AWARD NUMBER: W81XWH-18-1-0296

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.

REPORT DATE: August 2020

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2020	Annual	1 August 2019-31 July 2020

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0296

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Brandon DeKosky

E-Mail: dekosky@ku.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Kansas Center for Research, Inc.

2385 Irving Hill Road
Lawrence, KS 66045-7568

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

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. Most of the key methods have been developed and validated and we are regularly applying our single-cell emulsion devices for single T cell analysis. We are also utilizing new computational pipelines for analyzing TCR NGS data, and we are preparing 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 we are embarking on the heavy data acquisition and analysis phase of our studies in anti-cancer TCR research.

We have one postdoc, one technician, and one graduate student who are now focusing on this project, in addition to help from other undergraduate and postdoctoral students. We finalized our bioinformatic scripts for data analysis and we recently implemented one final measure for optimizing yield of our custom single-cell

workflows and developing methods for simpler single cell analysis. We completed optimization of library preparation strategies and have finalized our techniques for live cell co-culture for anti-cancer TCR identification.

One major publications from this work is nearing completion in terms of data acquisition, which will relate 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). We are also sample processing for applying this analysis to our first RCC patient samples (Aim 1 Task 2), with several libraries in ongoing phases of anti-cancer TCR screening.

2) Specific Objectives

We continue to refine our experimental methods for large-scale processing of T cell samples and library screening, and we are proceeding rapidly with methods for 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, which will be used for all samples to enhance the yield of our Valpha:Vbeta sequencing workflows by increasing the size and activation state of cell populations, and in the most recent project period we also established live cell co-culture methods for identifying anti-cancer T cells. We have now 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 are validating 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. We are now applying 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.

We are also advancing our technologies for rapid computational profiling of TCR immune responses. We finalized and optimized our methods for rapid interpretation of T cell receptor NGS data, including for the identification of antigen-specific TCRs. We have finalized pipelines for compiling and interpreting TCR prevalence after various library screening conditions, which is similar to a paper that we are currently preparing describing similar bioinformatic advances for the analysis of antibody repertoires.

Our major current objectives are to publish the current version of the TCR library generation and screening protocols (Aim 1 Task 1, Aim 2 Tasks 1 and 2), which will require another 6 months until final publication. We are now applying this technology in parallel to identify the anti-cancer TCRs in several RCC patients (Aim 1 Task 2), which is the next 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). For an unrelated project, we are in the process of writing a manuscript related to thecomputational prediction of T cell epitopes, which we may be able to use to help understand the neoantigen targets of the anti-cancer TCRs that we identify in our lymphoma TCR sample analysis.

3) Significant Results

We achieved major progress in developing 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 is currently under review in the *Proceedings of the National Academy of Sciences*, and we have already begun to leverage these technologies for the NGS-based analysis of T cell receptor affinity and specificitys as well.

We have 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 built on 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 validated it for use with human T cell receptor libraries.

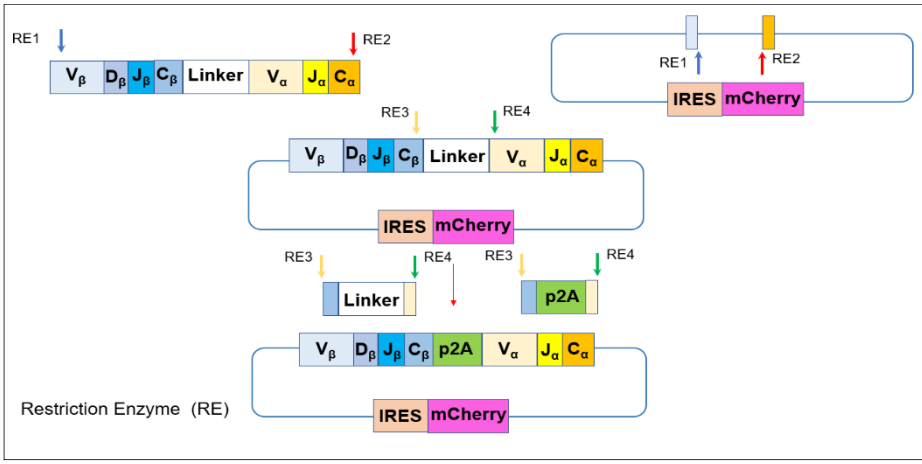

Figure 1: *Cloning strategy for display of our linked Valpha:Vbeta TCR genes. Two unique restriction enzymes at the outside and inside of the linked TCR gene amplicon are used to clone the amplicon into display plasmids. A p2a sequence is cloned into the center to allow for separate expression of alpha 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 have fully developed our sequencing and cloning workflow, and we have sequenced and displayed several human TCR repertoires (as shown in **Figure 3**). We are in the process of screening the T cell repertoires for the discovery of antigen-specific TCR genes, and we have identified some enriched cell populations after p:MHC staining and screening that contain multiple antigen-specific TCRs of varying affinities. We are VERY excited to prepare these results for publication in the next six months.

As we have validated our TCR display systems, have now shifted our focus onto library screening assays and live-cell killing and activation assays for the identification of cancer cell-specific TCRs. This will constitute the major focus of the next reporting periods of the project.

On the bioinformatic side, we also developed 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. We also applied them for the analysis of antigen-specific T cell receptors from sorted TCR libraries (**Figure 3**). Our next step is to apply NGS for screening affinity of pMHCs directly, which is ongoing.

