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TITLE: High-Throughput TCR Repertoire-Based Platforms for Antigen-Specific Cancer Immunotherapy

PRINCIPAL INVESTIGATOR: Brandon DeKosky

CONTRACTING ORGANIZATION: The University of Kansas Center for Research, Inc.
Lawrence, KS

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6. AUTHOR(S)

Brandon DeKosky

E-Mail: dekosky@ku.edu

5d. PROJECT NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Kansas Center for Research, Inc.
2385 Irving Hill Road
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We seek to develop new platform technologies that will help us to better understand why and how immune-based cancer treatments are effective, and to apply that fundamental knowledge to develop rapid, targeted cancer therapeutics and improve cancer care. Modern immune-based therapies have shown tremendous success for treating many different kinds of cancers, and T cells play a critical role in these treatments because they have a unique ability to specifically target and selectively destroy tumor cells. However, T cells are difficult to analyze in the laboratory because each T cell has multiple unique genes, and thus each T cell must be studied one cell at a time. In the prior reporting period, we have made progress toward establishing these new systems using both control TCRs and with patient immune libraries. We are excited to apply these technologies to understand the mechanistic features of cancer-specific T cell targeting, and apply that information to develop more precise and effective cancer therapeutics in future reporting periods.

15. SUBJECT TERMS

Key words or phrases identifying major concepts in the report

T cell receptor; single-cell analysis; T cell screening, Next-generation sequencing; renal cell carcinoma

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** We seek to develop new platform technologies that will help us to better understand why and how immune-based cancer treatments are effective, and to apply that fundamental knowledge to develop rapid, targeted cancer therapeutics and improve cancer care. Modern immune-based therapies have shown tremendous success for treating many different kinds of cancers, and T cells play a critical role in these treatments because they have a unique ability to specifically target and selectively destroy tumor cells. However, T cells are difficult to analyze in the laboratory because each T cell has multiple unique genes, and thus each T cell must be studied one cell at a time. This study will overcome these barriers and develop new ways to analyze T cell responses for millions of cells at once, allowing us to understand anti-cancer T cell responses at a much broader scale than is currently possible. We will apply these technologies to understand the mechanistic features of cancer-specific T cell targeting, and apply that information to develop more precise and effective cancer therapeutics.

2. **KEYWORDS:** T cell receptor; single-cell analysis; T cell screening, Next-generation sequencing; renal cell carcinoma

3. **ACCOMPLISHMENTS:**
    - **What were the major goals of the project?**
        - Specific Aim 1
            - Major Task 1: Develop TCRα:β cloning platforms for transducing patient naïve T cells
            - Major Task 2: Sort repertoires cloned into naïve T cells for activation by primary RCC tumor cell samples.
            - Major Task 3: Perform analysis and cloning of anti-tumor TCR responses using melanoma tumor samples.
        - Specific Aim 2
            - Major Task 1: Develop a workflow for cloning linked TCRα:β genes into TCR surface display expression vectors.
            - Major Task 2: Validate sort strategies using small numbers of known antigen-specific TCRs.
            - Major Task 3: Clone and transduce a large library from lymphoma patients for TCR panning, and quantify library size and efficiency. Sort against lymphoma BCR neoantigens to validate anti-cancer TCR sorting capabilities.
    - **What was accomplished under these goals?**

1) Major activities

Our laboratory has made steady progress on this CDMRP project, and the lab has continued to produce data at a rapid pace. We developed and validated our single-cell emulsion devices for single T cell analysis, and we published one manuscript on T cell response analysis, with a second manuscript under review after resubmission. We have established computational pipelines for analyzing TCR NGS data, and we published a research methods article to share these advances with the broader community. We have established the experimental, bioinformatic, and collaborative infrastructure necessary to implement these projects, and have completed substantial data acquisition and analysis for our studies in anti-cancer TCR research.

We have two postdocs who have focused on this project, in addition to help from other undergraduate and postdoctoral students. We have now established our bioinformatic scripts for data analysis and optimized yield of our custom single-cell workflows and developing methods for simpler single cell analysis. We finalized the

development of library preparation strategies and have been applying our techniques for live cell co-culture for anti-cancer TCR identification.

One major publication from this work has been published related to the development and application of natively paired T cell receptor functional screening pipelines (Aim 1 Task 1, Aim 2 Major Tasks 1 and 2), with another publication related to TCR sequence analysis published, and a third manuscript now under 2nd review. We also have analyzed our first RCC patient samples (Aim 1 Task 2), with several libraries showing positive data for anti-cancer TCR screening, and we analyzed the sequences of those samples for TCR generation and validation.

2) Specific Objectives

We refined our experimental methods for large-scale processing of T cell samples and library screening, and applied them to perform high-throughput functional analysis of natively paired alpha:beta T cell receptors in a variety of settings. We established our system for *in vitro* T cell stimulation as part of Valpha:Vbeta sequencing, published in 2022, and we also submitted a paper related to the establishment of live cell co-culture methods for needed to identify anti-cancer T cells. We developed a robust TCR library cloning and expression system, whereby we introduce silent and conservative mutations at the alpha and beta constant regions and leader regions, to allow for massively parallel cloning of natively paired and physically linked alpha:beta amplicons derived from single T cells. We validated our approach to dissect fine affinity features that will compare not just TCR affinity, but the on-rate and off-rate of T cells to peptide:MHC targets, as described in a paper published this year. We also applied these unique technologies for analyzing cell-based TCR activation using live cell co-culture using a samples from multiple RCC patients for anti-cancer TCR discovery, in collaboration with the Godwin laboratory at the University of Kansas Medical Center, and this work is ongoing.

