recognizing antigens located in the nucleus and nucleolus (compared to the cytoplasm) increased strongly during chronic rejection. These observations underscore the utility of segmenting autoantibody specificity.

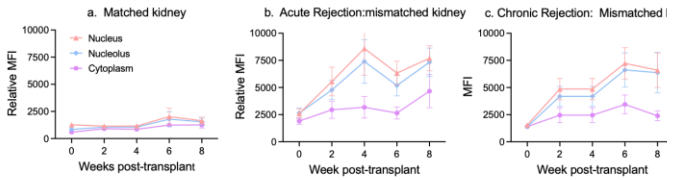

Fig 1. Quantification autoantibodies recognizing antigens located in the nucleus, nucleolus or cytoplasm of HEp2 cells on the indicated days post-transplant (mice) (a) Recipient-matched kidneys, (b) mismatched kidneys undergoing acute rejection, or (c) mismatched kidneys undergoing chronic rejection (treated with sub-optimal dose of clinically used anti-rejection therapy, CTLA-4Ig). Mean fluorescence intensity (MFI) of autoantibody binding was analyzed by CellProfiler software.

The overall project hypothesis is that *autoreactive B cells infiltrating kidney allografts differentiate into antibody-secreting plasma cells upon encounter with autoantigens and stimulation by pro-inflammatory signals within rejecting graft*[14,15]. *In contrast, DSA are produced by B cells in the spleen and lymph nodes.* In Aim 1, we will test the hypothesis these autoantibodies are detectable in the peripheral blood and can serve as a non-invasive biomarker of rejection. In Aim 2, we will test the hypothesis that rejection biopsies associated with autoantibodies are immunologically more complex than anti-HLA antibody-mediated rejection biopsies (**Fig 2**).

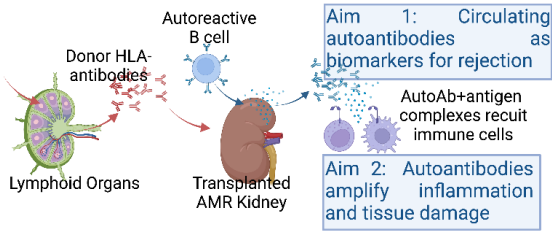

Fig 2. Pictorial summary of project Aims.

Aim 1, will test the hypothesis that autoantibodies can serve as a biomarker of rejection.

Study design is summarized in **Fig 3**. We will test already collected serial serum samples from 200 kidney transplant recipients experiencing rejection and 200 with no rejection. All recipients will have 2 baseline serum samples: pre-transplant and ~1 year post-transplant with *no* rejection. The 1 year time point is selected because the NW program performs protocol biopsies that allow the absence of rejection to be confirmed by pathology. Additional criteria for no rejection are serum creatine (sCr) levels <1.2 mg/dl and absence of DSA.

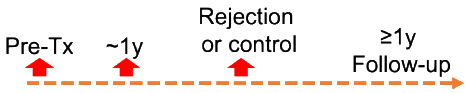

Fig 3. Timeline for test sera from kidney transplant recipients that experienced rejection or non-rejection control (red arrows: serum sample). Patients with ≥ 1 year follow-up after last serum collection will be selected.

The third time point will be sera collected at time of rejection diagnosis, and for the no rejection controls, sera will be collected at matched times post-transplant (**Fig 2**). Rejection is defined by pathology to include antibody-mediated, T cell-mediated or mixed rejection, and/or sCr >2.0 mg/dl. Only rejecting recipients that are negative for DSA will be included in this study to prevent their confounding the interpretation of the cellular assays. Recipients with >1 year follow-up after the last test serum will be included in this study, to allow for the assessment of graft outcomes.

In total, we will test 1200 serum samples, using the HEp2 immunofluorescence assay. We will quantify relative antibody binding to the nucleolus, nucleus and cytoplasm, which will be segmented using anti-Ki67 IgG, Hoechst stain, and anti-enolase1 IgG, respectively. Serum antibody staining will be detected with fluorochrome (AF647)-conjugated anti-human IgG, and images captured by confocal microscopy (Stellaris8). The relative mean fluorescence intensity (MFI) of anti-human IgG staining will be quantified by CellProfiler image analysis software. This analytical pipeline has already been established in the Chong-Clark labs (Fig 2). Statistical analysis integrating the lab and clinical data will be conducted in consultation with the UChicago Biostatistics Core.

To address the concern that HEp2 cells are epithelial tumor cells and that we may miss kidney-specific autoantigens, we propose to develop a similar immunofluorescence assay using a human kidney proximal epithelial cell line, HK-2 (ATCC). Initially, serum samples from rejecting patients will be tested to determine if the kidney epithelial cell assay improves the sensitivity and specificity of autoantibody detection, and strengthens the correlation between autoantibody and rejection.

Criteria of success. If our study demonstrates that autoantibodies are statistically more likely to be detected in kidney transplant recipients experiencing graft rejection, in future experiments we will test if this assay is *predictive* of graft failure. An assay with predictive ability will be more clinically useful. Furthermore, if the kidney epithelial cell assay is superior over the HEp2 cell assay, future experiments will use this cell assay. Finally, if autoantibodies recognizing nuclear or nucleolar antigens are most specific and sensitive to rejection, it will justify the immunofluorescence approach that we are using.

Aim 2, we will test the hypothesis that autoantibodies induce a phenotype of antibody-mediated rejection that is more immunologically complex compared to rejection mediated by anti-donor HLA antibodies. The rationale for this hypothesis is: (i) Circulating anti-HLA antibodies bind to predominantly to and induce damage to graft endothelial cells; in contrast, autoantibodies recognize intracellular autoantigens released by damaged graft cells (**Fig 2**). Consequently, autoantibodies can to bind to any area in the graft where there are damaged cells, and they induce further inflammation and cell damage. Thus, the impact of autoantibodies may be more widespread. (ii) Intrarenal B cells differentiate into antibody-secreting plasma cells upon encountering autoantigens in the graft, whereas anti-donor HLA antibodies are produced by B cells in the spleen/lymph nodes. Thus we anticipate the presence of productive T:B interactions resulting in the generation of antibody-secreting cells in biopsies from patients with circulating autoantibodies.

We propose to analyze 10 kidney biopsies diagnosed with classical AMR with DSA, and 10 kidney biopsies diagnosed with AMR with no DSA but with autoantibodies, identified from Aim 1. We will use highly multiplex immunofluorescence to interrogate the biopsies: the CODEX microfluidic system coupled with high-dimensional confocal microscopy, and an analytic suite that has been successfully applied to analyze diseased human tissue samples with outstanding sensitivity and specificity [14,16-18]. This approach is being optimized by the Clark lab, and can simultaneously detect 43 cell-specific and activation makers for T, B, NK, plasma & dendritic cells, monocytes, macrophages, neutrophils, granzyme, cytokines, iNOS, and collagen. The analytic suite is currently being optimized to handle multiplexed CODEX images. This high-resolution approach provides a compelling opportunity to test if *distinct* immunological cell networks characterize AMR associated with anti-HLA versus autoantibodies.

Criteria of success. Completion of Aim 2 should lead to *improved understanding of the mechanisms of anti-HLA-mediated rejection, versus autoantibody-mediated rejection*. These findings will prompt future experiments in mouse models to detail the mechanisms underlying the pathogenicity of autoantibodies, and to define therapies that prevent autoantibody production.

Nature of inter-institutional collaboration and how the success of the Catalyst project requires the expertise of each PI. The Northwestern team comprises Dr. Anat Tambur, Director of the HLA laboratory and Dr. Amishi Desai, a transplant nephrologist. They will identify patients, serum samples and biopsies, and participate in interpreting the laboratory data and patient transplant outcome data. The UChicago team comprising Dr. Anita Chong, a transplant immunologist, and Dr. Marcus Clark, basic and translational immunologist, have been working together for >4 years. Their labs have develop assays for quantifying autoantibodies, and the multiplex immunofluorescence and image analysis pipeline. Thus, each investigator brings essential and unique skill sets to this project.

Criteria for measuring success of the proposed project. Criteria for project success include validated assays for quantifying autoantibodies, and defining if autoantibodies can serve as a biomarker of graft rejection. In addition, our studies could lead to improved diagnosis of antibody-mediated rejection, and insights into the mechanism of rejection. We anticipate this study to result in at least 2 publications.

Long-term funding plan for continuation beyond the Catalyst Award including funding institution that will be solicited, grant category (i.e. R01, P series, U01) and tentative timeline. Submitting an R01 or a Program Project to NIH within 1 year of completion of the project.

Relevant Cited Scientific References

1 Loupy, A. & Lefaucheur, C. Antibody-Mediated Rejection of Solid-Organ Allografts. *N Engl J Med* **379**, 1150-1160, doi:10.1056/NEJMra1802677 (2018).

2 Lebraud, E., Eloudzeri, M., Rabant, M., Lamarthee, B. & Anglicheau, D. Microvascular Inflammation of the Renal Allograft: A Reappraisal of the Underlying Mechanisms. *Front Immunol* **13**, 864730, doi:10.3389/fimmu.2022.864730 (2022).

3 Sicard, A. *et al.* Outcome of kidney transplantations performed with preformed donor-specific antibodies of unknown etiology. *Am J Transplant* **14**, 193-201, doi:10.1111/ajt.12512 (2014).

4 Senev, A. *et al.* Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: Clinical presentation and implications for outcome. *Am J Transplant* **19**, 763-780, doi:10.1111/ajt.15074 (2019).

5 Anglicheau, D., Delville, M. & Lamarthee, B. Non anti-HLA antibodies and acute rejection: A critical viewpoint. *Nephrol Ther* **15 Suppl 1**, S53-S59, doi:10.1016/j.nephro.2019.03.003 (2019).

6 Delville, M. *et al.* Early Acute Microvascular Kidney Transplant Rejection in the Absence of Anti-HLA Antibodies Is Associated with Preformed IgG Antibodies against Diverse Glomerular Endothelial Cell Antigens. *J Am Soc Nephrol* **30**, 692-709, doi:10.1681/ASN.2018080868 (2019).

7 Jackson, A. M., Delville, M., Lamarthee, B. & Anglicheau, D. Sensitization to endothelial cell antigens: Unraveling the cause or effect paradox. *Hum Immunol* **80**, 614-620, doi:10.1016/j.humimm.2019.04.014 (2019).

8 Li, L. *et al.* Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and "antibodyome" measures. *Proc Natl Acad Sci U S A* **106**, 4148-4153, doi:10.1073/pnas.0900563106 (2009).

9 Pineda, S. *et al.* Novel Non-Histocompatibility Antigen Mismatched Variants Improve the Ability to Predict Antibody-Mediated Rejection Risk in Kidney Transplant. *Front Immunol* **8**, 1687, doi:10.3389/fimmu.2017.01687 (2017).

10 Sigdel, T. K. *et al.* Non-HLA antibodies to immunogenic epitopes predict the evolution of chronic renal allograft injury. *J Am Soc Nephrol* **23**, 750-763, doi:10.1681/ASN.2011060596 (2012).

11 Reindl-Schwaighofer, R. *et al.* Novel insights into non-HLA alloimmunity in kidney transplantation. *Transpl Int* **33**, 5-17, doi:10.1111/tri.13546 (2020).