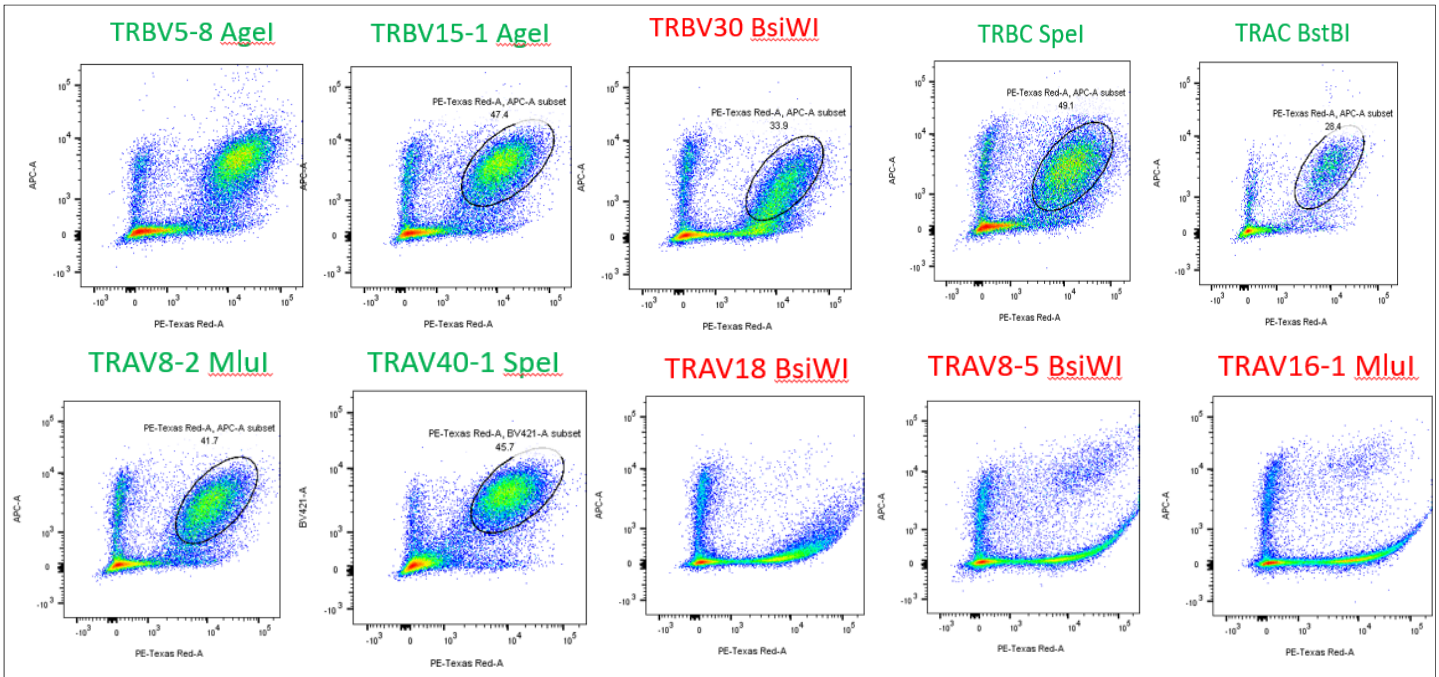

Figure 2: *A small panel of the restriction enzymes and leader peptides (for TRAV/TRBV) that we tested for allowing proper TCR display, using JRT3 cells displaying a known anti-HIV positive control antibody. mCherry expression is shown on the x-axis, while HIV peptide p:MHC binding is shown on the y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

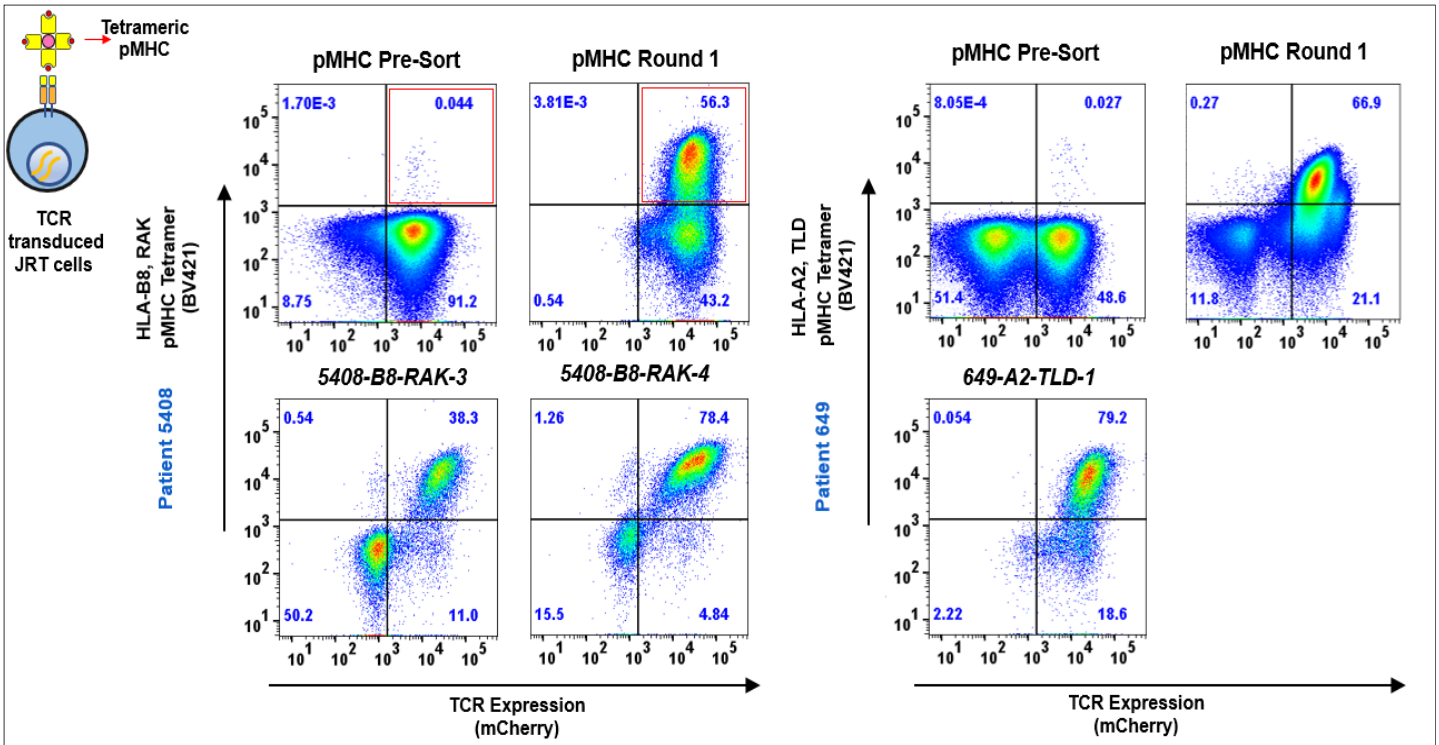

Figure 3: *Analysis of infectious mononucleosis patient samples for anti-EBV peptides as validation of our screening workflows. These data demonstrate the effective screening of immortalized TCR libraries from human patients using soluble peptide:MHC fluorescent screening antigens.*

We also established the platforms for detecting live cell activation in co-culture cell systems (**Figure 4**). We are now applying 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.