We advanced our technologies for rapid computational profiling of TCR immune responses, including several techniques for TCR response analysis in a recently published paper. We applied our methods for rapid interpretation of T cell receptor NGS data, including for the identification of antigen-specific TCRs. We finalized pipelines for compiling and interpreting TCR prevalence after various library screening conditions, as reported in a collaborative methods article released in 2022.

Our major current objectives were to publish the current version of the TCR library generation and screening protocols (Aim 1 Task 1, Aim 2 Tasks 1 and 2) in a series of publications, of which the first two have been published the next is under 2nd review. We have applied this technology in parallel to identify the anti-cancer TCRs in several RCC patients (Aim 1 Task 2), which is the current major objective that we are now focused on and generating strong data. Finally, once these pipelines are fully established with RCC patient samples, we will apply them for analysis of melanoma tumor samples (Aim 1 Task 3) and for lymphoma samples (Aim 2 Task 3).

3) Significant Results

We achieved major progress using our single cell platforms and strategies for advancing the bioinformatic analysis of these datasets to interpret immune function. Experimentally, in a different project we developed a new technology for precise interrogation of NGS datasets for immune receptor function, which was published in the *Proceedings of the National Academy of Sciences* (Madan & Zhang et al, PNAS 2021), and we have applied these technologies for the NGS-based analysis of T cell receptor affinity and specificity in a paper that was published in 2022 (Fahad & Chung, *Protein Engineering, Design, & Selection* 2022).

We also made major advances in workflows for high-throughput paired functional analysis of natively paired alpha:beta T cell receptor genes (Aim 1 Task 1, Aim 2 Tasks 1 & 2). We have applied our primer set and cloning strategy for the amplification of human T cell receptors and display on lentivirally transduced mammalian T cells (**Figure 1, Figure 2**), and we are now using it with human T cell receptor libraries, as described in our published paper.

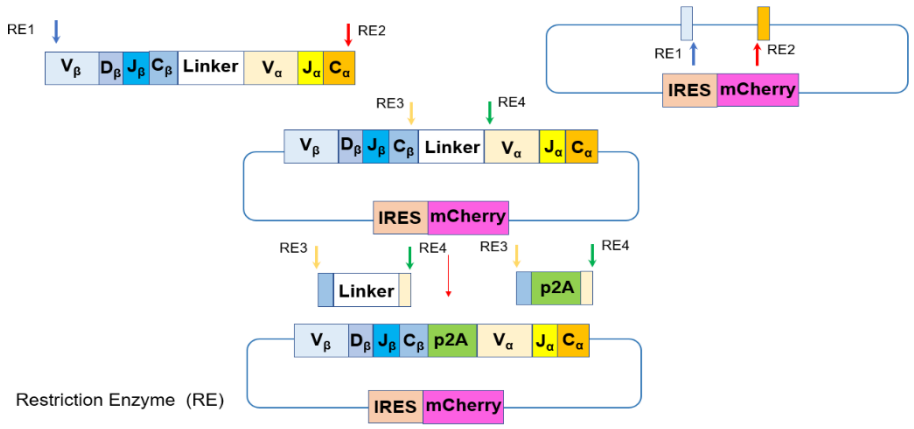

Figure 1: *Cloning strategy for display of our linked Valpha:Vbeta TCR genes. Two unique restriction enzymes at the outside and inside of the linked TCR gene amplicon are used to clone the amplicon into display plasmids. A p2a sequence is cloned into the center to allow for separate expression of alpha and beta chains, while an IRES and mCherry sequence is used to quantify TCR transcription. Surface staining for TCR constant region genes is used to determine surface TCR expression.*

We fully developed our sequencing and cloning workflow, and we have sequenced and displayed several human TCR repertoires (as shown in **Figure 3**). We applied the T cell repertoires for the discovery of antigen-specific TCR genes, and we identified some enriched cell populations after p:MHC staining and screening that contain multiple antigen-specific TCRs of varying affinities. These efforts are described in Fahad & Chung *PEDS* 2022.

We shifted our focus onto library screening assays and live-cell killing and activation assays for the identification of cancer cell-specific TCRs in the past year's efforts. We finalized publication related to cell-based activation screening methods, which is currently under 2nd review. We are also finalizing experiments for anti-RCC T cell immune responses, and we recently identified a set of TCRs that appear to target RCC cells in our NGS data.

On the bioinformatic side, we have applied new workflows to analyze T cell receptors and to track their prevalence across screening rounds, following cell sorting for either live-cell activation (e.g. against co-culture cancer cells) or peptide:MHC staining directly. We have used these methods to analyze the diversity of cloned natively paired alpha:beta chains, confirming the accuracy of our native T cell pairing workflows (Fahad & Chung *PEDS* 2022). We also applied them for the analysis of antigen-specific T cell receptors from sorted TCR libraries (**Figure 3**). We successfully applied NGS for screening affinity of pMHCs directly, with positive results.

