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

PRINCIPAL INVESTIGATOR: Brandon DeKosky

CONTRACTING ORGANIZATION: Massachusetts General Hospital

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Dr. Brandon J. DeKosky

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55 Fruit Street
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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

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

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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:** *Provide a brief list of keywords (limit to 20 words).*

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.

1) Major activities

Our laboratory has fulfilled our objectives on this CDMRP project,. We developed and validated our single-cell emulsion devices for single T cell analysis, and we published three manuscripts on T cell response analysis. 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 for high-throughput TCR screening, and have completed substantial data acquisition and analysis for our studies in anti-cancer TCR research.

We have three postdocs who have focused on this project, in addition to help from other undergraduate and postdoctoral students. We 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.

Two major publications 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 analyzed 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 three publications have been completed. We applied this technology in parallel to identify the anti-cancer TCRs in several RCC patients (Aim 1 Task 2). We are continuing to implement these technologies 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 papers.

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.

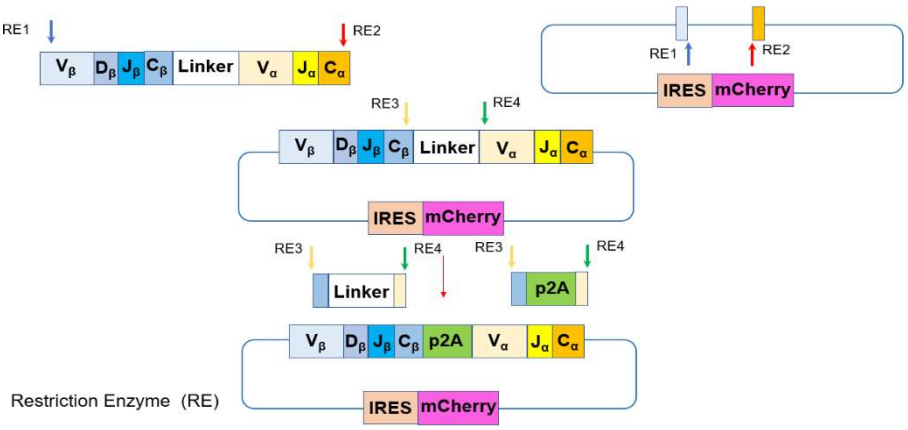

Figure 1: *Cloning strategy for display of our linked Valpha:Vbeta TCR genes. Two unique restriction enzymes at the outside and inside of the linked TCR gene amplicon are used to clone the amplicon into display plasmids. A p2a sequence is cloned into the center to allow for separate expression of alpha 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 were able to focus on library screening assays and live-cell killing and activation assays for the identification of cancer cell-specific TCRs in final stages of the project. We finalized publication related to cell-based activation screening methods, which is now published (Scientific Reports, 2023). We are also completing 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. The characterization of these TCRs is ongoing.

On the bioinformatic side, we 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.