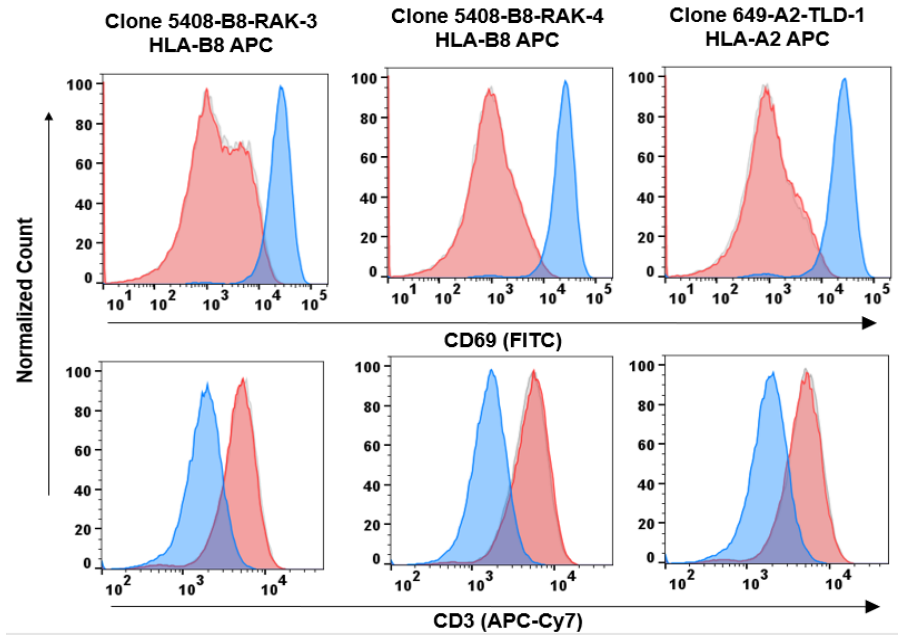

Figure 4: *Activation-based screening of T cell receptor libraries for antigen-specific T cell receptors. Antigen-specific T cell clones that we discovered using our single-cell TCR screening workflow were incubated with antigen-presenting cells (APCs) either with a targeted peptide pulse (blue), without 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.*

4) Key Outcomes or Other Achievements

We have achieved major progress on our research goals. In the next reporting period, we will begin to make public presentations about these exciting new platforms and prepare our findings for publication in 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, Matias Gutierrez, and Bharat Madan, and for technician Nicoleen Boyle. Andrew has been our project lead for advancing methods and techniques for T cell receptor analysis of anti-cancer immunity. Matias has assisted Andrew and also helped to develop new bioinformatic techniques, and Bharat Madan helped develop those bioinformatic methods as well. Nicoleen has been assisting with the cloning and sample analysis of TCR libraries.

This project provided for the training and professional development of 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. 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?**
  - *Dr. Andrew Chung presented at the American Society for Transplantation related to our analysis workflow, and related to the technologies developed in Specific Aim 1 and Specific Aim 2. Dr. DeKosky also delivered seminar presentations which discussed this work.*

Sills M, Chung CY, Zhou J, Ladi R, DeKosky BJ, "Evaluating Mammalian Cell Transfection Efficiency by Optimizing Electroporation Conditions for T-cell Receptor Expression," *KU Summer Undergraduate Research Symposium*, Lawrence, KS (July 2018). Poster Presentation

Chung CY, Fahad A, Moore S, Ladi R, Madan B, Fisher R, DeKosky BJ, "New Technologies to Analyze T Cell Responses to Epstein-Barr Virus", *K-INBRE DRP Core Meeting*, Kansas City, MO (January 2019). Podium presentation

Chung CY, Fahad A, Boyle N, Madan B, DeKosky BJ," Personalized T Cell Receptor Discovery for Treating PTLD and Other Pediatric Cancers", *Science is The Cure Research Symposium, The American Society of Transplantation Annual Meeting*, Orlando (February 2020). Podium presentation

Seminar presentations from Dr. DeKosky:

Gilead Sciences, Inc, Foster City, CA (Jan 2020)
The Wistar Institute / The University of Pennsylvania, Philadelphia, PA (Nov 2019)

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

We expect to advance both experimental work and prepare multiple publications in the coming year. We are currently screening anti-peptide:MHC TCR libraries from four different RCC clinical samples as the first test of our experimental and bioinformatic parameters for T cell receptor analysis. We anticipate that these works will contribute to a publication on high-throughput TCR screening that will be sent out in late 2020. In parallel, we are optimizing the efficiency of our single-cell technology platforms and attempting to finalize a fully-optimized workflow for patient sample processing and affinity analysis. We are now advancing experimental sample

analysis from Dr. Godwin's clinical samples for anti-cancer TCR discovery in a variety of settings. 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 optimizing our methods for computational interrogation of immune datasets. Once the data is obtained from our current TCR screening and the manuscript submitted for publication (late 2020 anticipated), we will generate automated scripts for simplified processing of experimental data based on those optimized parameters. We hope to prepare another bioinformatics-focused paper on TCR repertoire analysis of screened libraries as well.