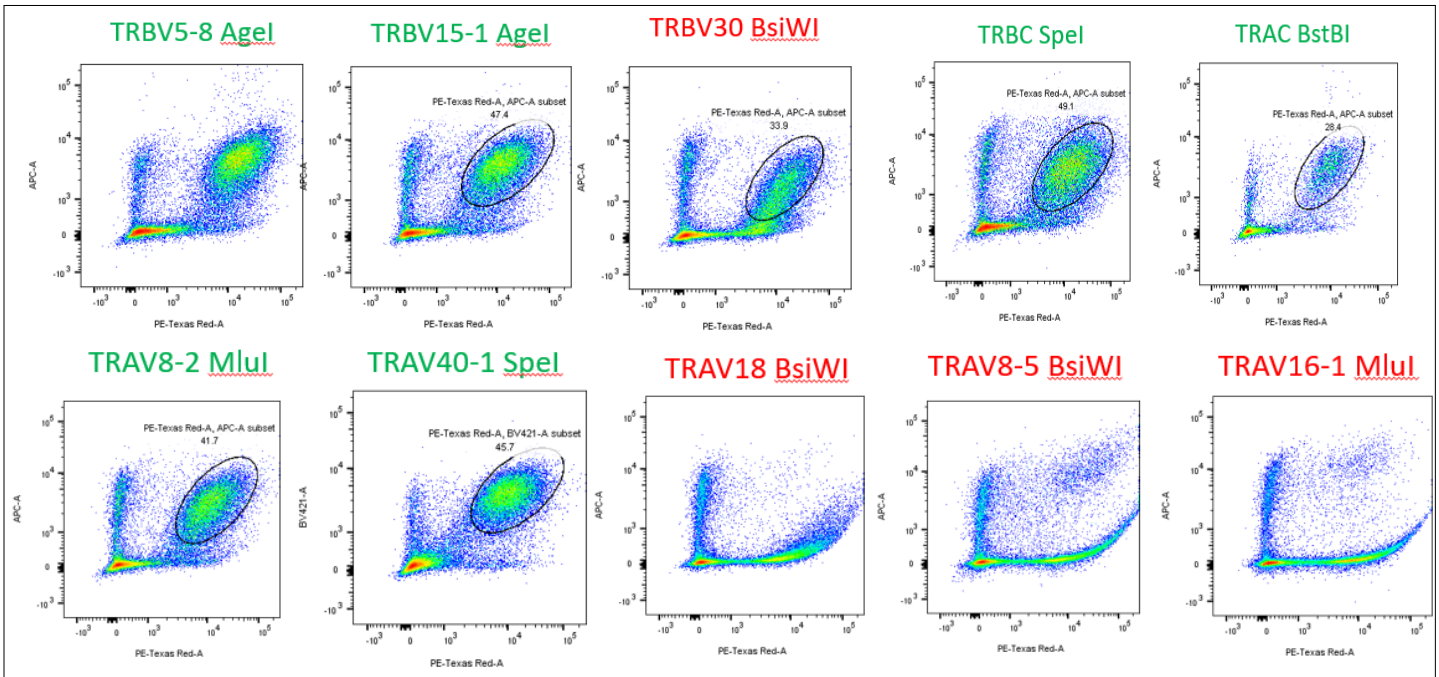

Figure 2: *A small panel of the restriction enzymes and leader peptides (for TRAV/TRBV) that we tested for allowing proper TCR display, using JRT3 cells displaying a known anti-HIV positive control antibody. mCherry expression is*

shown on the x-axis, while HIV peptide p:MHC binding is shown on the y axis. By performing this analysis individually, we were able to determine a set of mutations that allowed for proper TCR display (successful restriction enzymes for use in our cloning scheme are shown in green).