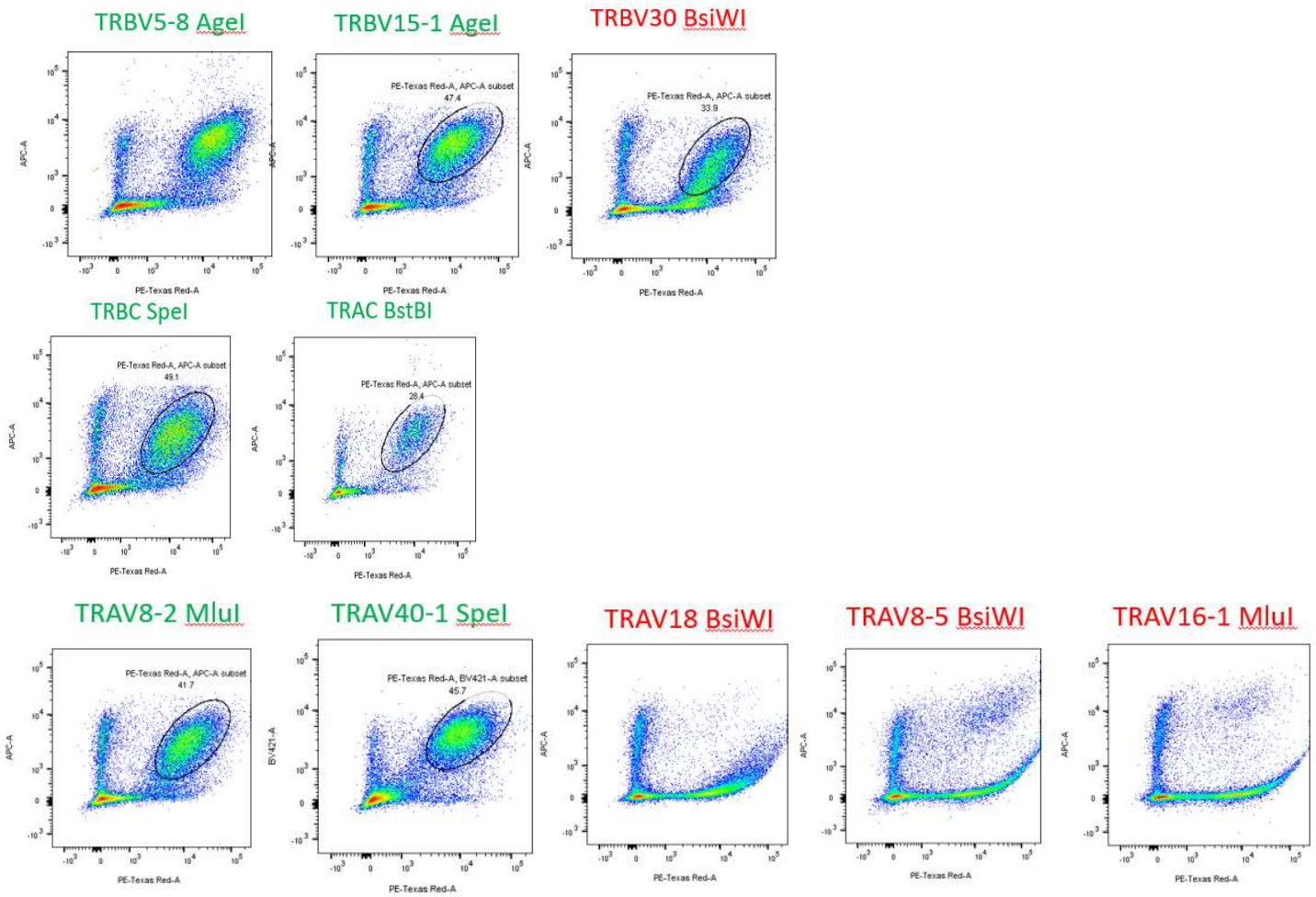

Figure 2: *A small panel of the restriction enzymes and leader peptides (for TRAV/TRBV) that we tested for allowing proper TCR display, using JRT3 cells displaying a known anti-HIV positive control antibody. mCherry expression is shown on the x-axis, while HIV peptide p:MHC binding is shown on the y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