4. **IMPACT:**
    - **What was the impact on the development of the principal discipline(s) of the project?**
        - While still at an early stage, the advances we have made in the first 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, once they are fully developed in future reporting periods.
    - **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 slight delays due to COVID-19 disrupting research facilities access in the current fiscal year, and from one of our lab members being away on maternity leave. We have mostly resolved COVID-19 barriers and anticipate full resumption of effort on this project in the coming fiscal year. We have also experienced another slight delay with the Sleeping Beauty transposon/transposase system, as the Sleeping Beauty system appears to have somewhat low efficiency compared to lentiviral transduction methods. Thus, we currently plan to optimize our systems using lentiviral transduction (as in currently used cell-based therapies), and adapt our systems to the Sleeping Beauty platform at a later date.
    - **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.** Nothing to report
        - **Books or other non-periodical, one-time publications.** Nothing to report
        - **Other publications, conference papers, and presentations.**

Sills M, Chung CY, Zhou J, Ladi R, DeKosky BJ, "Evaluating Mammalian Cell Transfection Efficiency by Optimizing Electroporation Conditions for T-cell Receptor Expression," *KU Summer Undergraduate Research Symposium*, Lawrence, KS (July 2018). Poster Presentation

Chung CY, Fahad A, Moore S, Ladi R, Madan B, Fisher R, DeKosky BJ, "New Technologies to Analyze T Cell Responses to Epstein-Barr Virus", *K-INBRE DRP Core Meeting*, Kansas City, MO (January 2019). Podium presentation

Chung CY, Fahad A, Boyle N, Madan B, DeKosky BJ," Personalized T Cell Receptor Discovery for Treating PTLD and Other Pediatric Cancers", *Science is The Cure Research Symposium, The American Society of Transplantation Annual Meeting*, Orlando (February 2020). Podium presentation

Seminar presentations from Dr. DeKosky:

Gilead Sciences, Inc, Foster City, CA (Jan 2020)
The Wistar Institute / The University of Pennsylvania, Philadelphia, PA (Nov 2019)

- **Website(s) or other Internet site(s)**
  Nothing to report
- **Technologies or techniques**
  We have validated new techniques for cloning T cell receptors, and we are in the process of validating those technologies for
- **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:	0.36 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:	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).*

Name:	Cheng Yu (Andrew) Chung
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.8 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:	Matias Fernando Gutierrez
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2 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:	Nicoleen Boyle
Project Role:	Assistant Researcher
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9.1 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).*

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

Previous/Current/Pending Support

DeKosky, Brandon – University of Kansas
Zero ended; three new

PREVIOUS (within last 5 years)

Title:	A New Experimental Platform to Analyze anti-gB Antibodies in Human B Cells
Effort:	1%
Supporting Agency:	NIH
Grants Officer:	Emily Tran, 301-451-7280, trane@mail.nih.gov
Performance Period:	01/11/16 – 04/30/18
Funding Amount	$116,933
Project Goals:	This project will establish a new pipeline to understand the comprehensive features of antibody-based immune protection from HCMV in human subjects in an effort to develop new therapeutics and design effective HCMV vaccines.
Specific Aims:	Aim 1: Establish an experimental platform for rapid discovery of human antibodies targeting the HCMV neutralization-sensitive glycoprotein B cell surface receptor. Aim 2: Develop a high-throughput robotic HCMV neutralization assay and apply it to discover HCMV-neutralizing antibodies.
Overlap:	none

Title:	New platforms for rapid and personalized TCR-based cancer immunotherapy
Effort:	2%
Supporting Agency:	NIH/University of Kansas Medical Center Research Institute, Inc. flowthru
Grants Officer:	240-276-5600, ncicenters-r@mail.nih.gov
Performance Period:	02/01/2018 – 01/31/2019
Funding Amount	$35,000
Project Goals:	
Specific Aims:	Aim 1: Develop a platform for rapid isolation of antigen-specific TCR repertoires as personalized, targeted cancer therapies for solid tumors. Aim 2: Develop a paired TCRα:β library display platform for rapid neoantigen-specific TCR library isolation and screening *in vitro.*
Overlap:	none

Title:	New technologies to analyze T cell responses to Epstein-Barr virus
Effort:	2%
Supporting Agency:	NIH/University of Kansas Medical Center Research Institute, Inc. flowthru
Grants Officer:	Christy Leake, 301-594-7706, Christy.leake@nih.gov
Performance Period:	05/01/2018 – 04/30/2019
Funding Amount	$75,000
Project Goals:	To establish new technologies and generate the research environment needed to analyze cellular development of T cell receptor (TCR) responses to Epstein-Barr virus (EBV) in transplant settings.
Specific Aims:	Aim 1: Establish a paired TCR alpha:beta library display platform to analyze T cell repertoire development in EBV-naïve transplant patients and EBV seropositive healthy donor controls. Aim 2: Establish technologies to express anti-EBV TCRs in naïve human T cells for PTLD therapies.
Overlap:	none

CURRENT

Title:	Development and application of antibody optimization technologies to improve public health
Effort:	2%
Supporting Agency:	Leidos Biomedical / NIH Flowthru
Grants Officer:	Joshua Wynne
Performance Period:	10/25/2017 – 08/30/2020
Funding Amount	$1,251,000
Project Goals:	To apply recent advances for in vitro antibody screening to optimize antibodies with high importance for advancing public health.
Specific Aims:	
Overlap:	none

Title:	Comprehensive analysis of human adaptive immune receptors to elucidate correlates of Epstein-Barr virus disease suppression
Effort:	50%
Supporting Agency:	NIH
Grants Officer:	Gabriel Hidalgo, 301-827-4630, gabriel.hidalgo@nih.gov
Performance Period:	09/19/2016 – 08/31/2021
Funding Amount	$1,830,316
Project Goals:	To apply new high-throughput immune profiling techniques to elucidate the features of effective Epstein-Barr virus (EBV) immune control.
Specific Aims:	Aim 1: Gain a comprehensive understanding of B-cell responses to EBV infection in clinical cohorts. Aim 2: Gain a comprehensive understanding of T-cell responses to EBV infection in clinical cohorts. Aim 3: Develop a machine-learning analytical workflow to rapidly identify immune correlates from high dimensional immune profiling data.
Overlap:	none

Title:	Antibody display libraries for precision screening of antibody immune responses to SARS-CoV-2
Effort:	12.5%
Supporting Agency:	NIH
Grants Officer:	Dan Coonfield
Performance Period:	09/01/2020 – 08/31/2021
Funding Amount	$376,545
Project Goals:	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:	Aim 1: Map antibody responses to broad SARS-CoV-2 proteins and epitopes in mild vs. severe cases to identify molecular immune correlates of COVID-19 disease severity. Aim 2: Apply a yeast display screening strategy to emulate the low pH endosomal environment and directly identify potent antibodies that prevent viral fusion.
Overlap:	none