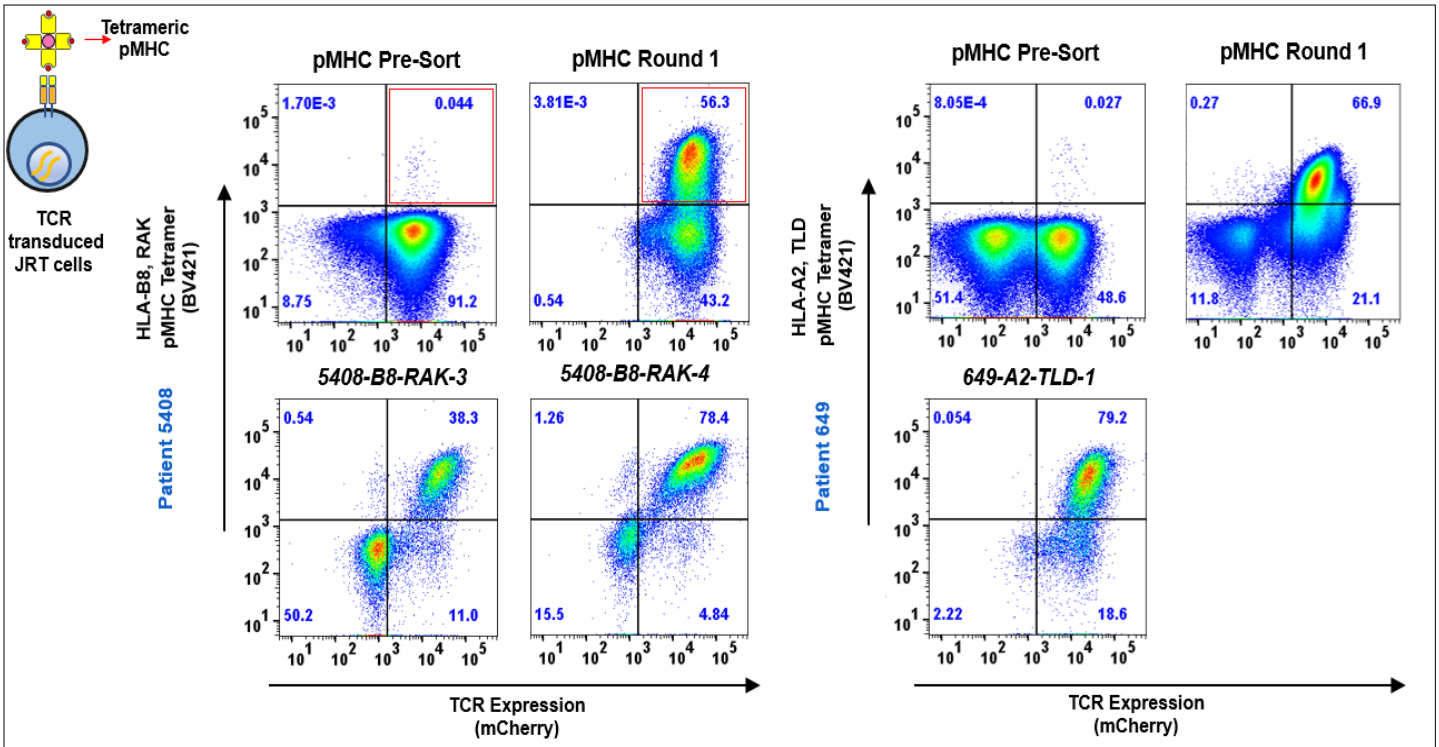

Figure 3: *Analysis of infectious mononucleosis patient samples for anti-EBV peptides as validation of our screening workflows. These data demonstrate the effective screening of immortalized TCR libraries from human patients using soluble peptide:MHC fluorescent screening antigens.*

We also established the platforms for detecting live cell activation in co-culture cell systems (**Figure 4**). These efforts are in a paper that is currently under 2nd review. We have also applied these technologies for the screening of for anti-RCC patient libraries from our collaborator, Dr. Andrew Godwin, and his team at the KU Medical Center (**Figure 5**).

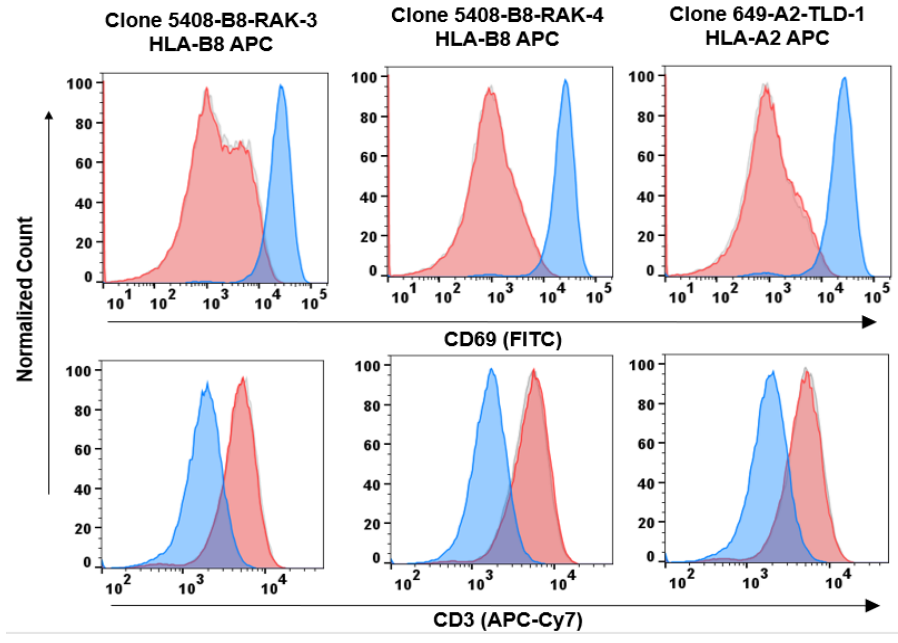

Figure 4: *Activation-based screening of T cell receptor libraries for antigen-specific T cell receptors. Antigen-specific T cell clones that we discovered using our single-cell TCR screening workflow were incubated with antigen-presenting cells (APCs) either with a targeted peptide pulse (blue), without any peptide pulse (red), or with a mismatched peptide (gray). These data show that activation-specific changes can be detected using the CD69 and CD3 fluorescent markers, for robust transduced library sorting. We will apply these methods for screening anti-cancer T cell libraries in the coming reporting period.*

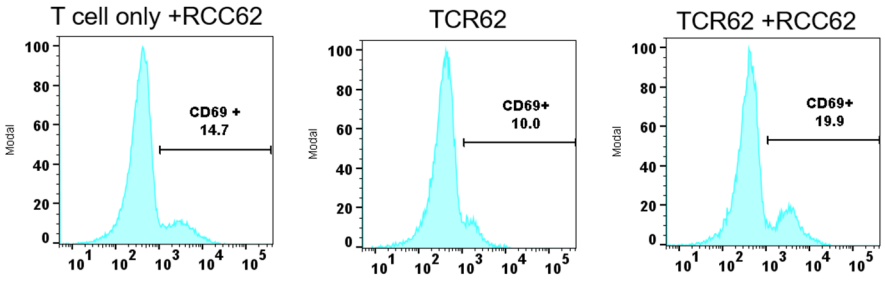

Figure 5: *Activation-based screening of human T cell repertoires against personalized cancer cell immune responses. TCR genes were captures from tumor-infiltrating lymphocytes and cloned into lentiviral display in human T cells. In parallel ("TCR62"). In parallel, patient-matched tumor cells were co-cultured with the cloned T cell libraries ("RCC62"). T cell activation was measured using the CD69 marker (x-axis). These data demonstrate higher responses in patient-matched T cell libraries co-cultured with cancer patient cells, presenting a new method to identify highly specific T cell responses against cancer cells.*

4) Key Outcomes or Other Achievements

We have achieved major progress on our research goals. In the next reporting period, we will continue to make public presentations about these exciting new platforms and submit our findings for publication to additional peer-reviewed journals. We look forward to isolating human anti-cancer T cells in the coming reporting period and evaluating their ability to target cancer cells *in vitro*.