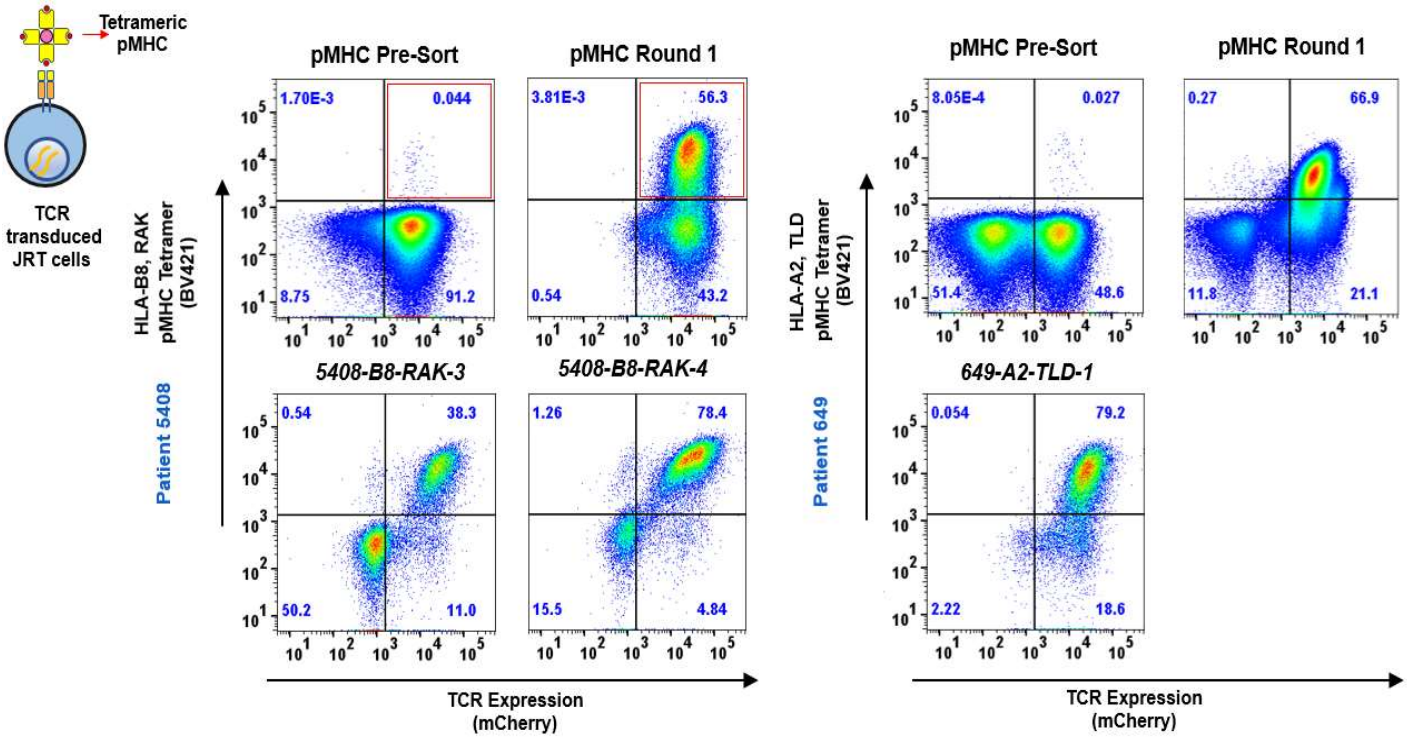

Figure 3: *Analysis of infectious mononucleosis patient samples for anti-EBV peptides as validation of our screening workflows. These data demonstrate the effective screening of immortalized TCR libraries from human patients using soluble peptide:MHC fluorescent screening antigens.*

We also established the platforms for detecting live cell activation in co-culture cell systems (**Figure 4**). These efforts were published in Fahad et al, Scientific Reports (2023). We have also applied these technologies for the screening of anti-RCC patient libraries from our collaborator, Dr. Andrew Godwin, and his team at the KU Medical Center (**Figure 5**).

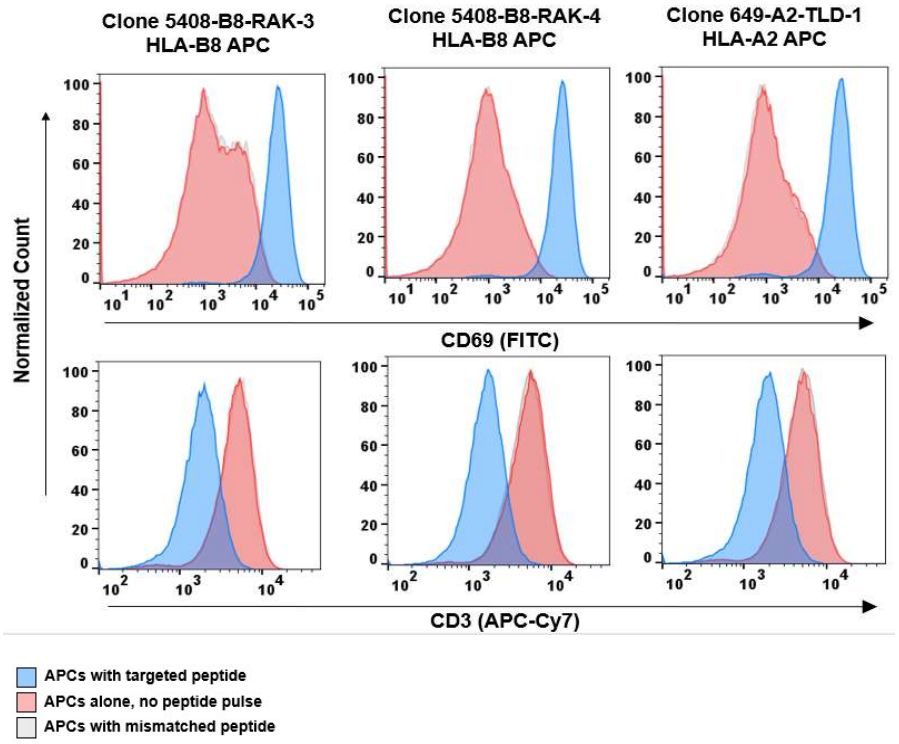

Figure 4: *Activation-based screening of T cell receptor libraries for antigen-specific T cell receptors. Antigen-specific T cell clones that we discovered using our single-cell TCR screening workflow were incubated with antigen-presenting cells (APCs) either with a targeted peptide pulse (blue), without 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 continue to apply these methods for screening anti-cancer T cell libraries in ongoing and future projects.*