12 Reindl-Schwaighofer, R. *et al.* Contribution of non-HLA incompatibility between donor and recipient to kidney allograft survival: genome-wide analysis in a prospective cohort. *Lancet* **393**, 910-917, doi:10.1016/S0140-6736(18)32473-5 (2019).

13 Lamarthee, B. *et al.* CRISPR/Cas9-Engineered HLA-Deleted Glomerular Endothelial Cells as a Tool to Predict Pathogenic Non-HLA Antibodies in Kidney Transplant Recipients. *J Am Soc Nephrol* **32**, 3231-3251, doi:10.1681/ASN.2021050689 (2021).

14 Asano, Y. *et al.* Innate-like self-reactive B cells infiltrate human renal allografts during transplant rejection. *Nat Commun* **12**, 4372, doi:10.1038/s41467-021-24615-6 (2021).

15 Liarski, V. M. *et al.* Cell distance mapping identifies functional T follicular helper cells in inflamed human renal tissue. *Sci Transl Med* **6**, 230ra246, doi:10.1126/scitranslmed.3008146 (2014).

16 Durkee, M. S. *et al.* Quantifying the effects of biopsy fixation and staining panel design on automatic instance segmentation of immune cells in human lupus nephritis. *J Biomed Opt* **26**, doi:10.1117/1.JBO.26.2.022910 (2021).

17 Durkee, M. S., Abraham, R., Clark, M. R. & Giger, M. L. Artificial Intelligence and Cellular Segmentation in Tissue Microscopy Images. *Am J Pathol* **191**, 1693-1701, doi:10.1016/j.ajpath.2021.05.022 (2021).

18 Liarski, V. M. *et al.* Quantifying in situ adaptive immune cell cognate interactions in humans. *Nat Immunol* **20**, 503-513, doi:10.1038/s41590-019-0315-3 (2019).

Program Director/Principal Investigator (Last, First, Middle): Chong, Anita & Clark, Marcus

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 06/01/2023 THROUGH 05/31/2025

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

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Anita Chong	PD/PI	0.13			265,000	2,812	712	3,524
Marcus Clark	Co-PI	0.10			387,997	3,220	815	4,035
Ismail Sayin	Pos-Doc	3.00			61,323	15,331	3,879	19,210
Deepjyoti Ghosh	Post-Doc	3.00			60,367	15,092	3,818	18,910
SUBTOTALS →						36,455	9,224	45,679

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* General lab and tissue culture supplies, kidney endothelial cells, HeP2 kits Biostatistics, Microscopy and Imaging Core services CODEX: antibodies, image data storage	45,350
TRAVEL Domestic, national and/or regional meetings	2,000
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Publication Costs	1,000

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 94,029
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 94,029

Program Director/Principal Investigator (Last, First, Middle): Chong, Anita & Clark, Marcus

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	45,679	45,679			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	45,350	50,500			
TRAVEL	2,000	2,000			
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	1,000	1,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	94,029	99,179			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	94,029	99,179			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 193,208

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See attached BUDGET JUSTIFICATION pages.

Budget Justification – The University of Chicago

The inclusion of faculty salary in this budget reflects the policy of the Division of the Biological Sciences (BSD) and the Pritzker School of Medicine. It is expected that investigators will recover reasonable and appropriate salary support from grants and contracts proportionate to the fraction of their time and effort devoted to the project. BSD faculty appointments are made on a twelve-month basis and the requested salary is based upon the investigator's total University compensation. If an award is made in a reduced amount, the investigator will be expected to retain an appropriate amount of salary support in the budget. Upon request, we will be pleased to provide institutional salary information. The current fringe rate is calculated at 25.3%.

Key Personnel
Anita Chong, PhD, Principal Investigator (0.13 Calendar Months) – Dr. Chong will be responsible for the overall direction of this project. She will supervise the post-docs, Ismail Sayin and Deep Ghosh, and will work closely with Drs. Marcus Clark, Anat Tambur and Amishi Desai to ensure timely progress on the project.

Anita Chong, PhD, Co-Principal Investigator (0.10 Calendar Months) – Dr. Clark will be responsible for Aim 2, and the computation aspects of the data analysis of Aim 1. He will supervise Deep Ghosh, and will work closely with Drs. Chong, Tambur and Desai to ensure timely progress on the project.

Other Personnel
Ismail Sayin, PhD, Post-doctoral fellow (3 Calendar Months) – Dr. Sayin will be primarily responsible for the quantification of autoantibodies using the HEp2 cell kits, and also develop new assay using kidney tubule epithelial cells. He has been responsible for the development of the autoantibody assay using HEp2 cell kit, as well as investigations into the production of autoantibodies in mouse kidney transplant models. Dr. Sayin is a trained human immunologist who received his PhD from Case Western, and has been a post-doc in the Chong lab for ~2 years.

Deepjyoti Ghosh, PhD, Post-doctoral fellow (3 Calendar Months) – Dr. Ghosh was responsible for the development of the algorithms for the quantification of autoantibody-stained HEp2 cells, and will be the lead investigator leading the computation analysis of CODEX images. Dr. Gosh received his PhD in Math from the University of Houston, and has been a post-doc in Bioinformatics in the Clark lab from ~1 year.

Supplies: (Year 1: $45,350; Year 2: $50.500,)
Tissue culture and general lab supplies ($23,500 Yr1 & $18,000 Yr2) – Funds are requested for purchase of tissue culture and general lab supplies necessary for an active immunology laboratory. This includes costs for the culture of human immortalized kidney tubule epithelial cells, and generating slides for the quantification of autoantibodies. We purchase commercial HEp2 cell kits for autoantibody quantification.

Integrated Light Microscopy and Biostatistics Cores ($9,850 Yr1 & $9,000 Yr2) – Funds are requested to use the laser-scanning confocal microscopes and digital scanning the slides for the quantification of autoantibody staining. In addition, we will consult with the Biostatistics Core for our data analysis of our cohort studies.

CODEX antibody panel and staining of biopsies ($12,000 Yr1 & $23,500 Yr2) – Funds are requested for the purchase of monoclonal antibodies for staining the biopsies, and for image data storage.

Travel: ($2,000, years 1-2)

Funds are requested for the Postdoctoral Fellow/Bioinformatician to each travel to 1 national and/or 1 regional meeting per year. The estimate includes airfare (coach), ground transportation, lodging, and per diems.

Publication Costs:
We have allocated $1,000 for publication costs per year.

Indirect Costs
Per Chicago Biomedical Consortium's guidelines, no indirect costs have been allotted.

Program Director/Principal Investigator (Last, First, Middle): Tambur, Anat & Desai Patel, Amishi

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM 6/1/2023 THROUGH 5/31/2025

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

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Anat Tambut	PD/PI	0.24				5,868	1,677	7,544
Amishi Desai Patel	MPI	0.24				5,804	1,658	7,462
Alessandra Antunes	Res Assoc	1.80				8,705	2,487	11,192
SUBTOTALS →						20,377	5,823	26,199

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

Plasticware/Gloves/Dry Ice — 500

TRAVEL

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

Publication Costs $500 NMEDW Query Build = $900
Shipping = $500 — 1,900

CONSORTIUM/CONTRACTUAL COSTS | DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* **$** 28,599

CONSORTIUM/CONTRACTUAL COSTS | FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD **$** 28,599

Program Director/Principal Investigator (Last, First, Middle): Tambur, Anat & Desai Patel, Amishi

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	26,199	26,693			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	500	500			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	1,900	1,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	28,599	28,193			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	28,599	28,193			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 56,792

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

See attached BUDGET JUSTIFICATION pages.

SCOPE OF WORK:

We will investigate a new platform to look at non-HLA antibodies in kidney transplant recipients and see whether this test can help identify patients in high risk for rejections or graft loss (in the absence of HLA-DSA).

BUDGET JUSTIFICATION

Northwestern University

PERSONNEL

Senior / Key Personnel

Anat R. Tambur, DMD, PhD, Principal Investigator, 0.24 Calendar Months
Dr. Tambur is a Research Professor of Surgery in the Division of Organ Transplantation. Dr. Tambur is a renowned expert in Transplant Humoral Immunology, directing our Transplant Immunology/Human Leukocyte Antigen (HLA) laboratory and biorepository. Dr. Tambur will be the lead PI at Northwestern, and will work with the University of Chicago team in the overall design of the study. She will work with Dr. Desai Patel to provide oversight on patient identification for the studies proposed and supervise the research associate in the identification of serum samples. She will meet with the University of Chicago team monthly or more regularly to review results of experiments executed in the proposal.

Amishi Desai Patel, DO, Co-Principal Investigator, 0.24 Calendar Months
Dr. Desai Patel is an Associate Professor in the Department of Medicine's Division of Nephrology. She is a practicing transplant nephrologist that has extensive experience in the care of kidney transplant recipients. She will go through the enterprise data warehouse (EDW) to identify the patients with biopsy-proven rejection, as well as healthy controls (2:1 matched controls). She will meet with the University of Chicago team monthly or more regularly to review results of experiments executed in the proposal.

Dr. Desai Patel has an appointment with Northwestern University (NU) and with the affiliated Northwestern Medical Group clinical practice plan (NMG). The institutional base salary used in this application represents the combined salary from both NU and NMG, which is paid by NU under a common paymaster. The number of person months in this application represents NU effort on the proposed project in relation to professional effort encompassed by the dual NU and NMG appointments.

Non-Key Personnel

Alessandra Armstrong, Research Associate, 1.80 Calendar Months
Alessandra Armstrong is currently a Research Associate in Dr. Tambur's laboratory and will be involved in the project under the direct supervision of Dr. Tambur. She has 15 years of experience, of which 11 were directly involved in HLA antibody testing and HLA typing. She further has 4 years working in Human Genetics, western blotting, immunohistochemistry and immunofluorescence techniques. Alessandra will assist with facilitating and coordinating daily administrative activities for the research study. She will work closely with Dr. Tambur and Desai to identify the patient serum samples based on serum Cr levels, biopsy-proven antibody-mediated rejection and anti-HLA antibody data. She will aliquot the serum samples, de-identify them, package them in dry ice for shipping to Dr. Chong's lab at The University of Chicago. She will also work with Dr. Desai in identifying biopsies for CODEX analysis.

Employee benefits have been calculated based on the following DHHS-approved rates:

- 09/01/22 – 08/31/23…. 27.9%

- 09/01/23 – 08/31/24 and thereafter…. 27.9% (provisional)

OTHER DIRECT COSTS

Materials and Supplies:

Plasticware, gloves, dry ice: $1,000

Services:

NMEDW Query Build $900 ($75/hour x 12 hours)

Publication Costs:

$1,000 total ($500 per NU investigator)

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: Chong, Anita S.