Title:	Comprehensive molecular and functional analyses of anti-HIV-1 broadly neutralizing antibody repertoires
Effort:	3%
Supporting Agency:	NIH
Grants Officer:	Chernay Rogers, 240-669-2992, Chernay.Rogers@nih.gov
Performance Period:	12/01/2018 – 11/30/2020
Funding Amount	$434,800

Project Goals:	To establish several technologies to comprehensively determine HIV-1 protective antibody responses and for enhanced understanding of HIV-1 protective antibodies.
Specific Aims:	Aim 1: Establish and validate technologies for epitope-specific characterization of anti-HIV antibody repertoires. Apply these technologies for bNAb discovery from HIV-1 patients. Aim 2: Develop a system for direct bNAb discovery from CAPRISA cohorts by directly identifying antibodies with broad affinity to many HIV strains. Aim 3: Establish a platform for rapid mammalian IgG secretion of antibody libraries to enable direct neutralization screening of anti-HIV-1 immune repertoires.
Overlap:	none

Title:	The influence of evolutionary landscapes on protective antibody development
Effort:	8%
Supporting Agency:	University of Colorado / NIH Flowthru
Grants Officer:	Laura Pone, 240-669-2951, laura.pone@nih.gov
Performance Period:	09/01/2018 – 12/31/2023
Funding Amount	$972,796
Project Goals:	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:	Aims 1: To determine *ontogeny* from germ line to mature human antibodies for two heterosubtypic HA stem binders. Aim 2: To determine *ontogeny* from germ line to mature human antibodies for four heterosubtypic and subtype- specific HA head binders. Aim 3: To determine **the** number **of evolutionary** trajectories from a representative germline Ab.
Overlap:	none

Title:	Probing antigen specificity and response of autoimmune B cells in Neuromyelitis Optica
Effort:	4%
Supporting Agency:	NIH
Grants Officer:	Jason Lundgren, 240-669-2973, jason.lundgren@nih.gov
Performance Period:	01/11/2019 – 12/31/2020
Funding Amount	$416,625
Project Goals:	To develop new technologies for immune response analysis and treatment of Neuromyelitis Optica.
Specific Aims:	Aim 1: Determine the molecular features of the anti-AQP4 antibody repertoire in NMO patients during active stages of NMO disease. Aim 2: Develop a set of AQP4 probes for capturing AQP4-specific B cells as targeted cell therapies.
Overlap:	none

Title:	High-throughput TCR repertoire-based platforms for antigen-specific cancer immunotherapy
Effort:	3%
Supporting Agency:	DoD
Grants Officer:	Jamie Shortall, 301-619-2393, Jamie.a.shortall.civ@mail.mil
Performance Period:	08/01/2018 – 07/31/2021
Funding Amount	$543,766
Project Goals:	To develop new technologies to advance antigen specific cancer therapies.
Specific Aims:	Aim 1: Develop a platform for rapid isolation of antigen-specific TCR repertoires as personalized, targeted cancer therapies for solid tumors. Aim 2: Develop a paired TCRα:β library display platform for rapid neoantigen-specific TCR library isolation and screening *in vitro.*
Overlap:	none

Title:	Columbia University's proposed response to the new coronavirus outbreak
Effort:	1%
Supporting Agency:	Columbia University/Jack Ma Foundation flowthru
Grants Officer:	Grants-office@columbia.edu
Performance Period:	04/15/2020 – 10/14/2020
Funding Amount	$100,000
Project Goals:	To investigate a range of different biotechnology solutions to interrupt the global COVID-19 pandemic.
Specific Aims:	Aim 1: Generate natively paired immortalized yeast display antibody libraries from convalescent coronavirus patients for use in analyzing the human response to coronaviruses. Aim 2: Perform high-throughput yeast display screening of natively paired antibody yeast display libraries and prepare those libraries for NGS analysis to enable deconvolution of genetic and functional anticoronavirus antibody molecular features. Aim 3: Perform bioinformatic analysis of NGS data to rapidly identify and discover new anti-coronavirus antibodies and enable antibody generation and recombinant antibody performance analysis. Aim 4: Evaluate key molecular features of humoral immune responses to coronavirus antigens via analysis of immune response data, and collaboratively apply those insights for the design of new pan-coronavirus vaccines and therapeutics.
Overlap:	none

Title:	COVID-19 Fast Grant
Effort:	1%
Supporting Agency:	Mercatus Center
Grants Officer:	Tyler Cowen, fastgrantscovid19@gmail.com
Performance Period:	04/15/2020 – 10/14/2020
Funding Amount	$75,00
Project Goals:	To rapidly identify protective SARS-CoV-2 neutralizing monoclonal antibodies and generate new biologic drugs as powerful medical countermeasures.
Specific Aims:	Aim: Mine immune responses in individuals who recovered from COVID-19 and to identify the antibody molecules they carry, and translate those antibodies into drugs for disease prevention and treatment.
Overlap:	none

Title:	Mining natively paired macaque antibodies for Marburg virus protective antibodies
Effort:	1%
Supporting Agency:	Integrated Biotherapeutics, Inc / SBIR NIH flowthru
Grants Officer:	Vandhana Khurana, 240-669-2966, khuranav@niaid.nih.gov
Performance Period:	04/01/2019 – 03/31/2021
Funding Amount	$129,990
Project Goals:	To discover new protective antibodies against Marburg virus from immunized rhesus macaques.
Specific Aims:	
Overlap:	none

Title:	Antibody response to protein drugs
Effort:	1%
Supporting Agency:	US-Israel Binational Science Foundation
Grants Officer:	Dr. Wine Yariv, 913-582-8239, bsf@bsf.org.il
Performance Period:	10/01/2018 – 09/30/2020
Funding Amount	$90,000
Project Goals:	To develop and apply a suite of new technologies for high-throughput analysis of anti-drug antibodies in human patients.
Specific Aims:	Aim 1: To establish a reliable, accurate and sensitive

	immunoassay for detecting all serum ADA while keeping a low background signal, two main factors are considered pivotal during the development of the immunoassay: i) retaining functionality of the MAb-F(ab')2 so that ADA epitopes are preserved, and ii) high purity of the MAb-F(ab')2 with no traces of Fc or undigested IgG that may contribute to the background level when using anti-Fc labeled antibody in the detection phase.
Overlap:	none