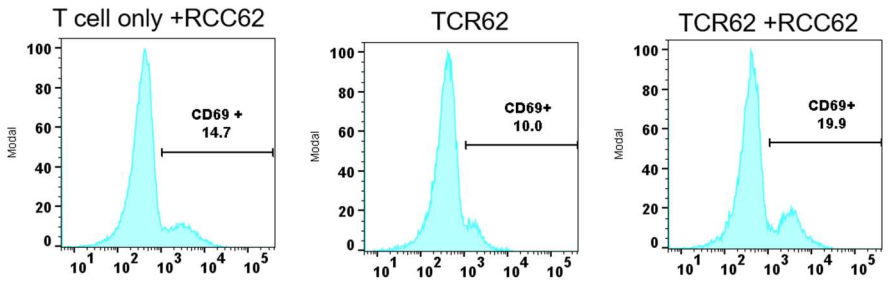

Figure 5: *Activation-based screening of human T cell repertoires against personalized cancer cell immune responses. TCR genes were captured from tumor-infiltrating lymphocytes and cloned into lentiviral display in human T cells. In parallel ("TCR62"). In parallel, patient-matched tumor cells were co-cultured 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.*

Finally, we implemented new approaches for cloning anti-cancer immune libraries into primary T cells using CRISPR/Cas9. We followed a published approach by Moosmann et al. (doi: 10.1016/j.xpro.2021.101031) to knock in first a control murine TCR (**Fig. 6**, left), and then a human TCR targeting a known peptide (LC13, **Fig. 6**, right). The advantage of such approaches is that we can assay single human TCRs, replaced in primary T cells, to understand TCR activation features

such as cytokine secretion and proliferation against cancer-associated antigens and neoantigens. We are now using these CRISPR-based approaches to clone human TCR libraries from cancer patients and screening against tumor cells and tumor antigens to identify protective anti-cancer TCRs.

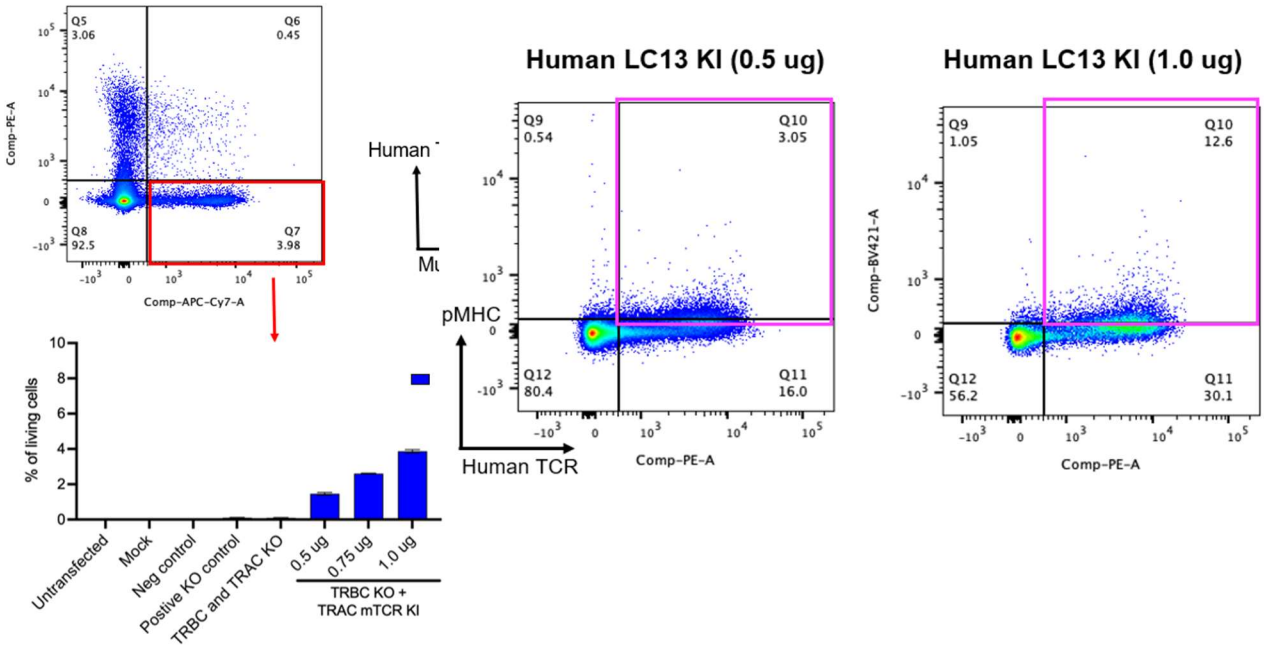

Figure 6: *CRISPR-based knock-in of TCRs into primary human T cells for TCR library screening in primary cells.* Left *A control TCR plasmid was used to clone a murine TCR into a human T cell population. With 1.0 mcg of plasmid, an efficiency around 4% was observed for TCR replacement.* Right *We replaced native human TCRs with the LC13 TCR and analyzed the population's capacity to bind LC13's known targeted pMHC. Flow cytometry demonstrated that over 10% of the treated cell population showed detectable binding to the pMHC, indicative of successful and efficient TCR replacement in human primary cells.*

4) Key Outcomes or Other Achievements

We have achieved our critical research goals, and developed these exciting new platforms and submit our findings for publication to additional peer-reviewed journals. We have also isolated human anti-cancer T cells based on their ability to target cancer cells *in vitro*, and we are continuing the characterization of those T cells as part of our ongoing work.