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

POSITION TITLE: Professor, Section of Transplantation, Department of Surgery, Committee on Immunology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Malaya	BSc	08/1981	Zoology-Ecology
Australian National University	PhD	05/1985	Cell Immunology
Tufts University	Post-Doc	04/1986	Immunology
University of Arizona	Post-Doc	05/1989	Cancer Immunology

A. Personal Statement

My research team has been investigating the behavior of memory alloreactive B cells and plasma cells in transplant recipients, with the goal of identifying pharmacological strategies that can control or deplete these clinically important pro-rejection cells. We conducted mechanistic studies in experimental rodent transplant models to identified a drug regimen that successfully reversed humoral immunity and antibody-mediated rejection (AMR). In collaboration with Dr. Ronald Pelletier (Rutgers University), we translated our findings to the successful treatment of kidney transplant patients diagnosed with AMR. More recently, I initiated a collaboration with Dr. Marlena Habal (NYU) to apply this protocol to desensitize patients with heart failure requiring a heart transplant. Most relevant to this proposal, we have collaborated with Dr. Marcus Clark (U Chicago) to investigate B cells in rejecting renal biopsies, and have develop complementary mouse models of kidney allograft rejection to identify the role of autoreactive antibodies in AMR.

My lab also has a long-standing interest in understanding the basis of immunological tolerance following allogeneic transplantation. We are continuing our close collaboration with Dr. Marisa Alegre (U Chicago) to study how infections and prior sensitization events prevent the induction of stable transplantation tolerance. We have developed approaches to track and analyze distinct populations of endogenous donor-reactive T and B cells, and are using multi-omics approach to define how these cells differentiate under conditions of rejection, memory/sensitization and tolerance. Our long-term goals are to develop better strategies to induce, maintain, and diagnose tolerance in transplant recipients, with an emphasis on how pregnancy-induced sensitization contributes to sex disparity in access to transplantation.

A third area of laboratory research is preventing respiratory infections through non-inflammatory and needle-free vaccination. My lab has an established collaborative research program of >10 years with Dr. Joel Collier, a bioengineer at Duke University, to develop adjuvant-free nanoparticulate vaccines. The current focus of my lab's research is to define the mechanisms by which antigen-displaying nanofibers induce dendritic cell activation and elicit systemic and tissue-specific $CD4^+$ and $CD8^+$ T cell responses.

Finally, I am actively involved in advocating the consideration of sex and gender in transplantation research and profession (Vinson, Chong et al. Kidney Int. 2021; Transplantation 2021; Reed, Chong et al. Front Immunol 2022). Notable publications most relevant to this proposal are listed:

1. Jain D, Rajab A, Young JS, Yin D, Nadasdy T, **Chong AS***, Pelletier RP*. 2020Reversing donor-specific antibody responses and antibody-mediated rejection with bortezomib and belatacept in mice and kidney transplant recipients. *Am J Transplant*. 10:2675-2685. PMCID: PMC8232017. (commentary in *Am J Transplant.* 2020)
2. Alishetti S, Farr M, Jennings D, Serban G, Uriel N, Sayer G, Vasilescu V, Restaino S, **Chong AS**, Habal MV. 2020. Desensitizing highly sensitized heart transplant candidates with the combination of belatacept and proteasome inhibition. *Am J Transplant*. 20:3620-30. PMCID: PMC8366746.

3. Asano Y, Daccache J, Jain D, Ko K, Kinloch A, Veselits M, Wolfgeher D, Chang A, Josephson M, Cunningham C, Tambur A, Khan A, Pillai S, **Chong AS**, Clark MR. 2021. Intrarenal B cells integrate *in situ* innate and adaptive immunity in human renal allograft rejection. *Nat Commun.* 12:4372. PMCID: PMC8285506. (highlighted in Lit Watch: *Am J Transplant*. 2021, Game Changer *Transplantation* 2020).
4. Suah A*, Tran D-KV*, Khiew SHW*, Andrade MS, Pollard JM, Jain D, Young JS, Yin D, Chalasani G, Alegre ML*, **Chong AS***. 2021. Humoral sensitization overrides pregnancy induced T cell tolerance to offspring-matched allografts. *J Clin Invest.* 131:e140715. PMCID: PMC7773355. (highlighted as a Game Changer: *Transplantation* 2021; Editor's Choice: *Sc Immunol* 2021).

* co-first or co-senior authors

Ongoing Research Support

R01 AI 148705-01A1 (Chong, Clark, PI) 02/2020-01/2025
NIH/NIAID
Title: Intrarenal B cells in acute kidney allograft rejection
Major Goal: The proposed study aims to understand in situ adaptive T and B cell immunity in human biopsies from rejecting kidney allografts, and in experimental renal transplantation in mice.
Role: PI

NIHAI201700100 (Esser-Khan, PI) 09/2019-09/2024
NIH/NIAID-DAIT
Title: Discovery of Adjuvants via Novel Modulation of Innate Immune Pathways for Influenza Vaccines
Major Goal: We propose to discover and develop adjuvants with unique properties that inhibit undesired sections of immune signaling pathways to control and enhance immunological outcomes.
Role: Co-Investigator

R01 AI 142747 (Chong, PI) 08/2019-08/2023
NIH/NIAID
Title: Deconstructing B cell transplantation tolerance
Major Goal: The research goals are to define the mechanisms of B cell tolerance, focusing on alloreactive B cell-intrinsic dysfunction and B cell-mediated suppression of donor-specific B cells.
Role: PI

Completed Research Support

P01AI097113 (Chong, PI) 07/2017-06/2022 (NCE)
NIH/NIAID
Title: Infections and the Stability of Transplantation Tolerance
Major Goal: Mechanistic studies on the mechanisms of robust life-long tolerance, how bacterial infections destabilize established transplantation tolerance, and how immunological memory generated by rejection or semi-allogeneic pregnancy induces metastable states of tolerance.
Role: PI for Project 2 & Core B

R01AI118182 (Chong, Collier, PI) 01/2015-12/2019
NIH/NIAID
Title: Supramolecular nanofiber vaccines
Major Goal: Application of bioengineering approaches coupled with mechanistic/immunological investigations into the immunogenicity of Q11 nanofibers.
Role: PI

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2007-Present	Professor, Section of Transplantation, Department of Surgery, Committee on Immunology, The University of Chicago, Chicago, IL
2002-2007	Associate Professor, Section of Transplantation, Department of Surgery, Committees on Immunology, and Molecular Metabolism & Nutrition, The University of Chicago, Chicago, IL
1995-2002	Associate Professor, Department of General Surgery, Adjunct Associate Professor, Dept. of Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL

Positions and Scientific Appointments

1989-1995	Assistant Professor, Department of General Surgery, Adjunct Assistant Professor, Dept. of Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL

Honors and Awards

2021	Francis Straus Mentorship Award, Division of Biological Sciences, University of Chicago
2020	Women in Transplantation Leader Award (Basic Science)
2020	Distinguished Fellow of American Association for the Advancement of Science (AAAS)
2015	AST Established Scientist Award (Basic Science)

Other Experience and Professional Memberships

2020-Present	Past-Chair, Chair, Pillar 1, Women in Transplantation Initiative of The Transplantation Society
2019-Present	Past-Chair, Chair and Co-chair of the Organizing Committee, Fellows Meeting, American Transplant Society
2018-Present	Past-chair, Chair, Co-chair, of Community of Transplant Scientists (COTS), American Transplant Society
2014-Present	Deputy Editor, Transplantation, The Official Journal of the Transplantation Society
2013-2018	Standing Member, NIAID Council (DIAT)
2009-2016	Chair, Transplantation Science Committee, The Transplantation Society
2009-2013	Member of Transplantation, Tolerance and Tumor Immunology Study Section

C. Contributions to Science

1. **Alloreactive B cells in rejection and tolerance**

It has become increasingly clear that alloantibodies mediate acute and chronic antibody-mediated rejection (AMR), and thus play an important role in determining the outcome of allografts in the clinic. Furthermore, pre-transplant donor-specific antibodies mediate acute AMR and often precludes access to transplantation. Understanding the signals controlling the *recall* antibody response and long-lived plasma cells that produce donor-specific antibodies may provide novel ways of overcoming the alloantibody barrier in sensitized recipients. Recent technical breakthroughs in our laboratory has allowed the visualization of endogenous alloreactive B cells in mouse transplant models has allowed us to probe the cellular and molecular basis of the B cell recall response (Chen et al. 2015; Young J et al. 2016; Yang et al. 2016). These studies led to the identification of a novel therapy (belatacept plus proteasome inhibition) for treating AMR in the clinic (Jain et al. 2020), and desensitizing patients on the wait-list for organ transplantation (Alishetti et al. 2020). In collaboration with Dr. Marcus Clark, we developed a human-to-mouse translational project to characterize the function and specificity of intrarenal B cells in rejecting allogeneic kidney biopsies (Asano et al. 2021). We developed complementary mouse models to study the development of autoantibody during kidney allograft rejection, mechanistic studies to define their role in rejection, and to identify therapeutic interventions. Finally, we studied B cell fate in transplantation tolerance, and showed that donor-specific B cell fate deviates away from plasma cells and into suppressors capable of inhibiting naive B cell responses in a donor-specific manner (Khiew et al. 2020).

a. Chen JJ, Wang Q, Yin D, Vu V, Sciammas R*, and **Chong AS***. 2015. Cutting Edge: CTLA-4Ig inhibits memory B cell responses and promotes allograft survival in sensitized recipients. *J Immunol.* 195:4069-73. PMCID: PMC4610858.

b. Young J, Chen J, Miller ML, Yin D, Vu V, Moon JJ, Sciammas R, Alegre ML, **Chong AS***. 2016. Delayed CTLA-4Ig treatment reverses established alloantibody responses and rescues allografts from acute allograft rejection. *Am J. Transpl*. 16:2312-23. PMCID: PMC4956497.

c. Yang J, Chen JJ, Young J, Wang Q, Yin D, Sciammas R*, and **Chong AS***. 2016. Tracing the fate of donor-MHC Class II reactive B cells in mouse cardiac transplantation: delayed CTLA-4Ig treatment prevents memory alloreactive B cell generation. *Transplantation* 100(8):1683-91. PMCID: PMC496160.

d. Khiew SH, Jain D, Chen J, Yang J, Yin D, Young JS, Dent A, Sciammas R, Alegre ML, **Chong AS**. 2020. Transplantation tolerance modifies donor-specific B cell fate to suppress de novo alloreactive B cells. *J Clin Invest.* 130:3453-3466. PMID: PMC7329196. (accompanying commentary in *J Clin Invest*. 2020)

2. **Impact of inflammation, infections and memory on immunological tolerance to allografts**

Success in drug-free allograft acceptance in limited numbers of human transplant recipients provides important proof-of-principle that the human immune response can be re-programmed to a state of functional tolerance to allografts. Thus, transplantation tolerance research is currently focused on defining mechanisms of tolerance, identifying barriers that prevent the induction and stable maintenance of transplantation tolerance, and developing novel approaches that can induce tolerance in memory T cells. The main contributions of our research to the field of transplantation research are: (i) showing that infections can overcome established states of transplant tolerance or erode the robustness of the tolerant state (Young et al, 2017); (ii) discovering that transplant tolerance is resilient; that while pro-inflammatory infections can override tolerance, the resolution of infection allows the tolerant state to spontaneously return (Miller et al. 2015); (iii) demonstrating that pregnancy as prior sensitizing event prevents the induction of transplantation tolerance because of fetus-specific B cells and antibodies. We also uncovered the unexpectedly pro-tolerogenic effects of pregnancy on alloreactive T cells that extends to offspring-matched heart allografts (Suah et al. 2021; Durgam et al. 2022); and (iv) highlighting that sensitization to a single donor antigen is sufficient to destabilize tolerance (Andrade et al. 2021).

a. Young J, Daniels MD, Miller ML, Wang T, Xu J, Wang Y, Yin D, Alegre ML*, **Chong AS***. 2017. Erosion of tolerance after infection. *Am J Transplant*. 17:81-90. PMCID: PMC5938732. (best basic science paper in *Am. J Transpl*. 2017).
b. Miller ML*, Daniels MD*, Wang T*, Chen J, Young J, Xu J, Wang Y, Yin D, Vu V, Husain AN, Alegre ML*, **Chong AS***. 2015. Spontaneous restoration of transplantation tolerance after acute rejection. *Nat Commun*. 6:7566. doi: 10.1038/ncomms8566. PMCID:PMC4498267
c. Durgam SS, Alegre ML, **Chong AS.** 2022. Toward an understanding of allogeneic conflict in pregnancy and transplantation. J Exp Med. 219(5):e20211493. doi: 10.1084/jem.20211493.
d. Andrade MS, Young JS, Pollard JM, Yin D, Alegre ML*, **Chong AS***. 2022. Linked sensitization by memory $CD4^+$ T cells prevents co-stimulation blockade induced transplantation tolerance. *JCI Insight* 8;7(11):e159205. doi: 10.1172/jci.insight.159205.