- **What opportunities for training and professional development has the project provided?**

This project has provided training for postdoctoral researchers Andrew Chung, Ahmed Fahad, Matias Gutierrez, Bharat Madan, Penny Timms, Viridiana Montessoro, and for technician Nicoleen Boyle. Andrew was our project lead at The University of Kansas, along with Ahmed Fahad, in advancing methods and techniques for T cell receptor analysis of anti-cancer immunity. Matias assisted Andrew and also helped to develop new bioinformatic techniques, and Bharat Madan helped develop those bioinformatic methods as well. Nicoleen assisted with the cloning and sample analysis of TCR libraries. After the laboratory's transition to Massachusetts General Hospital, the current efforts are now being implemented by Viridiana Montessoro and Penny Timms.

This project provided for the training and professional development of the (then) graduate student Ahmed Fahad, who has developed computational approaches for the rapid interrogation and analysis of TCR display functional data, and has also made major contributions to the live-cell sorting protocols of these experiments. Ahmed finished his Ph.D. in February 2022, published three papers related to this work (two published, one under 2nd revision), and is currently a post-doc at the lab. We have also trained undergraduate students Mattison Sills, and research technicians John Zhou and Shauna Moore who worked under the supervision of Dr. Andrew Chung with TCR transduction experiments.

- **How were the results disseminated to communities of interest?**

Conference presentations related to this work:

- *GRK 2504 / Friedrich-Alexander-Universität (FAU) 3rd Annual Collaborative Retreat,* The Ragon Institute, Cambridge MA (Sep 2022)
- *NIH Mechanistic Studies in Transplantation Workshop / CTOT*, Bethesda, MD (June 2022)
  -Also invited as expert panel discussant on new strategies in immunology
- *The Protein Engineering Summit (PEGS-Boston)*, Boston, MA (May 2022)

- *America's Antibody Congress, Festival of Biologics*, San Diego, CA (Mar 2022)
  -Also invited as expert panel discussant on next-generation antibody therapeutics
- *PepTalk: The Protein Science Week*, San Diego, CA (Jan 2022)
- *Antibody Engineering, Phage Display & Immune Repertoire Analysis Course*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (Nov 2021)
- *American Association of Pharmaceutical Scientists (AAPS)*, Philadelphia, PA (via Webinar, Oct 2021)

Academic & industrial seminar presentations related to this work:

- *NIAID Vaccine Research Center / NIH Main Campus,* Bethesda, MD (June 2022, *to reschedule*)
- *Seeker Biologics, Inc,* Boston, MA (Jun 2022)
- *Cell Profiling and Neoantigen Prediction Working Group, US Food & Drug Administration,* Silver Spring, MD (virtual visit, April 2022)
- *Department of Chemistry, The University of Nebraska at Omaha,* Omaha, NE (virtual visit, April 2022)
- *Department of Chemistry, The University of Kansas,* Lawrence, KS (virtual visit, March 2022)
- *Utrecht Molecular Immunology (UMI), University Medical Center Utrecht,* **Utrecht, Netherlands** (virtual visit, Sep 2021)

- **What do you plan to do during the next reporting period to accomplish the goals?**

We will continue to advance experimental work and prepare additional publications in the coming year. We screened anti-peptide:MHC TCR libraries from four different RCC clinical samples and achieved positive results on our experimental and bioinformatic parameters for T cell receptor analysis. We have now analyzed these data and will validate anti-RCC TCRs in the next reporting period. These efforts were published in two articles in 2022. Future studies will also investigate the use of PDX mouse models for evaluation of the protective effects of the anti-cancer TCRs that we discover.

In parallel with our experimental advances we are also continuing to optimize our methods for computational interrogation of immune datasets. We have prepared one bioinformatics-focused paper on quality control of TCR repertoire analysis of screened libraries in the past year, and we plan to prepare another paper focused on antigen-specific TCR repertoire analysis in the coming reporting period.

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - The advances we have made in the previous reporting period have enabled the large-scale interrogation of T cell receptors for anti-cancer immune responses. This will greatly impact future studies on T cell responses against cancer neoantigens, and may lead to new personalized and targeted cancer therapeutics.
   - **What was the impact on other disciplines?**
     - Our initial progress in T cell receptor screening technologies will also enable the analysis of viral infections and autoimmunity in other fields.
   - **What was the impact on technology transfer?**
     - Nothing to report
   - **What was the impact on society beyond science and technology?**
     - Nothing to report

5. **CHANGES/PROBLEMS:**
    - **Changes in approach and reasons for change**
        - There are no significant changes to objective or scope
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
        - We had delays in our research in the laboratory transitioning personnel and the project from The University of Kansas to Massachusetts General Hospital. No delays are anticipated in the coming reporting period.
    - **Changes that had a significant impact on expenditures**
        - Nothing to report
    - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
        - Nothing to report
    - **Significant changes in use or care of human subjects**
    - **Significant changes in use or care of vertebrate animals.**
    - **Significant changes in use of biohazards and/or select agents**
6. **PRODUCTS:**
    - **Publications, conference papers, and presentations**
        - **Journal publications.**

1. Fahad, A. S., Chung, C.-Y., Lopez Acevedo, S. N., Boyle, N., Madan, B., Gutiérrez-González, M. F., Matus-Nicodemos, R., Laflin, A. D., Ladi, R. R., Zhou, J., Wolfe, J., Llewellyn-Lacey, S., Koup, R. A., Douek, D. C., Balfour Jr, H. H., Price, D. A. & DeKosky, B. J. Immortalization and functional screening of natively paired human T cell receptor repertoires. *Protein Engineering, Design and Selection* **35,** gzab034 (2022).