The results of this project were also published in three important studies:

- Fahad, A. S. *et al.* Cell activation-based screening of natively paired human T cell receptor repertoires. *Sci Rep* **13**, 8011 (2023).
- Chung, C.-Y. *et al.* Quality Control: Chain Pairing Precision and Monitoring of Cross-Sample Contamination: A Method by the AIRR Community. *Methods Mol Biol* **2453**, 423–437 (2022).

- Fahad, A. S. *et al.* Immortalization and functional screening of natively paired human T cell receptor repertoires. *Protein Engineering, Design and Selection* **35**, gzab034 (2022).

- **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 efforts were 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:

- *Festival of Biologics, San Diego, CA (March 2023)*
- *FDA Single Cell Omics Symposium, Silver Spring, MD (virtual event, Feb 2023)*
- *Peptalk: The Protein Science Week, San Diego, CA (Jan 2023)*
- *Antibody Engineering & Therapeutics, San Diego, CA (Dec 2022)*
- *Antibody Engineering, Phage Display & Immune Repertoire Analysis Course, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (Oct 2022)*
- *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 (May 2023)
- NIAID Twinbrook Seminar Series, National Institutes of Health, Bethesda, MD (Apr 2023)
- UF Health Cancer Center, The University of Florida, Gainesville, FL (Apr 2023)
- Janssen Research and Development, Spring Hill, PA (Feb 2023)
- National Cancer Institute / NIH, Virtual Format (Feb 2023)
- Adimab, Lebanon, NH (Feb 2023)
- The Antibody Society, Virtual webinar format (Jan 2023)
- Dept. of Microbiology and Immunology, Georgetown University, Washington, D.C. (Nov 2022)
- 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)
- Department of Chemical Engineering, Massachusetts Institute of Technology / Ragon Institute of MIT/MGH/Harvard, Boston, MA (virtual visit, Mar 2021)
- Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX (virtual visit, Feb 2021)
- Center for Vaccines and Immunology, The University of Georgia, Athens, GA (virtual visit, Dec 2020)
- Biological Standards Working Group, Adaptive Immune Receptor Repertoire Community (Dec 2020)
- Research in Progress Seminar Series, KU Medical Center, Kansas City, KS (Aug 2020)

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **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**
  - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - Nothing to report
- **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: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **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).
3. Fahad, A. S. et al. Cell activation-based screening of natively paired human T cell receptor repertoires. Sci Rep 13, 8011 (**2023**).

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

Conference presentations related to this work:

- *Festival of Biologics, San Diego, CA (March 2023)*
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- Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX (virtual visit, Feb 2021)
- Center for Vaccines and Immunology, The University of Georgia, Athens, GA (virtual visit, Dec 2020)
- Biological Standards Working Group, Adaptive Immune Receptor Repertoire Community (Dec 2020)
- Research in Progress Seminar Series, KU Medical Center, Kansas City, KS (Aug 2020)

- **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:	0.96 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:	2.5 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).*

Name:	Penny Timms
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.5 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).

Name:	Cheng Yu (Andrew) Chung
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	9.4 calendar months
Contribution to Project:	Lead experiment design and laboratory data collection for TCR screening.
Funding Support:	none

Name:	Matias Fernando Gutierrez
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.45 calendar months
Contribution to Project:	Assist with laboratory data collection for TCR screening.
Funding Support:	none

Name:	Nicoleen Boyle
Project Role:	Assistant Researcher
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	18.1 calendar months
Contribution to Project:	Assist with laboratory data collection for TCR screening.
Funding Support:	none

Name:	Fnu Bharat
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	BHARATFNU
Nearest person month worked:	2.64 calendar months
Contribution to Project:	Perform bioinformatic analysis of TCR sequence data
Funding Support:	*(Complete only if the funding support is provided from other than this award).*

Name:	Chen Chung
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5.85 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).*

Name:	Fernando Gutierrez
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.81 calendar months
Contribution to Project:	Assist with laboratory data collection for TCR screening.
Funding Support:	*(Complete only if the funding support is provided from other than this award).*

Name:	Bharat Madan
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	BHARATFNU
Nearest person month worked:	0.08 calendar months
Contribution to Project:	Perform bioinformatic analysis of TCR sequence data
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?

Yes.

ACTIVE SUPPORT

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.