3. **Novel non-inflammatory vaccines based on engineered peptide-based nanoparticles**

Vaccines are enormously successful at preventing diseases such as smallpox, polio, seasonal influenza, and COVID-19, but many diseases still remain untreatable with vaccines. The long-term goal of our studies is to develop non-inflammatory sub-unit vaccines that avoid the need for reactogenic adjuvants. Over the past >10 years, my laboratory has collaborated with Collier laboratory in investigating an engineered peptide system that self-assembles into nanofibers capable of displaying peptide or protein antigens, and raising strong immune responses when delivered by subcutaneous, intraperitoneal, intranasal and sublingual routes. We reported that peptide or protein antigens linked to the Q11 peptide and self-assembled into nanofibers (AgQ11) raise durable T cell and B cell responses without the need for additional adjuvants, and in the absence of measurable inflammation at the injection site (Hudalla et al. 2013, Chen et al. 2013). By titrating the density of T cell epitopes displayed by Q11 nanofibers, we were able to specific T cell and antibody outputs (Pompano et al. 2014). Finally, we reported that Q11 nanofibers could be formulated into intranasal vaccines capable of eliciting lung-resident immunity (Si et al. 2020).

a. Hudalla G, Sun T, Gasiorowski J, Han H, Tian Y, **Chong A**, and Collier J. 2014. Gradated assembly of multiple proteins into supramolecular nanomaterials. *Nature Materials*. 13:829-36. PMCID:PMC4180598.
b. Chen JJ*, Pompano R*, Santiago FW, Maillat L, Sciammas R, Sun T, Han H, Topham DJ, **Chong AS*** and Collier JH*. 2013. The use of self-adjuvanting nanofiber vaccines to elicit high-affinity B cell responses to peptide antigens without inflammation. *Biomaterials* 34:8776-85. PMCID: PMC3814015.
c. Pompano R*, Chen JJ*, Verbus, EA, Zhao, F, Han H, Fridman A, McNeeley T, Collier JH* and **Chong AS*** 2014. Titrating T cell Epitopes within Self-Assembled Vaccines Optimizes $CD4^+$ Helper T Cell and Antibody Outputs. *Adv Healthcare Mater.* 3:1898-908. PMCID: PMC4227912.
d. Si Y, Tian Q, Kelly SH, Shores LS, Camacho DF, Sperling AI, Andrade MS, Collier JH* and **Chong AS***. 2020. Adjuvant-free nanofiber vaccine induces in situ lung dendritic cell activation and TH17 responses. *Sci Adv.* 6: eaba0995. PMCID: PMC7413739.

4. **Immunobiology of xenograft rejection and xenotransplantation**

Transplantation is an effective approach for the treatment of end-stage organ failure that is limited by organ availability. Xenotransplantation was, and continues to be, a promising alternative approach to bridge the gap between the supply and demand of organs, tissues, and cells. However, immunological barriers prevent successful clinical xenotransplantation; in particular T-independent antibody responses to xenogeneic sugar epitopes are unique to xenotransplantation. From 1994-2008, my laboratory investigated the immunobiology of xenograft rejection, and published 30 articles in this area. We focused on the critical xenoantigen, alpha1,3-gal and anti-Gal IgG production in mouse models using galactosyltransferase (GT)-KO mice, to show their potency in inducing endothelial cell activation that is mediated not only by complement activation; my lab was the first to implicate the contribution of FcγR-expressing NK cells in antibody-mediated rejection of xenografts (Yin et al. 2004, Xu et al 2003). We also investigated potential immunosuppressive strategies for promoting xenograft acceptance using co-stimulation blockade and leflunomide, and how the graft might increase expression of complement regulatory proteins to mediate graft accommodation (Ding et al. 2008, Yin et al. 2002).

a. Ding JW, Zhou T, Ma L, Yin D, Shen J, Ding CP, Tang IY, Byrne GW, **Chong AS.** 2008. Expression of complement regulatory proteins in accommodated xenografts induced by anti-alpha-Gal IgG1 in a rat-to-mouse model. *Am J Transplant*. 8:32-40. PMID: 17973967
b. Yin D, Zeng H, Ma L, Shen J, Xu H, Byrne GW, **Chong AS.** 2004. NK cells mediate IgG1-dependent hyperacute rejection of xenografts. *J Immunol*. 172:7235-8. PMID: 15187097.
c. Xu H, Yin D, Naziruddin B, Chen L, Stark A, Wei Y, Lei Y, Shen J, Logan JS, Byrne GW, **Chong AS.** 2003. The in vitro and in vivo effects of anti-galactose antibodies on endothelial cell activation and xenograft rejection. *J Immunol*. 170:1531-9. PMID: 12538718.
d. Yin D, Ma L, Shen J, Byrne GW, Logan JS, **Chong AS.** 2002. CTLA-4Ig in combination with anti-CD40L prolongs xenograft survival and inhibits anti-gal ab production in GT-KO mice. *Am J Transplant*. 2:41-7. PMID: 12095055.

5. **<u>Leflunomide as an immunosuppressive agent with anti-viral properties for transplantation</u>**

Life-long pharmacological immunosuppression is necessary to prevent rejection of transplanted allograft, and there continues to be a need to develop more effective agents that can control both T and B cell/antibody responses. Major side effects of continued immunosuppression are increased susceptibility to infections and cancers, and drug-specific toxicities. For approximately 16 years, my laboratory (1990-2006) worked on a novel immunosuppressive/anti-inflammatory drug, leflunomide, and published 43 papers defining its efficacy in preventing allograft and xenograft rejection (Williams et al. 1994; Chong et al. 1996) and its mechanism of action (Xu et al. 1995). Remarkably, we discovered that leflunomide also had potent anti-viral effects, inhibiting human cytomegalovirus (Waldman et al. 1999), and subsequently, polyoma BK virus. We proposed that leflunomide is an ideal drug for transplant recipients, and our studies paved the way for clinical trials of leflunomide in kidney transplantation for simultaneously controlling CMV and polyoma virus infections and preventing rejection.

a. Williams JW, Xiao F, Foster P, Clardy C, McChesney L, Sankary HN, and **Chong AS.** 1994. Leflunomide in Experimental Transplantation: Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. *Transplantation* 57: 1223-1231.
b. **Chong AS**, Shen J, Xiao F, Blinder L, Liu W, Sankary H, Foster P, and Williams JW. 1996. Delayed xenograft rejection in the concordant hamster heart into Lewis rat model. *Transplantation* 62: 90-96.
c. Xu XL, Williams JW, Finnegan A, Bremer E and **Chong AS.** 1995. The novel immunosuppressive agent leflunomide is an inhibitor of tyrosine phosphorylation in T cells. *J. Biol. Chem.* 270: 12398-12403.
d. Waldman JW, Knight DA, Lurain NS, Miller DM, Sedmack DD, Williams JW, and **Chong AS.** 1999. Novel mechanism of inhibition of human cytomegalovirus by the experimental immunosuppressive agent, leflunomide. *Transplantation* 68: 814-825.

Complete list of published work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/anita.chong.1/bibliography/public/

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: Marcus R. Clark, M.D.

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

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 California, Riverside, CA	B.S.	06/1981	Biological Sciences
University of California, Los Angeles, CA	M.D.	06/1984	Medicine
University of Michigan, Ann Arbor, MI		06/1987	Residency, Medicine
University of California, San Francisco, CA		06/1990	Fellowship, Rheumatology

A. Personal Statement

I have over thirty years of experience as a NIH-funded investigator in mentoring, team building, leadership and publishing rigorous, durable and impactful science. Our laboratory pursues an integrated research program focused on B cells and autoimmunity. In mouse-based studies, we have elucidated underlying fundamental molecular mechanisms of both B cell development and peripheral activation. In our human research, we are focused on understanding *in situ* adaptive immune responses that destroy tissue. Most of these latter studies are on lupus nephritis and renal allograft rejection.

In B cell development, we and others have elucidated how signals initiated through the pre-B cell receptor, together with those delivered through the IL-7 receptor, coordinate cell cycle progression with immunoglobulin light chain gene recombination. The studies resulted in discovering the epigenetic reader BRWD1 as critical for both regulating *Igκ* accessibility (a) and in coordinating broad transcriptional programs in early and late B lymphopoiesis. Current evidence from our laboratory reveals that *Brwd1* mutations lead to immunodeficiency in humans and BRWD1 plays a role in peripheral adaptive immunity. Most recently, we have demonstrated that the pre-BCR initiates an IRF4-CXCR4 feedforward loop and it is CXCR4 that directly signals to open both *Igκ* and *Igλ* to recombination (b). These latter findings fundamentally rewrite the canonical model of B lymphopoiesis. Furthermore, they are the first demonstration of a direct and independent role for CXCR4 in driving an important biological process. In the periphery, we have focused on two areas. First, in our studies of germinal centers (GC), we have recently defined two novel populations within the dark zone that both allow compartmentalization of fundamental GC functions and reveal the molecular programs of the GC cycle (c). These data provide a fundamentally new GC paradigm. We have also defined molecular mechanisms of receptor endocytosis and endocytic trafficking and how the mechanisms influence BCR trafficking and cell fate.

We have been applying our knowledge of immunology to understand how *in situ* adaptive immune responses drive renal tubulointerstitial inflammation (TII). In lupus, we have demonstrated the prognostic importance of TII and that it is associated with intrinsic adaptive cell mechanisms not reflected in the peripheral blood. This has led us to use and develop novel technologies to understand immunity in human tissue samples. Most notably, we have developed a new image analysis technology, Cell Distance Mapping, which allows, for the first time, identification of cognate adaptive cell networks in human tissue (d). In all research areas, we have used sophisticated bioinformatic techniques, and multiple technical approaches, to obtain a comprehensive understanding of important immunological processes in human biopsies. This area of research is most relevant to the current CBC proposal.