2. Chung, C.-Y., Gutiérrez-González, M., López Acevedo, S. N., Fahad, A. S., DeKosky, B. J., & AIRR Community. Quality Control: Chain Pairing Precision and Monitoring of Cross-Sample Contamination: A Method by the AIRR Community. *Methods Mol Biol* **2453,** 423–437 (2022).

- **Books or other non-periodical, one-time publications.** Nothing to report
- **Other publications, conference papers, and presentations.**

Conference presentations related to this work:

- *GRK 2504 / Friedrich-Alexander-Universität (FAU) 3rd Annual Collaborative Retreat,* The Ragon Institute, Cambridge MA (Sep 2022)
- *NIH Mechanistic Studies in Transplantation Workshop / CTOT*, Bethesda, MD (June 2022)
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- *PepTalk: The Protein Science Week*, San Diego, CA (Jan 2022)
- *Antibody Engineering, Phage Display & Immune Repertoire Analysis Course*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (Nov 2021)
- *American Association of Pharmaceutical Scientists (AAPS)*, Philadelphia, PA (via Webinar, Oct 2021)

Academic & industrial seminar presentations related to this work:

- *NIAID Vaccine Research Center / NIH Main Campus,* Bethesda, MD (June 2022, *to reschedule*)
- *Seeker Biologics, Inc,* Boston, MA (Jun 2022)
- *Cell Profiling and Neoantigen Prediction Working Group, US Food & Drug Administration,* Silver Spring, MD (virtual visit, April 2022)
- *Department of Chemistry, The University of Nebraska at Omaha,* Omaha, NE (virtual visit, April 2022)
- *Department of Chemistry, The University of Kansas,* Lawrence, KS (virtual visit, March 2022)
- *Utrecht Molecular Immunology (UMI), University Medical Center Utrecht,* **Utrecht, Netherlands** (virtual visit, Sep 2021)

- **Website(s) or other Internet site(s)**
  Nothing to report
- **Technologies or techniques**
  We have established new techniques for cloning T cell receptors, and we are in the process of applying those technologies for anti-cancer TCR discovery.
- **Inventions, patent applications, and/or licenses**
  Nothing to report
- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Brandon DeKosky
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	BDEKOSKY
Nearest person month worked:	0.12 calendar months
Contribution to Project:	Scientific lead, coordinate with collaborators, and directly supervise lab staff
Funding Support:	*(Complete only if the funding support is provided from other than this award).*

Name:	Ahmed Saeed Fahad
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.68 calendar months
Contribution to Project:	T cell sorting, sequence, and analysis of antibody sequences following functional screening studies.

Funding Support:	*(Complete only if the funding support is provided from other than this award).*

Name:	Viridiana Montessoro
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.0 calendar months
Contribution to Project:	Lead experiment design and laboratory data collection for TCR screening.
Funding Support:	*(Complete only if the funding support is provided from other than this award).*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

OTHER SUPPORT

Other Support – Project/Proposal

ACTIVE

Massachusetts General Hospital

Title: Rapid antibody screening systems to identify and engineer antiviral protection

*Major Goals: The goals are to establish a new single-cell assay to map immortalized human antibody immune repertoires for their antiviral neutralization properties. Apply directed evolution to improved antiviral antibodies and engineer exquisite neutralization breadth and potency.
*Status of Support: Active
Project Number: R21AI166396
Name of PD/PI: DeKosky
Role: PI
*Source of Support: National Institutes of Health
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 3/15/22-3/14/24
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	.18
2. 2024	.18

***Title: High-throughput TCR repertoire-based platforms for antigen-specific cancer immunotherapy**

*Major Goals: The goal is to develop new technologies to advance antigen specific cancer therapies.
*Status of Support: Active
Project Number: W81XWH-18-1-0296
Name of PD/PI: DeKosky
Role: PI
*Source of Support: Department of Defense/CDMRP
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: 08/2018 – 07/2022 (MGH: 3/15/22 – 3/14/23) (NCE)

* Total Award Amount (including Indirect Costs):
* Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
4. 2023	0.12 (NCE)

***Title: Mining Precise Multi-Mutation Data Networks for AI-Guided Antibody Design**
*****Major Goals This project will link experimental and computational approaches to enable improved AI/ML-guided antibody design.
*****Status of Support: Active
Project Number: Ragon Institute Schwartz AI/ML/Immunology Initiative
Name of PD/PI: DeKosky / Gómez-Barbarellos Co-PIs
Role: Co-PI
*****Source of Support: Ragon Institute Schwartz AI/ML/Immunology Initiative
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2022-06/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.01

***Title: Comprehensive analysis of human adaptive immune receptors to elucidate correlates of Epstein-Barr virus disease suppression**
*****Major Goals: The goal is to apply new high-throughput immune profiling techniques to elucidate the features of effective Epstein-Barr virus (EBV) immune control.
*****Status of Support: Active
Project Number: 5DP5OD023118-05
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: National Institutes of Health/Office of the Director
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2016 – 08/2023 (NCE)
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Months
6. 2022	0.01 (NCE)