Title:	T cell responses shared among triple negative breast cancer patients
Effort:	0.5%
Supporting Agency:	University of Colorado, Denver / NIH Flowthru
Grants Officer:	Mutema Nyankale, 240-276-5987, nyankalem@mail.nih.gov
Performance Period:	09/01/2018 – 08/31/2020
Funding Amount	$22,725
Project Goals:	To develop new technologies to identify TCRs targeting triple negative breast cancer cells.
Specific Aims:	Aim 1: To identify **T cell** *Receptors* of **shared** memory **T** cells from the *Blood* of *TNBC* survivors. Aim 2: To determine longitudinal changes in the memory **T cell** repertoire that correlate with a *Pathologic* complete response after treatment.
Overlap:	none

PENDING

Title:	Droplet-based single B and T cell receptor screening for multi-parameter adaptive immune monitoring
Effort:	8.34%
Supporting Agency:	NIH
Grants Officer:	Dan Coonfield
Performance Period:	09/01/2020 – 08/31/2025
Funding Amount	$3,755,291
Project Goals:	To establish a suite of technologies that maximize the information collected from cell samples and enable new approaches in antibody and T cell receptor drug discovery.
Specific Aims:	Aim 1: Develop new natively paired BCR heavy:light screening technologies for repertoire-scale functional molecular analyses of B cell immune responses. Aim 2: Establish natively paired TCRα:β gene sequencing and screening for repertoire-scale T cell functional analysis of human clinical samples. Aim 3: Apply a commercially available single-cell platform to link BCR/TCR immune receptor function to single-cell transcriptomes in adaptive immune cells.
Overlap:	none

Title:	Comprehensive analyses of anti-SARS-CoV-2 antibody responses in COVID-19 patients
Effort:	25%
Supporting Agency:	National Institutes of Health
Grants Officer:	Dan Coonfield
Performance Period:	09/01/2020 – 08/31/2025
Funding Amount	$3,441,721
Project Goals:	To reveal key biomarkers of COVID-19 disease severity and identify potent SARS-CoV-2 protection mechanisms that will catalyze new medical interventions to treat and prevent COVID-19.
Specific Aims:	Aim 1: Identify repertoire-scale features of anti-SARS-CoV-2 antibody responses that correlate with COVID-19 symptom severity, or with immune protection. Aim 2: Characterize the set of SARS-CoV-2 neutralizing antibodies that prevent endosomal virus entry. Aim 3: Evaluate antibody recognition of SARS-CoV-2 membrane proteins using lipid nanodisc antigens.
Overlap:	none

Title:	Comprehensive analyses of antibody responses to broad coronavirus antigens in COVID-19 patients
Effort:	4.17%
Supporting Agency:	National Institutes of Health
Grants Officer:	Dan Coonfield
Performance Period:	09/01/2020 – 08/31/2022
Funding Amount	$416,013
Project Goals:	To reveal the comprehensive antibody-based vulnerabilities of SARS-CoV-2 and catalyze new vaccine and therapeutic discovery against COVID-19 and other emergent respiratory coronaviruses.
Specific Aims:	Aim 1: Achieve a comprehensive molecular understanding of antibody responses against the entire SARS-CoV-2 proteome in convalescent COVID-19 patients. Aim 2: Identify cross-reactive antibodies and targeted epitopes against broad human and animal coronaviruses to inform new COVID-19 therapeutics and vaccine designs.
Overlap:	none

Title:	Development of a rapid throughput platform for COVID-19 B cell epitope identification and multiplex immunoprofiling
Effort:	10%
Supporting Agency:	New York University School of Medicine / NIH flow thru
Grants Officer:	Dan Coonfield
Performance Period:	09/30/2020 – 09/29/2025
Funding Amount	$944,148
Project Goals:	This project will generate a highly multiplex assay platform for immunoprofiling of anti-SARS-CoV-2 antibodies.
Specific Aims:	Aim 1: To develop and validate a highly multiplex Ab profiling assay system. Aim 2: To characterize responses in the blood and compared to the lung, the primary site of symptomatic disease, epitope-defined anti-COVID-19 Ab profiles, and in matched plasma samples.
Overlap:	none

Title:	Novel Mutated Ovarian Cancer Specific Cell Surface Protein as Target for Ovarian Cancer Immunotherapy
Effort:	6.7%
Supporting Agency:	KU Medical Center Research Institute, Inc. / DoD flow thru
Grants Officer:	Dan Coonfield
Performance Period:	05/01/21 – 04/30/23
Funding Amount	$39,535
Project Goals:	This project seeks to identify a new antibody targets and antibodies for precision ovarian cancer treatment.
Specific Aims:	Aim 1: Discover the identity of mutated ovarian cancer specific cell surface proteins from multiple ovarian cancer cell lines. Aim 2: Develop monoclonal antibodies for mutated ovarian cancer specific cell surface proteins and evaluate the specificity of newly developed antibodies.
Overlap:	none

Title:	Bridge Proposal: Droplet-based single B and T cell receptor screening for multi-parameter adaptive immune monitoring
Effort:	4.17%
Supporting Agency:	KU Medical Center Research Institute, Inc. / NIH flow thru
Grants Officer:	Dan Coonfield
Performance Period:	10/01/2020 – 04/30/2021
Funding Amount	$40,000
Project Goals:	To develop innovative cell-based assays that mine human immunity with molecular detail, and to accelerate progress in human immune research and drug discovery.