In addition to research, my other focus is on mentoring and education. I have extensive experience in research mentoring including serving as the primary advisor for eleven graduate students and over 30 postdoctoral fellows. As Chief of the Section of Rheumatology, I have mentored and developed the careers of dozens of faculty and fellows. For the last eleven years, I have been the Director of the University of Chicago Medical Scientist Training Program. This position allows me to apply my expertise in fundamental research, translational biology and clinical care to the training of the next generation of leaders in academic medicine.

a. Mandal M, Hamel KM, Maienschein-Cline M, Tanaka A, Teng G, Tuteja, JH, Bunker JJ, Eppig JJ, Schatz DG and **Clark MR**. 2015. Histone reader BRWD1 targets and restricts recombination to the

Igκ locus. Nature Immunology 16:1094-1103. Reviewed in News and Views, 16: 1007-1009. PMC4575638

b. Mandal M, Okoreeh MK, Kennedy DE, Maienschein-Cline M, Junting A, Mclean KC, Kaverina N, Veselits M, Aifantis I, Gounari F, **Clark MR**. 2019. CXCR4 signaling directs *Igk* recombination and the molecular mechanisms of late B lymphopoiesis. Nature Immunology.20:1393-1403. PMC6754289

c. Kennedy DE, Okoreeh MK, Maienschein-Cline M, Ai J, Veselits M, McLean KC, Dhugana Y, Wang H, Peng J, Chi H, Mandal M, **Clark MR**. 2020. Novel specialized cell state and spatial compartments within the germinal center. Nature Immunology. 21:660-670. PMC7255947 Reviewed in News and Views 21:599.

d. Liarski VM, Sibley A, van Panhuys N, Junting A, Chang A, Kennedy D, Merolle A, Germain RN, Giger M. and **Clark MR**. Convolutional neural networks identify in situ adaptive immune cell architectures in humans. 2019. Nature Immunology. 20:503-513. PMC6474677 Reviewed in News and Views: 20:382-383

Ongoing Research Support

NIH U19 AI082724 (Clark, PI) 06/2009-05/2024
University of Chicago Autoimmunity Center of Excellence
The major goal of this project is to establish a center of excellence focused on the role of adaptive immunity in the pathogenesis of human autoimmune diseases.

DoD LRI180083 (Clark, PI) 06/2019-09/2024
Quantifying In Situ Adaptive Immunity in Human Tubulointerstitial Lupus Nephritis
The goal of this project is to apply CDM to obtain a quantitative understanding of in situ immunity in lupus nephritis.

NIH T32 GM007281 (Clark, PI) 07/2013-06/2023
Medical Scientist National Research Service Award
This is the training grant of University of Chicago MD/PhD training program.

NIH R01 AI148705 (Clark, Chong, PI) 02/2020-1/2025
Intrarenal B cells in acute kidney allograft rejection
The goal of the project is to define the role of B cells in allograft rejection in both humans and mouse models.

NIH R01 AI143778 (Clark, PI) 04/2019-03/2024
BRWD1 in peripheral adaptive immunity
The aim of this project is to define the role of BRWD1 epigenetic regulation in germinal center responses.

NIH R01 AI150860 (Clark, PI) 03/2021-03/2026
Role of CXCR4 in immunoglobulin light chain recombination
The aim of this project is to understand how CXCR4 directs late B lymphopoiesis

Alliance for Lupus Research (Clark, PI) 12/2020-11/2023
In situ adaptive immunity in Lupus tubulointerstitial inflammation

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2011-Present	Director, University of Chicago Medical Scientist Training Program
2008-Present	Co-Director, Knapp Center for Lupus Research
2007-Present	Professor, Departments of Medicine and Pathology, Committee on Immunology
2000-Present	Chief, Section of Rheumatology
2000-2007	Associate Professor, Departments of Medicine and Pathology, Committee on Immunology,
1993-2000	Assistant Professor, Departments of Medicine and Pathology, Committee on Immunology
1990-1993	Instructor, National Jewish Center for Immunology and Respiratory Medicine

Scientific Appointments (selected)

2010-2016	Member, NIAMS Board of Scientific Councilors
2005-2009	Member, Cellular and Molecular Immunology–B

Honors and Awards

2014	Elected to American Association of Physicians (AAP)
2005	Elect to Henry Kunkel Society
2004	Elected to American Society for Clinical Investigation (ASCI)

Other Experience and Professional Memberships

2012-2015	Co-chair, Lupus Research Institute Study Section
2001-2006	Associate Editor, Journal of Immunology
2001-2005	Ad hoc reviewer, Immunobiology Study Section
2010-	Reviewer, Immunity,
2014-	Reviewer, Cell,
2016-	Reviewer, Nature
2008-	Reviewer, Nature Immunology
2016-	Reviewer, Nature Medicine
2020-	Reviewer, Science Immunology
2000-	Reviewer, Journal of Experimental Medicine
2015-	Reviewer, Science Translational Medicine
2000-	Reviewer, Journal Clinical Investigation
1995-	Reviewer, Molecular and Cellular Biology
1994-	Reviewer, Proceedings of the National Academy of Sciences

C. Contributions to Science

1. Regulation of Igκ recombination: Thirty years ago, it was proposed by Alt that accessibility of immunoglobulin genes to the RAG proteins regulated recombination. However, despite intense investigation, the mechanisms controlling immunoglobulin gene accessibility have remained unclear. In 2011, we demonstrated that downstream of the IL-7 receptor, active STAT5 recruited PRC2 to the *Igκ* intronic enhancer and repressed Jκ recombination (a). This was the first description of both a mechanism of *Igκ* repression and that STAT molecules can be direct transcriptional repressors. We then demonstrated that Jκ accessibility was regulated epigenetically and that a very specific set of epigenetic marks at Jκ enabled recruitment of the epigenetic reader BRWD1 that remodeled chromatin structure and enabled RAG protein recruitment (a above). Subsequent studies demonstrated that BRWD1, in mice and humans, also orchestrated the transcriptional program of late B lymphopoiesis (b). Finally, we have recently demonstrated that Vκ is transcribed by transcription factories and that this is regulated by cyclin D3 (c-d). This was the first demonstration of a cell cycle molecule mediating repression by preventing access to RNA polymerase II. Together, these studies reveal novel, lineage and stage specific mechanisms regulating *Igκ* accessibility to RAG-mediated recombination.

a. Mandal M, Powers SE, Maienschein-Cline, M, Bartom ET, Hamel KM, Kee BL, Dinner A and **Clark MR.** 2011. Epigenetic repression of the Igκ locus by STAT5-mediated Ezh2 recruitment. Nature Immunology 12:1212-1220. Reviewed in News and Views, 12:1139-1140, Nature Immunology featured paper of the month, F1000. PMC3233979
b. Mandal M, Maienschein-Cline M, Maffucci P, Veselits M, Kennedy DE, McLean, KC, Okoreeh MK, Karki S, Cunningham-Rundles C, **Clark MR**. 2018. BRWD1 orchestrates epigenetic landscape of late B lymphopoiesis. Nature Communications. 9:3888-3899. PMC6155124
c. Powers SE, Mandal M, Matsuda S, Miletic AV, Cato MH, Tanaka A, Rickert RC, Koyasu S and **Clark MR**. 2012. Subnuclear cyclin D3 compartments and the coordinated regulation of proliferation and immunoglobulin variable gene repression. J Experimental Medicine. 209:2199-2213. PMC3501354
d. Karki S, Kennedy DE, McLean K, Grzybowski AT, Maienschein-Cline M, Banerjee S. Xu H, Davis E, Mandal M, Labno C, Powers SE, Le Beau MM, Dinner AR, Singh H, Ruthenburg AJ and **Clark MR**. 2018. Regulated capture of Vκ gene topologically associating domains by transcription factories. Cell Reports 24:2443-2456. PMC6310487

2. Signaling mechanism of B lymphopoiesis: Numerous studies have demonstrated the critical importance of the IL-7 receptor and the pre-BCR in B lymphopoiesis. However, the molecular functions of these receptors have been unclear. In 2006, we demonstrated that the IL-7 receptor drove proliferation by inducing expression of cyclin D3 (a) and inhibits recombination, in part, by activating PI-3 kinase. In contrast, the pre-BCR inhibits proliferation by repressing cyclin D3 and inducing E2A which enhance Igκ accessibility to recombination (b). These data demonstrate that the IL-7 receptor drives proliferation while the pre-BCR orchestrates recombination. Furthermore, molecular antagonisms downstream of each receptor ensure that in any one cell proliferation or Igκ recombination predominates. This general paradigm has provided a framework for understanding a broad range of observations in the field (c). Most recently, we have demonstrated that CXCR4 is critical for both Igk recombination and for receptor editing (d). These studies establish a new, three receptor model of B lymphopoiesis.

a. Cooper AB, Sawai CM, Sicinska E, Powers SE, Sincinski P, **Clark MR*** and Aifantis I* 2006. A unique role for cyclin D3 in early B cell development. Nat Immunology 7:489-497. *co-senior and corresponding authors.
b. Mandal M, Powers SE, Ochiai K, Georgopoulos K, Kee BL, Singh H, **Clark MR**. 2009, Ras orchestrates exit from the cell cycle and light-chain recombination during early B cell development. Nature Immunology. 10:1110-7. PMC3057509
c. **Clark MR**, Mandal M, Ochiai K, Singh H. 2014.Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signaling. Nat Immunol Rev 14:69-80. PMC4276135
d. Okoreeh M*, Kennedy DE, Emmanuel AO, Veselits M, Moshin A, Ladd RH, Erickson S, McLean KC, Madrigal B, Nemazee D, Maienschein-Cline M, Mandal M, **Clark MR**. 2022. Asymmetrical forward and reverse developmental trajectories determine molecular programs of B cell antigen receptor editing. Science Immunology 7(74):eabm1664. PMID: 35930652.