***Title: Comprehensive molecular and functional analyses of anti-HIV-1 broadly neutralizing antibody repertoires**
*****Major Goals: The goal is to establish several technologies to comprehensively determine HIV-1 protective antibody responses and for enhanced understanding of HIV-1 protective antibodies.
*****Status of Support: Active
Project Number: 5 R21AI143407-02
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: National Institutes of Health/NIAID
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2018 – 11/2022 NCE
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
4. 2022	0.01 (NCE)

***Title: Antibody display libraries for precision screening of antibody immune responses to SARS-CoV-2**

*Major Goals: The goal is to determine the antibody-based immune features in COVID-19 patients to accelerate the development of new medical interventions. SARS-CoV-2 causes asymptomatic or mild disease in many individuals, demonstrating that an effective human immune response can fully prevent disease.
*Status of Support: Active
Project Number: 3DP5 OD023118-05S1
Name of PD/PI: DeKosky
Role: PI
*Source of Support: National Institutes of Health/Office of the Director
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2020 – 08/2023 (NCE)
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
2. 2022	.01 NCE

***Title: The influence of evolutionary landscapes on protective antibody development**
*****Major Goals: The goals are to provide a new, powerful method to map and manipulate rules of *in vivo* antibody affinity maturation to develop vaccines against refractory pathogens of high interest to public health including Influenza, Dengue, and HIV.
*****Status of Support: Active
Project Number: 1R01AI141452
Name of PD/PI: Whitehead
Role: PI of subaward
*****Source of Support: University of Colorado / NIH Flow through
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2019 – 08/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
4. 2022	0.96
5. 2023	0.96

***Title: Anti-malarial antibody improvement project**
*****Major Goals: The goals are to work with the foundation to improve anti-malarial antibodies as more potent preventive drugs against malaria infection.
*****Status of Support: Active
Project Number: INV-043182
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: Bill and Melinda Gates Foundation
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022 – 04/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period

Year	Summer Months
1. 2022	0.12
2. 2023	0.06

***Title: Dissecting the mechanisms of HIV resistance in vivo to broadly neutralizing antibodies**
*Major Goals: The major goals of this project are to apply high-throughput platforms to understand HIV-1 antibody resistance mechanisms and identify evolutionary pathways for the development of broadly neutralizing antibody variants resistant to HIV-1 escape.
*Status of Support: Active
Project Number: 1U01AI169587
Name of PD/PI: Herschhorn

Role: PI of subaward
*Source of Support: University of Minnesota / NIH Flow through
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2022 – 05/2027
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.25
2. 2024	0.25
3. 2025	0.25
4. 2026	0.25
5. 2027	0.25

PENDING (active transferring)

All grants that have start dates prior to 8/31/2021 started at University of Kansas, and are in the process of transferring to Ragon Institute, or there will be a subaward to Ragon Institute for a portion of the work on grants that remain at University of Kansas, as noted in the **Status** line.

***Title: Antibody discovery against RSV and protein targets**
*****Major Goals: The major goal is to sequence antibody libraries targeting protein drug targets.
*****Status of Support: Active/To be transferred to Massachusetts General Hospital
Project Number: BSA22052
Name of PD/PI: DeKosky
*****Source of Support: University of Kansas / Sanofi S.A.
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2022 – 10/2022
*****Current Period Direct Award Amount:
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Months
1. 2022	0.01

MIT

***Title: Effective Antibody Discovery Against Difficult High-Value Drug Targets**
*****Major Goals This project will develop new droplet-based screening technologies for antibody discovery against complex protein drug targets.
*****Status of Support: Active
Project Number: 6948532
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: MIT Deshpande Center for Technical Innovation
*****Primary Place of Performance: Massachusetts Institute of Technology
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022-08/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	no salary effort
2. 2023	no salary effort

***Title: Library-scale platforms for personalized anti-cancer TCR discovery**
*****Major Goals This project will develop new library-scale T cell receptor screening platforms and in vivo models for personalized T cell receptor drugs against cancer.
*****Status of Support: Active
Project Number: TBD
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: Koch Institute / MIT
*****Primary Place of Performance: Massachusetts Institute of Technology
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2022-06/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.01

***Title: Rapid Functional Screening of Anti-HIV-1 Antibodies for Potent Immune Drug Development**
*****Major Goals This project will develop high-throughput platforms for anti-HIV-1 antibody drug screening.
*****Status of Support: Active
Project Number: TBD
Name of PD/PI: DeKosky
Role: PI
*****Source of Support: MIT Research Support Committee
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2022-06/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.01

PENDING (New grant proposals under review)

MIT

***Title: CAREER: Mining Multi-Mutation Improvement Pathways to Accelerate Antibody Design**
*****Major Goals: The purpose of this project is to establish new experimental and computational frameworks to study and predict antibody gene mutation profiles and advance antibody drug development.
*****Status of Support: Pending
Project Number: 23010201
Name of PD/PI: DeKosky
*****Source of Support: NSF
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2023-04/2028
Current Year Direct Costs:
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	1

***Title: BWF-PATH: Mapping the evolution of protective antibody repertoires against diverse viruses**
*Major Goals: This project will establish new experimental and computational frameworks to study and predict antibody development and co-evolution against viruses.
*Status of Support: Pending
Name of PD/PI: DeKosky
*Source of Support: Burroughs Wellcome Fund
*Primary Place of Performance: Massachusetts General Hospital