*Specific Aims: This project will establish a new single-cell assay to map immortalized human antibody immune repertoires for their antiviral neutralization properties, and apply directed evolution to improved antiviral antibodies and engineer exquisite neutralization breadth and potency. Viral neutralization is the best known correlate for protection against viral infections in most clinical studies, and the discovery of highly potent neutralizing antibodies has recently inspired new waves of protective vaccines and antibody-based drugs.

*Status of Support: Active

Project Number: R21AI166396

Name of PD/PI: DeKosky
Role: PI
*Source of Support: National Institutes of Health
*Name and address of the funding agency's procuring Contracting/Grants Office:
Bianca R. Steele, NIAID, 5601 Fishers Lane MSC 9806, Bethesda, MD 20892-9806,
*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	1.71
2. 2024	1.45

Overlap: None

***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.

*Specific Aims: This project will generate new knowledge regarding antibody structure and mutational improvement pathways to accelerate antibody drug discovery. We will apply precision functional screening to map the effect of hundreds of thousands of antibody multi-mutation sequence combinations, and apply those data to inform the AI-guided design of improved antibody drugs.

*Name of Individual: DeKosky, Brandon J

*Status of Support: Active
Project Number: Ragon Institute Schwartz AI/ML/Immunology Initiative
Name of PD/PI: DeKosky / Gómez-Bombarelli Co-PIs
Role: Co-PI
*Source of Support: Ragon Institute Schwartz AI/ML/Immunology Initiative
*Name and address of the funding agency's procuring Contracting/Grants Office:
Mark Schmeissing, The Ragon Institute, 400 Technology Square 1st Fl, Cambridge, MA 02139
*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

Overlap: None

***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.
*Specific Aims: This project seeks to gain new insight regarding the molecular mechanisms for adaptive immune control of Epstein-Barr virus (EBV). New mechanistic knowledge will be leveraged to develop
novel immune-based strategies to treat and prevent EBV-associated diseases.
*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
*Name and address of the funding agency's procuring Contracting/Grants Office:
Gabriel Hidalgo, NIH, 9000 Rockville Pike, Bethesda, MD 20892
*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
1. 2017	2.4
2. 2018	2.4
3. 2019	2.4
4. 2020	2.4
5. 2021	2.4
6. 2022	0.01 (NCE)
7. 2023	0.01 (NCE)

Overlap: None

*Name of Individual: DeKosky, Brandon J

***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.
*Specific Aims: This project will determine antibody-based molecular correlates of COVID-19 disease severity to improve mechanistic understanding and accelerate medical intervention development.
*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
*Name and address of the funding agency's procuring Contracting/Grants Office:
Gabriel Hidalgo, NIH, 9000 Rockville Pike, Bethesda, MD 20892
*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
1. 2021	.18
2. 2022	.01 NCE
3. 2023	.01 NCE

Overlap: None

***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.
*Specific Aims: This project aims to 1) Determine ontogeny from germ line to mature human antibodies for two heterosubtypic HA stem binders; 2) Determine ontogeny from germ line to mature human antibodies for four heterosubtypic and subtype- specific HA head binders; 3) Determine the number of evolutionary trajectories from a representative germline Ab.
*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
*Name and address of the funding agency's procuring Contracting/Grants Office:
Kevin Roy Heath, NIH, 9000 Rockville Pike, Bethesda, MD 20892
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2021 – 12/2023
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

*Name of Individual: DeKosky, Brandon J

Year	Summer Months
1. 2019	0.96
2. 2020	0.96
3. 2021	0.96
4. 2022	0.96
5. 2023	0.6

Overlap: None

***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.
*Specific Aims: This project will implement an optimized high-throughput system for rapid antibody library generation and screening of 2 antibodies targeting the malaria circumsporozoite protein (CSP).
*Status of Support: Active
Project Number: INV-043182
Name of PD/PI: DeKosky
Role: PI
*Source of Support: Bill and Melinda Gates Foundation
*Name and address of the funding agency's procuring Contracting/Grants Office:
Jacqueline Kirchner
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2022 – 04/2024
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period

Year	Summer Months
1. 2023	0.12
2. 2024	0.01

Overlap: None

***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.
*Specific Aims: This project will 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*Name and address of the funding agency's procuring Contracting/Grants Office:
*Name and address of the funding agency's procuring Contracting/Grants Office:

*Name of Individual: DeKosky, Brandon J

Jenna L. Briggs, NIAID, 5601 Fishers Lane MSC 9806, Bethesda, MD 20892-9806,

*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	1.0
2. 2024	1.0
3. 2025	1.0
4. 2026	1.0
5. 2027	1.0

Overlap: None

***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.
*Specific Aims: This project applies a new T cell receptor (TCR) library display platform for high-throughput functional analysis of anti-tumor immune responses, and to develop rapid autologous T cell therapies for precise cancer treatment.
*Status of Support: Pending
Project Number: RSG-22-134-01-IBCD
Name of PD/PI: DeKosky
*Source of Support: American Cancer Society
*Name and address of the funding agency's procuring Contracting/Grants Office:
Alice L. Pomponio
*Primary Place of Performance: Massachusetts General Hospital
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2023-12/2026
*Total Award Amount (including Indirect Costs):
*Person Months (Calendar/Academic/Summer) per budget period.