3. Peripheral adaptive immunity: We have a long history of studying the biochemical and molecular mechanisms of B cell activation and selection in the periphery. In mice, we have recently applied current molecular and imaging technologies to understand B cell activation in germinal centers (GCs). These studies have identified a new GC B cell population. The resulting novel three population model of germinal centers segregates important GC functions (selection, somatic hypermutation and proliferation) into distinct B cell populations and niches (a). Furthermore, this new model reveals the underlying complex epigenetic mechanisms controlling GC responses. In humans, we have focused on understanding *in situ* B cell selection in renal inflammation. In lupus nephritis, we first demonstrated that severe TII is associated with tertiary lymphoid neogenesis and *in situ* antigen-mediated B cell selection and differentiation into plasmid cells (b). We subsequently demonstrated that vimentin, a molecular pattern of inflammation, commonly drives this *in situ* antigen-mediated selection (c). Most recently, we have paired scRNA-Seq to antibody repertoire expression to demonstrate that in renal allograft rejection, a unique innate-like B cell population is selected *in situ* by molecular patterns of inflammation and damage (d). These data suggest that, in both autoimmune and non-autoimmune inflammation, a break in tolerance to inflammation drives local B cell activation and selection. It also suggests that comparison across human diseases can reveal common mechanisms subverting B cell tolerance and driving inflammation.

a. Kennedy DE, Okoreeh MK, Maienschein-Cline M, Ai J, Veselits M, McLean KC, Dhugana Y, Wang H, Peng J, Chi H, Mandal M, **Clark MR**. 2020. Novel specialized cell state and spatial compartments within the germinal center. Nature Immunology. 21:660-670 PMC7255947
b. Chang A, Henderson S, Liu N, Guttikonda R, Hsieh C, Utset T, MD, Meehan S, Quigg R, Meffre E, Weigert, M and **Clark MR**. 2010. *In situ* B cell mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol. 186(3):1849-60. PMC3124090 Featured on the cover and Selected for F1000
c. Kinloch AJ, Chang A, Ko K, Henry-Dunand CJ, Henderson S, Maienschein-Cline M, Kaverina N, Rovin BH, Ferrer MS, Wolfgeher D, Liarski V, Haddon DJ, Utz PJ, Wilson PC and **Clark MR**. 2014. Vimentin is a dominant target of in situ humoral immunity in human lupus tubulointerstitial nephritis. Arthritis Rheum 66:3359-3370. PMC4264660
d. Asano Y, Daccache J, Jain D, Ko K, Kinloch A, Veselits M, Wolfgeher D, Chang A, Josephson M, Cunningham P, Tambur A, Kahan AA, Pillai S, Chong A and **Clark MR**. 2021. Infiltrating innate-like, self-reactive B cells in human allograft rejection. Nature Communications. 12:4372, PMID: 34272370

4. Role of *in situ* adaptive immunity in human lupus nephritis: Systemic lupus erythematosus is thought of as the prototypical systemic autoimmune disease and that nephritis, with immune complex deposition and inflammation in glomeruli, is a manifestation of this systemic autoimmunity. However, we have demonstrated

that tubulointerstitial inflammation (TII) is a more severe manifestation of lupus nephritis than glomerulonephritis and, furthermore, it appears to be a manifestation of *in situ* adaptive immunity. Using novel computational approaches, we demonstrated that *in situ* B cell responses are driven by T follicular helper cells (a) and that the *in situ* B cell repertoire is primarily selected on molecular patterns of inflammation. We have also demonstrated that IgE responses can predominate *in situ* and that selective dysregulation of BCL-2 in TII makes it an attractive therapeutic target (b). Recently, we have developed novel methodologies to identify and quantify cognate T cell:antigen presenting interactions in human tissue (c). Using this approach, Cell Distance Mapping, we have identified plasmacytoid dendritic cells as an important *in situ* antigen presenting cell in lupus nephritis. CDM represents the first technique ever developed that could capture and quantify *in situ* adaptive immunity in human tissue. Finally, we have expanded the capabilities of CDM to high dimensional imaging to demonstrate that in lupus TII there are distinct immunological states each associated with a different response to conventional therapy and prognosis (d). These data demonstrate the importance of TII, and of *in situ* adaptive immune responses, in the pathogenesis of human lupus nephritis. Furthermore, they illustrate a novel approach to the study of *in situ* autoimmunity.

a. Liarski VM, Kaverina N, Chang A, Brandt D, Yanez D, Talasnik L, Carlesso G, Herbst R, Utset TO, Labno C, Peng Y, Jiang Y, Giger, ML and **Clark MR**. 2014. Cell Distance Mapping identifies functional T follicular helper cells in inflamed human renal tissue. Science Translational Medicine. 6:230ra46. PMC4129446
b. Ko K, Wang J, Perper S. Jiang Y, Yanez Y, Kaverina N, Junting A, Liarski VM, Chang A, Peng Y, Lan L, Westmoreland S, Olson L, Giger ML, Wang LC, **Clark MR**. 2016. BCL-2 as a therapeutic target in human tubulointerstitial inflammation. Arthritis Rheum. 68:2740-2751. PMC5083145
c. Liarski VM, Sibley A, van Panhuys N, Junting A, Chang A, Kennedy D, Merolle A, Germain RN, Giger M. and **Clark MR**. Convolutional neural networks identify in situ adaptive immune cell architectures in humans. 2019. Nature Immunology. 20:503-513. PMID: 30778242 Reviewed in News and Views: 20:382-383
d. Abraham R*, Durkee MS, Ai J, Veselits M, Casella G, Asano Y, Chang A, Ko K, Oshinsky C, Peninger E, Giger ML, **Clark MR**. 2022. Specific in situ inflammatory states associate with progression to renal failure in lupus nephritis. J Clinical Investigation. 132:e155350. PMID: 35608910. Commentary: Li H, Tsokos MG and Tsokos GC. 2022. Lymphocytes in the neighborhood: good or bad for the kidney? 132:e160657 PMID: 35775489.

5. Interrelationships between B cell antigen receptor signaling and endocytic trafficking: The BCR performs two fundamental functions, signaling with the activation of downstream transcriptional networks and endocytic trafficking to specialized late endosomes (MIIC) containing MHC class II and the toll-like receptors 7 and 9. The later function is required for recruitment of second signals required for full B cell activation and differentiation. In a series of papers, we demonstrated that receptor signaling and endocytic trafficking are intimately related (a-b). BCR-dependent signals control entry into the MIIC and that this trafficking mechanism is regulated in anergy (c). Conversely, receptor endocytosis modulates BCR signaling and fine-tunes signaling responses (d). Before the publication of these papers, BCR endocytic trafficking was thought to be an invariant, constitutive process and not a point of intersection, and regulation, by signaling pathways.

a. Hou P, Araujo E, Zhao T, Massenburg D, Veselits M, Doyle C, Dinner AR and **Clark MR**. 2006. B cell antigen receptor signaling and internalization are mutually exclusive events. PLoS Biology. 4:e200. *Note: featured in Nature Immunology Reviews June 2006.*
b. O'Neill SK, Veselits M, Zhang M, Labno C, Cao Y, Finnegan A, Uccellini M, Alegre M-L, Cambier J, and **Clark MR**. 2009. Endocytic sequestration of the B cell antigen receptor and toll-like receptor 9 in anergic cells. Proc Natl Acad Sci, USA. 106(15):6260-7. PMC2662959
c. **Clark MR**, Tanaka A, Powers SE, and Veselits M. Receptors, subcellular compartments and the regulation of peripheral B cell responses: the illuminating state of energy. 2011. Mol Immunol. 48(11):1281-6. PMC3089810
d. Veselits M, Tanaka A, Chen Y, Hamel K, Mandal M, Kandasamy M, Manicassamy B, O'Neill SK, Wilson P, Sciammas R, **Clark MR**. Ig-beta ubiquitination activates PI-3 kinase signals required for endosomal sorting. 2017. J Exp Med. 214(12):3775-3790. PMC5716028.

Complete list of published work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/marcus.clark.1/bibliography/public/

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: Tambur, Anat R

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

POSITION TITLE: Research Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
The Hebrew University - Hadassah School of Dental Medicine, Jerusalem, Israel	B. Med. Sci.	06/1984	Basic Science Dentistry
The Hebrew University - Hadassah School of Dental Medicine, Jerusalem, Israel	DMD	06/1987	Dental Medicine
The Hebrew University - Hadassah School of Dental Medicine, Jerusalem, Israel	PhD	06/1993	Immunology
Rush University, Chicago Illinois	Post Doctoral	06/1998	Histocompatibility

A. Personal Statement

I have over 25 years of experience in HLA antigen and antibody testing in the context of solid organ as well as stem cell transplantation. From early on in my career I devoted time to promote education and quality of HLA antigen and antibody testing. I was involved in spearheading the credentialing exam for the American Board of Histocompatibility and served as President of that Board as well as President of the American Society for Histocompatibility and Immunogenetics. Driven by my passion to teach, I have established one of the very few fellowships training young HLA laboratory directors more than 10 years ago. I am the founding member of STAR (Sensitization in Transplantation, Assessment of Risk), an on-going AST/ASHI workgroup which became the leading authority on HLA related testing for solid organ transplantation. My main contributions to advancements and understanding of HLA antibody testing include improving depth of data interpretation for single antigen bead testing; and specifically, interrogation of the concept of HLA epitopes as it relates to histocompatibility and immunogenicity. A common thread for many research projects headed by me is understanding the role of HLA-DQ in transplant outcome. In addition, I am currently serving on multiple advisory boards of pharmaceutical companies invested in designing drugs to address unmet needs of the very highly sensitized patients, guiding study design for goals and endpoints revolving around HLA antibodies. Most of my input relates to studies addressing desensitization of highly sensitized patients or treatment of antibody mediated rejection driven by HLA donor specific antibodies. My other role is to oversee all testing relevant for our Stem Cell Transplant program, where I utilize my knowledge in analyzing deep-sequencing of the HLA encoding regions as well as chimerism analysis.

1. **Tambur AR**, Rosati J, Roitberg S, Glotz D, Friedewald JJ, Leventhal JR. Epitope analysis of HLA- DQ antigens: What does the antibody see? Transplantation 2014;98(2):157-166. PMID: 25003284.
2. **Tambur AR**. HLA Epitope Matching or Eplet Risk Stratification – the Devil is in the Details. Frontiers in Immunology, 2018:9:2010, PMID: 30233594.
3. **Tambur AR**. Human leukocyte antigen matching in organ transplantation: What we know and how can we make it better (Revisiting the past, improving the future). Curr Opin Organ Transplant 2018;23(4):470-476. PMID: 29750676.
4. **Tambur AR**, McDowell H, Hod-Dvorai R, Abundis MAC, Pinelli DF. The quest to decipher HLA immunogenicity: Telling friend from foe. Am J Transplant 2019;19(10):2910-2925. PMID: 31165546.

Ongoing Research Support

Vanguard Charitable Endowment Program (Tambur, PI) 03/2018-02/2023
HLA Epitopes in Organ Transplantation – Clinical Applications

Completed Research Support

Vanguard Charitable Endowment Program (Tambur, PI) 09/2018-08/2021
HLA-DQ Immunogenicity and Its Role in Antibody Mediated Rejection in Solid Organ Transplantation

Vanguard Charitable Endowment Program (Tambur, PI) 12/2019-11/2020
Analyzing the Immunogenicity of HLA-DQ in solid organ transplantation

Vanguard Charitable Endowment Program (Tambur, PI) 01/2021-12/2021
Analyzing the Immunogenicity of HLA-DQ in solid organ transplantation: CRISPR Cas 9 edited singly expressed HLA class II cells for the study of HLA DQ imunogenicity

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-Present	Ad-hoc Faculty at The Graduate School, Northwestern University
2020-Present	Founder and Chair – STAR 2022 workgroup
2010-Present	Research Professor, Department of Surgery, Northwestern University, Chicago, IL
2004-Present	Director, Transplant Immunology laboratory, Comprehensive Transplant Center, Northwestern Memorial Hospital
2004-2010	Research Associate Professor, Department of Surgery, Northwestern University, Chicago
2003-2008	Senior Investigator and Head of Transplant Immunology Laboratory, Visiting Associate, Professor, Neurosurgery Department, University of Illinois, Chicago, IL
2001-2004	Director, HLA laboratory, Rush Medical Laboratories, Department of Pathology, Rush University Medical Center, Chicago, IL
1998-2003	Assistant Professor, Heart Failure and Cardiac Transplant Program, Section of Cardiology, Internal Medicine; and the Department of Immunology/Microbiology Rush Medical College, Chicago, IL