Specific Aims:	Aim 1: Develop new natively paired BCR heavy:light screening technologies for repertoire-scale functional molecular analyses of B cell immune responses.
Overlap:	none

Title:	Molecular identification and precise therapeutic targeting of anti-insulin B cells in Type I diabetes
Effort:	4%
Supporting Agency:	National Institutes of Health
Grants Officer:	Dan Coonfield
Performance Period:	04/01/2021 – 03/31/2023
Funding Amount	$416,656
Project Goals:	This project represents the first step of a broad research program in the guided development and analysis of targeted molecular therapeutics to treat human autoimmune diseases, beginning with T1D.
Specific Aims:	Aim 1: Determine the molecular features of the anti-insulin, anti-proinsulin, and anti-pre-proinsulin antibody repertoire in Type 1 Diabetes patients at the initial stages of T1D onset. Aim 2: Develop a series of insulin probes to capture insulin-specific B cells for targeted cell therapies.
Overlap:	none

Title:	Mechanisms of Brain Clearance of Monoclonal Antibodies
Effort:	8.34%
Supporting Agency:	National Institutes of Health
Grants Officer:	Dan Coonfield
Performance Period:	04/01/2021 – 03/31/2023
Funding Amount	$416,625
Project Goals:	This project is aimed at improving brain delivery and controlling brain clearance of anti-amyloid-beta monoclonal.
Specific Aims:	Aim 1: To study the brain delivery and mechanisms of brain clearance of anti-Aβ mAb. Aim 2: To engineer Fc regions with distinct pH-based FcRn binding features and evaluate BBB based clearance after high-concentration brain delivery.
Overlap:	none

Previous/Current/Pending Support

Ellington, Andrew - University of Texas at Austin
Six ended, eleven new

PREVIOUS (within last 5 years)

1541244 (Simmel; Germany) 08/01/2015 – 07/31/2018 0.24 CAL
ERASynBio $539,997
ERASynBio: A Unified Nucleic Acid Computation System for Organisms
Major Goals: To generate dCas9 and T7 RNA polymerase variants that can be used as parts in the UNACS system for cellular programming.
Aim 1: Engineering next-generation riboregulators for a Unified Nucleic Acid Computation System. Aim 2: Development of a "universal join' between RNA programming and protein function. Aim 3: Extending RNA sensing and regulation to eukaryotes.
Program Official: Larry Halverson

DBI-0939454 (Ellington;Wichman) 08/01/2016 – 07/31/2018 0.12 CAL
NSF/Michigan State $138,535
Rapid analysis of parasite dynamics and evolution in arthropod populations
Major Goals: Create a point of contact diagnostic to monitor arboviral diseases and to better understand the evolutionary dynamics of these diseases.
Aim 1. Develop LAMP-based POC diagnostics system. Aim 2. Test system on Drosophila viruses.

R43 NS105463 (Hall) 04/01/2018 – 09/30/2018 0.12 CAL
NIH $38,862
Development of a Unified Information Rich Test for CNBP and DMPK to Improve Assessment of Myotonic Dystrophy Type 1 and 2
The goal of this project is to develop a comprehensive molecular diagnostic test for Myotonic Dystrophy Type 1 and 2.

HDTRA1-16-1-0001 (Anslyn) 10/28/2015 – 10/27/2018 0.12 CAL
DoD-DTRA $303,059
Rapid, Selective, and Sensitive Sensors for Nerve Agents
Major Goals: To create a series of colorimetric, fluorescent, and chemiluminescent detection methods for nerve agents using oximes as the relative moiety.
Task 1: Synthesis and immobilization of receptor-nerve agent surrogate species 6 & 7. Task 2: Systematic Evolution of Ligands by eXponential Enrichment (SELEX) process to produce aptamers for target compounds 6 & 7. Task 3: Aptamer testing in the context of nerve agent surrogate samples. Task 4: Synthesis of signal cascade complexes 9 & 10 for signal cascade reactions. Task 5: Optimization and testing of signal cascade complexes with fluorides and thiols. Task 6: Compatibility studies of kit components. Task 7: Kit integration with handheld fluorescence reader.

Unknown (Arimoto) 11/01/2017 – 10/31/2018 0.12 CAL
Military Infectious Diseases Research Program $28,619
Evaluation of novel assay for field-deployable molecular diagnostic platform for rapid identification of mosquitoes, pathogens, and insecticide resistance status for military vector control operations
The goal of this project is to evaluate the LAMP-OSD assay for use in rapid vector identification, pathogen detection, and insecticide resistance detection and conduct an in depth assessment of the potential utility of this tool in military vector control operations and identify improvements for optimum field readiness.
Task 1: Shipment of (RT)-LAMP-OSD assays. Task 2: Mosquito sampling. Task 3: Mosquito identification. Task 4: Mosquito processing for LAMP-OSD assay. Task 5: Development of new LAMP-OSD assays.

SOMNOGHP (Ellington) 03/01/2016 – 11/30/2018 0.12 CAL
Templeton Foundation $325,924
Balancing reactivity and replicability with phosphorothiorates
Major Goals: Develop a short replicator that relies on foldback priming and use this replicator and its accompanying replication mechanism as a ‘breadboard’ to examine origins problems.
Aim 1. Developing a simple replicator that can serve as a ‘breadboard’ for origins questions. Aim 2. Direct visualization of sequence and fitness landscapes. Aim 3. Coupling replication and catalysis.
Program Officer: Linda Jackson, Foundation for Applied Molecular Evolution, 13709 Progress Blvd Box 7, Alachua, FL 32615, 386-418-8085

F-1654 (Ellington) 06/01/2016 – 05/31/2019 0.12 CAL
Welch Foundation $240,000
Kinetic and structural characterization of the first error-correcting reverse transcriptase
Major Goals: Determine important residues and perform kinetic analyses for the error-correcting function of an evolved reverse transcriptase.
Aim 1. Determining the residues responsible for substrate specificity and generality. Aim 2. Kinetic analyses of engineered reverse transcriptases. Aim 3. Kinetic X-ray crystallography of the engineered reverse transcriptase.
Program Officer: Norbert Dittrich, The Welch Foundation, 5555 San Felipe, Suite 1900, Houston, TX 77056-2730, 713-961-5168

W911-NF-17-2-0091 (Davies) 05/11/2017 – 09/09/2019 0.0 CAL
Army Research Labs $185,485.37
Synthetic assembly of bacterial communities
The goal of this project is the production of peptide and protein materials that contain non- standard amino acids that can improve the biophysical properties of the materials.

Ajinomoto, Inc. 227 (Ellington) 08/01/2017 – 11/27/2019 0.12 CAL
Ajinomoto $159,746
0.12 cal.mo.

Metabolic engineering of E. coli for high-level production of L-DOPA
Major Goals: Use directed evolution to create E. coli strains that can produce large amounts of enantiomerically pure L-DOPA.