Year	Summer Months
1. 2023	0.20
2. 2024	0.16
3. 2025	0.16
4. 2026	0.16

Overlap: None

MIT

***Title: Effective Antibody Discovery Against High-Value Membrane Drug Targets**

*Major Goals This project will develop new droplet-based screening technologies for antibody discovery against complex protein drug targets.

*Name of Individual: DeKosky, Brandon J

*Specific Aims: NA
*Status of Support: Active
Project Number: 6948532
Name of PD/PI: DeKosky
Role: PI
*Source of Support: MIT Deshpande Center for Technical Innovation
*Name and address of the funding agency's procuring Contracting/Grants Office:
Eric Olivieri, 292 Main Street, E38-676, Cambridge, MA 02142
*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	0.01

Overlap: None

***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.
*Specific Aims: NA
*Status of Support: Active
Project Number: TBD
Name of PD/PI: DeKosky
Role: PI
*Source of Support: Koch Institute / MIT
*Name and address of the funding agency's procuring Contracting/Grants Office:
Emma Westling, 77 Massachusetts Ave. Cambridge, MA 02139
*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

Overlap: None

***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.
*Specific Aims: NA
*Status of Support: Active
Project Number: TBD
Name of PD/PI: DeKosky
Role: PI
*Source of Support: MIT Research Support Committee

*Name and address of the funding agency's procuring Contracting/Grants Office:
Ting Ting Li, 77 Massachusetts Ave. Cambridge, MA 02139
*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

Overlap: None

IN-KIND

None

What other organizations were involved as partners?

Collaboration with Dr. Andrew Godwin at the University of Kansas Medical Center, and with Dr. David Price at Cardiff University, Wales, UK.

8. SPECIAL REPORTING REQUIREMENTS

1. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** n/a
   - **QUAD CHARTS:** attached in appendices
2. **APPENDICES:** n/a

9. APPENDICES:

a. Special Reporting Requirement – Award Chart
b. DD882 Patent/Invention closure form

Award Log Number: Award Title

PI: Brandon J. DeKosky, Massachusetts General Hospital, Massachusetts **Budget:** $543,766

Topic Area: Kidney Cancer **Mechanism:** W81XWH-17-PRCRP-CDA

Research Area(s): SCS Coding **Award Status:** 08/01/2018 – 03/14/2023

Study Goals: This project will develop a new technological pipeline to enable autologous TCR-based, targeted cellular immunotherapies as personalized cancer treatments against a broad range of malignancies.

Specific Aims: Aim 1: Develop a platform for rapid transformation of antigen-specific TCR repertories from solid tumors for personalized antigen-specific TCR-based cancer therapies. Aim 2: Develop a paired TCRα:β library display platform for rapid TCR library isolation and screening in vitro.

Key Accomplishments and Outcomes:

Publications:

Fahad, A. S., Chung, C. Y., López 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, H. H., Price, D. A. & DeKosky, B. J. Cell activation-based screening of natively paired human T cell receptor repertoires. *Sci Rep* **13,** 8011 (2023).

2.

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).

Patents: none to date

Award Log Number: Award Title

PI: Brandon J. DeKosky, Massachusetts General Hospital, Massachusetts **Budget:** $543,766

Topic Area: Kidney Cancer **Mechanism:** W81XWH-17-PRCRP-CDA

Funding Obtained:

*Library-scale platforms for personalized anti-cancer TCR discovery*Koch Institute Frontier Research Program
06/01/2022-5/31/2023
$200,000
Role: Co-PI with Michael Birnbaum

Droplet-based single B and T cell receptor screening for multi-parameter adaptive immune monitoring
NIGMS P20 GM103418 (PI: D. Wright)
K-INBRE Bridge Award
10/01/2020-04/30/2021
$37,875
Role: PI

High-throughput functional screening of personalized anti-cancer T cell receptor repertoires
American Cancer Society, Research Scholar Grant
RSG-22-134-01-IBCD
01/01/2023-12/31/2026
$792,000
Role: PI