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2023-04/2028
Annual Direct Costs:
* Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.07
2. 2024	0.07
3. 2025	0.07
4. 2026	0.07
5. 2027	0.07

***Title: Biochemical Determinants of Protective Human Immune Memory**
*****Major Goals: This project will use antibody- and T-cell based techniques to study the biochemical interactions in human immune memory.
*****Status of Support: Pending
Name of PD/PI: DeKosky
*****Source of Support: Sloan Foundation
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2023-04/2026
Annual Direct Costs:
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.12
2. 2024	0.12

Massachusetts General Hospital

***Title: The role of NKG2D in autoimmune diabetes**
*****Major Goals: The major goals of this project are to establish systems for T cell receptor expression in engineered cells and to collaborate with researchers at KUMC to develop T cell tolerance-based approaches for autoimmune diabetes.
*****Status of Support: Pending
Project Number: R01
Name of PD/PI: Markiewicz
*****Source of Support: University of Kansas Medical Center Research Institute/National Institutes of Health
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2022 – 1/2027
*****Total Award Amount (Indirect costs to be calculated at time of award per RFA):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.03
2. 2024	0.03
3. 2025	0.03
4. 2026	0.03
5. 2027	0.03

***Title: Enabling functional and transcriptional analysis of donor-specific T cells in transplant recipients**
*Major Goals: This study will analyze T cell receptor genes in transplant patients to understand the mechanisms of long-lived transplant tolerance.
*Status of Support: Pending

Project Number: R21
Name of PD/PI: Cravedi
*Source of Support: Icahn School of Medicine at Mount Sinai/National Institutes of Health
*Primary Place of Performance: Ragon Institute, Massachusetts General Hospital Project/
Proposal Start and End Date: (MM/YYYY) (if available): 04/2023 – 03/2025
*Total Award Amount (Indirect costs to be calculated at time of award per RFA):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	0.03
2. 2025	0.03

***Title: Detailed molecular analysis of post-transplant immune responses**
*****Major Goals: This study will analyze T cell receptor genes in transplant patients to understand the mechanisms of long-lived transplant tolerance.
*****Status of Support: Pending
Project Number: U19
Name of PD/PI: DeKosky
*****Source of Support: The University of Pennsylvania
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/2023 – 01/2024
*****Total Award Amount (Indirect costs to be calculated at time of award per RFA):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	0.03

***Title: Efficient discovery and characterization of antibodies targeting GPCRs**
*****Major Goals: This project will establish and implement a new approach for the discovery of antibody research reagents and potential therapeutics against G-protein coupled receptors.
*****Status of Support: Pending
Project Number: R01
Name of PD/PI: DeKosky
*****Source of Support: National Institutes of Health
*****Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2023 – 06/2028
*****Total Award Amount (Indirect costs to be calculated at time of award per RFA):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	0.5
2. 2025	0.5
3. 2026	0.5
4. 2027	0.5
5. 2028	0.5

***Title: Molecular-scale mapping of human antibody immunity**
*Major Goals: The goal is to develop improved platforms to understand and interrogate human antibody immunity in health and disease.
*Status of Support: Pending
Project Number: DP1
Name of PD/PI: DeKosky
*Source of Support: National Institutes of Health

*Primary Place of Performance: Ragon Institute, Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2023 – 07/2028
*Total Award Amount (Indirect costs to be calculated at time of award per RFA):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	1.53

***Title: High-Throughput Platforms for Rapid & Personalized Immune Therapy Drug Discovery in Cancer Patients**
*****Major Goals This project will develop T cell receptor screening platforms and in vitro models for new personalized T cell receptor drugs against cancer.
*****Status of Support: Pending
Project Number: TBD
Name of PD/PI: DeKosky
*****Source of Support: American Cancer Society
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/2022-9/2026
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.2

***Title: Immune repertoire remodeling following B cell-targeted CAR T cells for induction of islet allograft tolerance in non-human primates.**
*****Major Goals This project will apply high-throughput platforms to determine the mechanisms of transplant tolerance and rejection in pre-clinical transplant models.
*****Status of Support: Pending
Project Number: U19
Name of PD/PI: Naji / DeKosky sub-award
*****Source of Support: NIH/The University of Pennsylvania
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/01/2023 - 02/28/2028
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	1.73

***Title: Antibody Discovery Platform.**
*****Major Goals This project will apply high-throughput platforms to identify antibodies with a potential protective activity against Alzheimer's disease.
*****Status of Support: Pending
Project Number: TBD
Name of PD/PI: DeKosky
*****Source of Support: MassCATS / The Massachusetts Life Sciences Center
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/2022-9/2023
*****Total Award Amount (including Indirect Costs):
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	.01

***Title: Bruton's Tyrosine Kinase and Immune Tolerance in Type 1 Diabetes**
*****Major Goals The aim of this project is to understand how Bruton's Tyrosine Kinase works through B cells and B cell receptors to influence immune tolerance relevant to Type I Diabetes antigens.
*****Status of Support: Pending
Project Number: R01
Name of PD/PI: DeKosky
*****Source of Support: Washington University School of Medicine
*****Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/2023-04/2028
*****Current Period Direct Award Amount:
*****Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2024	.48

IN-KIND

None

***Overlap**

There is no scientific, budgetary or commitment overlap.

- **What other organizations were involved as partners?**
  - N/a

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** n/a
   - **QUAD CHARTS:** n/a
9. **APPENDICES:** n/a