Honors and Awards

2022	Recipient of the *Bernard Amos Distinguished Scientist Award* (American Society of Histocompatibility and Immunogenetics)
2022	Research Mentor Award for excellence as a research mentor – Department of Surgery, Northwestern University

Other Experience and Professional Memberships

2018-2019	Founder and Chair – STAR 2019 workgroup
2019-Present	AST Scientific Review Committee - member
2019	Chair – 50 years of innovation – in memory of Dr. Paul I Terasaki
2018-Present	Editorial Board member, Expert Review of Clinical Immunology
2017-Present	Editorial Board member, Tissue Antigens / HLA
2016-2018	Chair - National Clinical Affairs Committee 2015-2017 UNOS board member
2016-2017	Founder and Chair – STAR workgroup (Sensitization in Transplantation: Assessment of Risk – The North American workgroup). Collaboration between the American Society for Histocompatibility and Immunogenetics and the American Society for Transplantation
2016-2017	Immediate past President and Chair of the annual meeting planning committee – American Society for Histocompatibility and Immunogenetics
2015-Present	Associate Editor, American Journal of Transplantation
2015-Present	Associate Editor, Clinical Transplantation
2015-2016	*President* - American Society for Histocompatibility and Immunogenetics
2014-Present	Reviewer, Frontiers in Immunology
2014-2015	*President Elect* – American Society for Histocompatibility and Immunogenetics
2013-Present	Reviewer, JASN

Other Experience and Professional Memberships

2013-2015	Director in Training Review Committee – DTRC – *committee member*
2012-Present	Editorial Board member, Human Immunology
2010-Present	American Society of Transplant Surgeons (ASTS)
2010-Present	The Transplantation Society (TTS)
2010-2012	NMDP Laboratory Advisory team member
2010	Scientific Reviewer for the CIBMTR (Center for International Blood and Marrow Transplant Research) Consensus Conference – Antibody Mediated Rejection (AMR) in Heart Transplantation (ISHLT) – Chicago - invited participant
2009-Present	Founding member [invited] Women Leaders In Transplantation, sponsored by The Transplantation Society
2005	Guest Editor, Neurological Research, October 2005 volume 27(7)
2004-Present	American Society of Transplantation (AST)
2003-Present	Reviewer, Transplantation
2003-2006	Chair of the Proficiency Testing Program committee – (ASHI)
2003-2005	Scientific and Educational Affairs – ASHI. Committee member
2003-2005	President of the American Board for Histocompatibility and Immunogenetics (ABHI)
2001-2007	HLA laboratory director exam committee – ABHI. Committee member
2000-Present	International Society for Heart and Lung Transplantation (ISHLT)
2000-2003	Chair – ABHI – Laboratory Director Exam, Credential committee
1993- Present	American Society for Histocompatibility and Immunogenetics (ASHI)

C. Contributions to Science

1. My research in the last few years focused on understanding the role of HLA-DQ antibodies in antibody mediated rejection (ABMR). Our publications clearly demonstrate that HLA-DQ antibodies are the most frequent antibodies in ABMR but more importantly, provide some insight into the reasons for that. We have also published on physiologic approaches to analyze HLA-DQ antigens and antibodies such that better prediction of virtual crossmatching can take place. Moreover, the results of our studies can now be translated into more equitable means of assigning cPRA point for the highly sensitized patients as well as providing them with better and broader opportunities of identifying HLA compatible donors and minimizing the risk for developing ABMR. The group of four publications listed below that relate to this work, have been cited 73 times by authors in 20 different countries, indicating extensive dissemination of this body of work (Scopus).
    a. **Tambur AR**, Kosmoliaptsis V, Claas FHJ, Mannon RB, Nickerson P, Naesens M, Significance of HLA-DQ in kidney transplantation: time to reevaluate human leukocyte antigen-matching priorities to improve transplant outcomes? An expert review and recommendations. Kidney Int 2021, 100:1012- 1022. PMID:34416097
    b. **Tambur AR**. Human Leukocyte Antigen matching in organ transplantation: what we know and how can we make it better (Revisiting the past, improving the future). Curr Opin Organ Transplant 2018, 23:470-476. PMID: 29750676.
    c. **Tambur AR.** HLA-DQ antibodies: are they real? Are they relevant? Why so many? Currently Opinion in Organ Transplantation. 2016;21(4):441-446. PMID: 27200497.
    d. **Tambur AR**, Rosati J, Roitberg S, Glotz D, Friedewald JJ, Leventhal JR. Epitope analysis of HLA- DQ antigens: what does the antibody see? Transplantation, 98(2):157-166, 2014. PMID: 25003284
2. Recent studies from my laboratory paved the way for new approaches to study and analyze HLA epitopes. The outcome of this research not only will help minimize the risk of developing ABMR but will also minimize the risk of developing de-novo HLA antibodies, with ramification for minimizing the likelihood of patients becoming highly sensitized (for future need for organ re-transplantation). This is particularly true for the pediatric patients who inevitably require additional transplants in their life span. When evaluating the group

of three publications below that discuss this work through the lens of the NIH, the Weighted RCR is noted to be 5.03, indicating it is a highly influential set of articles relative to NIH- funded papers (iCite).

a. Garcia-Sanchez C, Usenko CY, Herrera ND, **Tambur AR**. The shared epitope phenomenon – a potential impediment to virtual crossmatch accuracy. Clin Transplant 2020;34(8). PMID: 32418254.
b. Senev A, Coemans M, Lerut E, Van Sandt V, Kerkhofs J, Daniëls L, Vanden Driessche L, Compernolle V, Sprangers B, Van Loon E, Callemeyn J, Claas F, **Tambur AR**, Verbeke G, Kuypers D, Emonds M-P, Naesens M. The impact of eplet mismatch load on de novo occurrence of donor- specific anti-HLA antibodies, rejection, and graft failure after kidney transplantation: an observational cohort study. J Am Soc Nephrol 2020;31(9):2193-2204. PMID: 32764139, PMCID: PMC7461684.
c. **Tambur AR**, McDowell H, Hod-Dvorai R, Casillas Abundis MA, Pinelli DF. The quest to decipher HLA immunogenicity: Telling Friend from Foe. Am J Transplant. 2019: 19:2910-2925, PMID: 31165546.

3. As a clinician I strive to improve patient care by better assessment of HLA antibodies during the time frame leading to transplantation and during monitoring post transplantation. I have founded an AST-ASHI workgroup that launched the STAR (Sensitization in Transplantation: Assessment of Risk) series, already published two practice recommendations and am currently working on the third. I also participate in other consensus meetings such as for the TTS and further providing guidelines for clinical recommendation. The articles referenced below ranked highly for citation-impact with each in the 93rd percentile or higher when benchmarked against documents published in the same year, subject category and document type (InCites).

a. **Tambur AR**, Camplbell P, Class FH, Nickerson P. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. AJT 2018 18:1604-1614, PMID: 29603613.
b. **Tambur AR**, Campbell P, Chong AS, Feng S, Ford ML, Gebel H, Gill RG, Kelsoe G, Kosmoliaptsis V, Mannon RB, Mengel M, Reed EF, Valenzuela NM, Wiebe C, Dijke IE, Sullivan HC, Nickerson P. Sensitization in Transplantation: Assessment of Risk (STAR) 2019 Working Group Meeting Report. AJT 2020 20:2652-2668, PMID: 32342639, PMCID: PMC7586936.
c. Schinstock CA, Mannon RB, Budde K, Chong AS, Haas M, Knechtle S, Lefaucheur C, Montgomery RA, Nickerson P, Tullius SG, Ahn C, Askar M, Crespo M, Chadban SG, Feng S, Jordan SC, Man K, Mengel M, Morris RE, O'Doherty I, Ozdemir BH, Seron D, **Tambur AR**, Tanabe K, Taupin J-L, O'Connell PJ. Recommended treatment for antibody mediated rejection after kidney transplantation: 2019 expert consensus from The Transplant Society working group. Transplantation *104:911-922,* PMID: 31895348, PMCID: PMC7176344.
d. Pinelli DF, **Tambur AR**. Virtual Cross Matching for Deceased Donor Transplantation: One Size Does Not Fit All. Kidney International. 2020 Apr;97(4):659-662, PMID: 32200862.

4. My laboratory help identify approaches to improve the clinical utility of the single antigen bead assay – by means of providing granular information regarding antibody-strength and recommendation on how to use this information in clinical practice. The subject matter of this contribution has garnered attention outside of the clinical world, with the 2018 publication listed below being mentioned in 37 news stories and in 34 tweets from 27 users, with an upper bound of 27,634 followers (Altmetric).

a. Pinelli DF, Zachary AA, Friedewald JJ, Gjertson DW, Evans MD, Chatroop EN, Leffell MS, Vo AA, Jordan SC, Montgomery RA, **Tambur AR**. Prognostic tools to assess candidacy for and efficacy of antibody removal therapy. Am J Transplant 2019;19(2):381-390. PMID: 29981209.
b. **Tambur AR**, Schinstock C, Maguire C, Lowe D, Smith B, Stegall M. Estimating alloantibody levels in highly sensitized renal allograft candidates: using serial dilutions to demonstrate a treatment effect in clinical trials. Am J Transplant 2021;21(3):1278-1284. PMID: 33078553.
c. Maguire AH, Schinstock CA, **Tambur AR**. Measuring HLA alloantibodies – beyond a binary decision. Curr Opin Organ Transplant 2020;25(6):529-535. PMID: 33055530.
d. Schinstock C, **Tambur A**, Stegall M. Current Approaches to Desensitization in Solid Organ Transplantation. Front Immunol 2021;12. PMID: 34046044, PMCID: PMC8144637.

5. The lack of high-resolution data for HLA typing led many investigators to use imputation, or in other words - assumed substitution for missing data, which can lead to use of inaccurate information. We have counseled against the use of imputation supported by data and mathematical explanation. The articles listed below are published in two highly influential journals with impact factors of 11.2 and 8.1, and are noted to be in the first quartile in the subject areas of Surgery, Transplantation, and Multidisciplinary Sciences (JCR).

a. Manski CF, **Tambur AR**, Gmeiner M. Predicting kidney transplant outcomes with partial knowledge of HLA mismatch. Proc Natl Acad Sci U S A 2019;116(41):20339-20345. PMID: 31548419, PMCID: PMC6789916.
b. Engen RM, Jedraszko AM, Conciatori MA, **Tambur AR**. Substituting imputation of HLA antigens for high resolution HLA typing: Evaluation of a multiethnic population and implications for molecular mismatch evaluation for clinical decision making. In transplantation. Am J Transplant 2021;21(1):344-352. PMID: 32430947.
c. Engen RM, Tambur AR, Accurate eplet identification is necessary for accurate risk assessment. Am J Transplant 2021, PMID: 33866684

Complete list of published work in MyBibliography:

https://www.ncbi.nlm.nih.gov/mync bi/1r5T4YaWrn9Au/bibliography/public/

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: Amishi Desai, DO

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

POSITION TITLE: Associate Professor of Medicine

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 Illinois, Champaign-Urbana, IL	B.S.	2000	Microbiology/Political Science
Midwestern University CCOM, Downers Grove, IL	D.O.	2005	Medicine

A. Personal Statement

I am a transplant nephrologist at Northwestern Medicine. Prior to joining Northwestern Medicine in September of 2022, I was the Medical Director of Kidney/Pancreas Transplantation and Division Director for Transplant Nephrology at Loyola University Medical Center. In both of my current roles, I make daily decisions about the care of kidney transplant recipients and am keenly aware of the shortcomings of our current tools to monitor patients and individualize care. It is well known that kidney transplantation treats end-stage kidney disease but despite advances in pharmacological immunosuppression, antibody mediated rejection (AMR) continues to limit the longevity of the renal allograft. The kidney biopsy remains the 'gold standard' for diagnosing rejection and more specifically, in AMR, pathology plus the presence of human leukocyte antigens (HLA) helps to diagnose AMR. Unfortunately, greater than 50% of AMR biopsies are not associated with detectable donor HLA-specific antibodies (DSA). For the physician, the presence of AMR on the biopsy without DSA presents a management conundrum. Are there non-HLA antigens mediating AMR in the patient?