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Jan 31, 2008

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE ABOVE ORGANIZATION. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT *(X one)*
Massachusetts General Hospital	W81XWH-18-1-0296	USA MED RESEARCH ACQ ACTIVITY	W81XWH-18-1-0296	☐ a. INTERIM ☒ b. FINAL
b. ADDRESS *(Include ZIP Code)* 55 Fruit Street, Boston, MA 02114-2696	**d. AWARD DATE** *(YYYYMMDD)* 20220315	**b. ADDRESS** *(Include ZIP Code)* 820 CHANDLER ST, FORT DETRICK MD 21702-5014	**d. AWARD DATE** *(YYYYMMDD)* 20220315	**4. REPORTING PERIOD** *(YYYYMMDD)* a. FROM 08/01/2018 b. TO 03/14/2023

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) *(Last, First, Middle Initial)* a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS *(X)* d. (1) UNITED STATES (a) YES	(1) UNITED STATES (b) NO	(2) FOREIGN (a) YES	(2) FOREIGN (b) NO	CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER *(X)* e. (a) YES	e. (b) NO
Not Applicable	Not Applicable	Not Applicable		X		X		X

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR *(Last, First, Middle Initial)* Not Applicable	(b) NAME OF EMPLOYER Not Applicable	(1) TITLE OF INVENTION Not Applicable	(2) FOREIGN COUNTRIES OF PATENT APPLICATION Not Applicable
(b) NAME OF EMPLOYER	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*	(2) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d. (1) CLAUSE NUMBER	d. (2) DATE *(YYYYMM)*	DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES *(YYYYMMDD)* f. (1) AWARD	f. (2) ESTIMATED COMPLETION
Not Applicable	Not Applicable	Not Applicable			Not Applicable		

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* ☐ **SMALL BUSINESS or** ☒ **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED *(YYYYMMDD)*
Foster, Charlene	Authorized Signing Official Manager, Post Award	Charlene Foster	07/14/2023

DD FORM 882 INSTRUCTIONS

GENERAL

This form is for use in submitting INTERIM and FINAL invention reports to the Contracting Officer and for use in reporting the award of subcontracts containing a "Patent Rights" clause. If the form does not afford sufficient space, multiple forms may be used or plain sheets of paper with proper identification of information by item number may be attached.

An INTERIM report is due at least every 12 months from the date of contract award and shall include (a) a listing of "Subject Inventions" during the reporting period, (b) a certification of compliance with required invention identification and disclosure procedures together with a certification of reporting of all "Subject Inventions," and (c) any required information not previously reported on subcontracts containing a "Patent Rights" clause.

A FINAL report is due within 6 months if contractor is a small business firm or domestic nonprofit organization and within 3 months for all others after completion of the contract work and shall include (a) a listing of all "Subject Inventions" required by the contract to be reported, and (b) any required information not previously reported on subcontracts awarded during the course of or under the contract and containing a "Patent Rights" clause.

While the form may be used for simultaneously reporting inventions and subcontracts, it may also be used for reporting, promptly after award, subcontracts containing a "Patent Rights" clause.

Dates shall be entered where indicated in certain items on this form and shall be entered in six or eight digit numbers in the order of year and month (YYYYMM) or year, month and day (YYYYMMDD). Example: April 2005 should be entered as 200504 and April 15, 2005 should be entered as 20050415.

1.a. Self-explanatory.

1.b. Self-explanatory.

1.c. If "same" as Item 2.c., so state.

1.d. Self-explanatory.

2.a. If "same" as Item 1.a., so state.

2.b. Self-explanatory.

2.c. Procurement Instrument Identification (PII) number of contract (DFARS 204.7003).

2.d. through 5.e. Self-explanatory.

5.f. The name and address of the employer of each inventor not employed by the contractor or subcontractor is needed because the Government's rights in a reported invention may not be determined solely by the terms of the "Patent Rights" clause in the contract.

Example 1: If an invention is made by a Government employee assigned to work with a contractor, the Government rights in such an invention will be determined under Executive Order 10096.

Example 2: If an invention is made under a contract by joint inventors and one of the inventors is a Government employee, the Government's rights in such an inventor's interest in the invention will also be determined under Executive Order 10096, except where the contractor is a small business or nonprofit organization, in which case the provisions of 35 U.S.C. 202(e) will apply.

5.g.(1) Self-explanatory.

5.g.(2) Self-explanatory with the exception that the contractor or subcontractor shall indicate, if known at the time of this report, whether applications will be filed under either the Patent Cooperation Treaty (PCT) or the European Patent Convention (EPC). If such is known, the letters PCT or EPC shall be entered after each listed country.

6.a. Self-explanatory.

6.b. Self-explanatory.

6.c. Self-explanatory.

6.d. Patent Rights Clauses are located in FAR 52.227.

6.e. Self-explanatory.

6.f. Self-explanatory.

7. Certification not required by small business firms and domestic nonprofit organizations.

7.a. through 7.d. Self-explanatory.