Presently, there is a movement in kidney transplantation towards more non-invasive kidney monitoring with pursuit of donor-derived cell-free DNA (dd-cfDNA) testing. I have participated in many studies regarding the varying industry-derived product but all remain inferior when it comes to AMR. This project aims to look for non-HLA antigens that may be mediating AMR. I am asked to collaborate in this study given my clinical expertise and background in kidney transplantation. I have collaborated with and been a Co-Investigator in NIH-sponsored and Industry sponsored clinical trials. Recently and specific to ongoing non-invasive rejection monitoring, I was the Principal Investigator and Co-Investigator for industry-sponsored trials in dd-cfDNA use in our transplant recipients. In addition, I have extensive experience in the management and care of patients with AMR. With my role and experience, I will be in an excellent position to support Dr. Chong as a clinician partner for this grant proposal. I am committed to working with her to facilitate and execute all proposed portions of the grant proposal.

<u>The following displays my key ongoing and completed research projects from the past three years:</u>

2021-2022 AlloVir
Principal Investigator, Industry-sponsored/funded
NCT04605484
Study of Posoleucel in Kidney Transplant Patients with BK Viremia: multicenter, randomized, double-blinded, placebo-controlled, multiple dosing interval, 3-period study of the safety, tolerability and effectiveness of adoptively transferred ALVR105 multivirus-specific T cells

2021-2022 Natera: Multi-Organ Transplant Rejection Detection (MOTR)

NCT04091984
Co-Investigator, Industry-sponsored/funded
Use of dd-cfDNA in multi-organ recipients to detect rejection

2019-2022 Natera: The PROspera Kidney Transplant ACTIVE Rejection Assessment Registry (ProActive) study
Co-Investigator, Industry-sponsored/funded
Use of prospera dd-cfDNA to prospectively monitor patients post-transplantation for allograft dysfunction

2019-2022 Care Diagnostics: Outcomes from the Kidney Allograft Outcomes Allosure Registry (OKRA)
Principal Investigator, Industry-sponsored/funded
Evaluation of patient outcome from the KOAR data registry

2019-2022 APOL-1 Long-Term Kidney Transplantation Outcomes Network – APOLLO
NCT03615235
Principal Investigator, NIH funded
Longitudinal trial assessing the impact of the novel APOL-1 gene impact on African American donor and transplant recipients

2018-2022 Medeor Therapeutics: HLA-matched Living Donor Kidney Transplant
NCT03363945
Co-Investigator, Industry-sponsored/funded
Phase 3, randomized, multi-Center, open-label, controlled trial to assess the efficacy and safety of cellular immunotherapy with MDR-101 for induction of immune tolerance in recipients of HLA-matched, living donor kidney transplants

2018-2022 Care Diagnostics: Kidney Allograft Outcomes AlloSure Registry (KOAR)
NCT03326076
Principal Investigator, Industry sponsored/funded
Evaluation of patient outcomes from longitudinal dd-cfDNA monitoring in kidney transplant recipients

B. Positions, Scientific Appointments, and Honors

Positions:

2022-Present Associate Professor of Medicine, Divisions of Nephrology/Hypertension and Organ Transplant, Northwestern University Feinberg School of Medicine, Chicago, IL
2019-2022 Unit Medical Director Transplant/Hepatology Floor, Loyola University Medical Center Maywood, IL
2017-2022 Director Division of Transplant Nephrology, Loyola University Medical Center, Maywood, IL
2018-2022 Associate Professor of Medicine, Division of Transplant Nephrology, Loyola University Medical Center, Maywood, IL
2015-2022 Medical Director, Kidney and Pancreas Transplantation, Loyola University Medical Center Maywood, IL
2015-2018 Assistant Professor of Medicine, Division of Transplant Nephrology, Loyola University Medical Center, Maywood, IL
2014-2015 Director of Transplant Fellowship, University of Illinois Medical Center, Chicago, IL
2012-2015 Assistant Professor of Medicine, Division of Nephrology, University of Illinois Medical Center, Chicago, IL
2011-2012 Clinical Instructor Medicine, Division of Nephrology, University of Chicago Medicine, Chicago, IL
2010-2011 Fellowship in Transplant Nephrology, University of Chicago Medicine, Chicago, IL
2008-2010 Fellowship in Nephrology, University of Chicago Medicine, Chicago, IL

2005-2008 Medicine Residency, Internal Medicine, McGaw Medical Center of Northwestern University,

Evanston, IL

Other Experience and Professional Memberships:

2022	ASN DO Community of Practice Leader
2022	SRTR People Driven Transplant Metrics Consensus Conference
2021-Present	National Kidney Foundation Member
2021-2022	Elected to Loyola University Medical Center Faculty Council
2020-2022	UNOS/OPTN Nominating Committee Member
2020-2022	UNOS/OPTN at Large - Board Member
2019- Present	Women in Transplantation (WIT) Pillar 1 Member
2017-2020	Group lead for Socio-Economic Disparities Work Group – UNOS
2016-Present	UNOS/OPTN Hospital and Regional Representative
2016-2022	Gift of Hope Advisory Council Member
2016-2020	NKFI Professional Advisory Board Member
2016-2019	UNOS/OPTN Region 7 Representative for Minority Affairs Committee
2016	NKFI Nephrology Debate Mentor for Loyola Team
2015-Present	National Kidney Foundation of Illinois (NKFI) Member
2015-2021	NKFI Professional Advisory Executive Committee member
2015-2016	Past Chair for Trainee and Young Faculty Community of Practice – AST
2015	National Kidney Foundation of Illinois (NKFI) Nephrology Debate Chair
2014-2015	Chair for Trainee and Young Faculty Community of Practice – AST
2013-2016	Editor of Trainee and Young Faculty Quarterly Newsletter – AST
2013-2014	Co-chair for Trainee and Young Faculty Community of Practice – AST
2011-2015	Trainee and Young Faculty Community of Practice member – AST
2011-2012	Young Trainee/Fellow representative for Women's Health Community of Practice – AST
2010-Present	American Society of Transplantation
2010-Present	Women in Nephrology member – ASN
2008-Present	American Society of Nephrology

Honors:

2022-Present	ASN Osteopathic Nephrologist Community of Practice Leader
2021-2022	Loyola University Medical Center Faculty Council (elected)
2020-2022	UNOS/OPTN Board Member at Large (elected)
2016-2019	UNOS/OPTN Region 7 Representative for Minority Affairs Committee (elected)
2013-2016	Chair of Trainee and Young Faculty Community of Practice, AST (elected)
2011-2012	Young Trainee/Fellow Representative for AST Women's Health Community

C. Contributions to Science

1. Methods to monitor allograft function are limited during acute infection where biopsies may not be feasible. Non-invasive biomarkers of renal allograft status would greatly advance knowledge of the renal allograft in the critical care setting. We trialed the use of non-invasive biomarkers in patients with SARS-CoV-2 to assess allograft injury.
   a. Reusing JO, Yoo JW, **Desai A,** Brossart K, McCormick S, Malashevich AK, Blook MS, Fehringer G, White R, Billings PR, Tabriziani H, Demko ZP, Cauthier P, Akkina SA, David-Neto E. Association between total cell free DNA and SARS-CoV-2 in Kidney Transplant Patients: A Preliminary Study. Transplant Proceedings. March 2022

2. Access to transplantation and health disparity play a significant role within recipient kidney transplantation. Bridging the gap in education, social support and post-transplant care is critical to allograft longevity. I have served on communities to address minority disparities, spoken on many platforms about health disparity and participated in trials comparing minority populations.
   a. **Desai A,** Sodhi R, Akkina S. Kidney Transplant Outcomes in Minority Populations: Can We Close the Gap? Current Opinion in Organ Transplantation. October 2021. 26:5

b. **Desai A,** Chon WJ, Arwindekar D, Tang IY, Josephson MA, Akkina S. Impact of Maintenance Steroids versus Rapid Steroid Withdrawal in African-American Kidney Transplant Recipients: Comparison of Two Urban Centers. Int J Clin Med. 2016. 7:204-216
c. Robinson JK, Friedewald JJ, **Desai A,** Gordon EJ. A Randomized Controlled Trial of a Mobile Medical App for Kidney Transplant Recipients: Effects on Use of Sun Protection. Transplantation Direct. 2016. Jan 2(1):e51
d. Robinson JK, Kwasny ScD, Friedewald JJ, **Desai A,** Gordon EJ. A randomized controlled trial of sun protection education delivered with tablet personal computers: response across the health literacy spectrum of kidney transplant recipients. JMIR Cancer. 2015. 1(2):e8

3. Immunosuppression as it relates to long-term health risk and immune responses is continually being studied. I have participated in trials looking at long term health risks of steroid use as well as the immune response as it relates to vaccines and infection.
   a. Reusing JO, Yoo JW, **Desai A,** Brossart K, McCormick S, Malashevich AK, Blook MS, Fehringer G, White R, Billings PR, Tabriziani H, Demko ZP, Cauthier P, Akkina SA, David-Neto E. Association between total cell free DNA and SARS-CoV-2 in Kidney Transplant Patients: A Preliminary Study. Transplant Proceedings. March 2022
   b. Cowan M, Chon WJ, **Desai A,** Andrews S, Bai Y, Veguilla V, Katz JM, Josephson MA, Wilson PC, Sciammas R, Chong AS. Impact of Immunosuppression on Recall Immune Responses to Influenza Vaccination in Stable Renal Transplant Recipients. Transplantation. 2013. 97(8): 846–853
   c. Edwards BJ, **Desai A,** Tsai J, Du H, Edwards GR, Bunta AD, Hahr A, Abecassis M, Sprague SM. Elevated incidence of fractures in solid organ transplant recipients on glucocorticoid-sparing immuno-suppressive regimens. Journal of Osteoporosis. 2011. 2011: 591793

4. Access preservation in chronic kidney disease is essential in long term renal replacement therapy. All too often care is not taken in avoiding peripheral access to preserve vasculature for dialysis access. Not only is access preservation critical in our chronic kidney disease population but it is important to be mindful of this in our kidney transplant recipients to assure future access if the allograft fails.
   a. Hammes M, **Desai A,** Pasupneti S et al. Avoidance of Peripherally Inserted Central Catheters in CKD: Incidence and Predictive Factors of Venous Thrombosis. Clin Nephrol. 2015. 84(7):21-8