I-Corps (Ellington) 06/15/2019 – 11/30/2019 0.12 CAL
National Science Foundation $50,000
Structure Based Machine Learning Aided Protein Engineering
Major Goals: Evaluate the market potential of structure based machine learning algorithms for protein design

Asuragen 300439 (Ellington) 04/30/2018 - 03/31/2020 0.12 CAL
Asuragen $38,882
Development of a unified information-rich test for CNBP and DMPK to improve assessment of Myotonic Dystrophy Type 1 and 2
Major Goals: Production, purification and quality control of enzymes, and for providing enzymes to Asuragen.

HR001117S002-IA-FP-008 (Barrick) 07/01/2017 – 05/30/2020 0.6 CAL
DARPA $ 236,860
0.6 cal.mo.
AEPHID: Aphid Endosymbionts for Plant Host Immunization and Defense
Major Goals: Engineer therapeutic viruses to be carried by aphid vectors into plant hosts in order to protect them from pathogens and deliver beneficial traits.

NSF Beacon (PI) 08/01/2019 – 07/31/2020 0.12 CAL
NSF $96,716
Evolution of synthetic plant viruses
Major Goals: We will create and observe the evolution of a fully synthetic plant virus to study how synthetic biological entities interface with hosts.

CURRENT
1 R01 CA186132 (Co-I; Richards-Kortum) 09/01/2015 – 08/31/2020 0.12 CAL
NIH $267,885
High-Resolution Imaging and HPV Oncoprotein Detection for Global Prevention of Cervical Cancer
Major Goals: The goal of this project is the development of robust, affordable technologies to enable early detection of cervical cancer in low-resource settings.

HDTRA1-18-1-0030 (Co-PI) 3/22/2018 – 8/31/2020 0.6 CAL
DTRA $536,889
Immunization with transition state analogues
Major Goals: To create a series of transition state antibodies for the neutralization of nerve agents.

HR00111820048 (PI) 04/1/2019 – 09/30/2020 1.0 CAL
DARPA $1,721,136 (PhII)
Sensor Plants that Communicate via High Amplification Volatile Organic Channels
Major Goals: Develop sensor plants that communicate via volatile organic channels.

UTAP-EXPL/NTec/0015/2017(Peterson) 11/05/2018 – 11/04/2020 0.12 CAL
Portuguese Science and Technology Foundation $86,077.00
International Collaboratory for Emerging Technologies-CoLab
Major Goals: DREAM seeks to use the actual lines of research of the national and international groups involved (which already have a strong background in the development of nanosystems), deepening it to develop a novel vehicle-ligand with antiviral and antitumoral effect in precancerous cells infected by HPV.

FAB-SUB81XWH-20-P-0037 (Chin) 02/12/2020 – 11/11/2020 0.12 CAL
Fabrico (Through DoD) $49,909
Ratiometric Quantification of Targeted Methylated DNA sites using MM-LAMP-OSD & Detection Devices

Major Goals: To develop a novel isothermal nucleic acid amplification method called Mismatch Methyl Loop Mediated Isothermal Amplification-One-step Strand Displacement (MM-LAMP-OSD) and automate it on a field-ready device for quantitative identification of methylated nucleotides at targeted sites in the human genome.

R21 AI135576 (PI) 04/01/2017 – 11/30/2020 0.6 CAL
NIH $425,547
Rapid, cellphone-based POC detection of Borrelia species in field-caught ticks
Major Goals: Develop a point-of-care test to identify tick species and the presence of Borrelia
Aim 1. Develop a one-pot, multiplex TAO-PS-LAMP assay that transduces genetic signatures of I. scapularis and Borrelia species into color-coded fluorescent signals readable by smartphones. Aim 2. Develop a POC device for identification of Borrelia-infected field-caught I. scapularis ticks.

R21FY2019-075 (DeKosky PI) 12/01/2018 – 11/30/2020 0.24 CAL
NIH(University of Kansas Pass-through) $165,889
Comprehensive molecular and functional analyses of anti-HIV antibody repertoires
Establish a platform for rapid IgG secretion of antibody libraries for direct neutralization screening.

FAB-SUB81xWH-18-C-0147-001 (PI) 06/01/2018 – 11/30/2020 0.12 CAL
Fabrico Technology (DHA-SBIR) $349,998
Field portable coliform bacteria & E. coli RNA biomarker LAMP-OSD system
Major Goals: Complete an EPA ATP application for the verification of a RT-LAMP-OSD system capable of distinguishing between viable and nonativen-viable coliform bacteria and E. coli yielding a 'Yes/No' (presence/not presence).

Water Lens SRA (PI) 02/01/2020 – 12/31/2020 0.12 CAL
Water Lens $49,789
Rapid molecular test for sulfate reducing bacteria in fracking water
Major Goals: We will develop an assay which can indicate presence or absence of SRB in wastewater from fracking sites via fluorescent output.

HR001118S0041 (Silberg PI) 10/01/2019 – 01/28/2021 0.6 CAL
Willam Marsh Rice University (DARPA) $282,563
Subterranean surveillance of chemicals in soil using a microbial relay network that transmits using rare indicator gases
Major Goals: We are developing peptide sender:receiver systems that will be capable of lateral communication between bacteria in soil, and that will amplify signals sent from subterranean sources.

090165-16882 (Co-I) 10/01/2019 – 01/28/2021 0.12 CAL
University of Illinois Urbana-Champaign (DARPA) $150,000
Native DNA-Based Data Storage and Computing
Major Goals: Development of tools and methods for DNA information storage, based on increasing the scale of so-called "DNA punch cards." One aspect of this project will be to develop higher throughput methods for the preparation, execution, and analysis of punch cards, using chemical modifications and high-throughput sequencing approaches.

NNX15AF46G (PI) 02/17/2015 – 02/16/2021 2.4 CAL
NASA $2,500,000
Expanded Alphabets for Constructing Evolutionary Machines
Major Goals: To identify nucleic acid molecules that could have evolved in alien environments.

2027169 (Co-PI) 04/01/2020 – 3/31/2021 0.24 CAL
National Science Foundation $200,000
RAPID: Development of Rapid POC SARS-2019-nCoV LAMP-OSD Assay System
Major Goals: Development of a low cost easy to use point of care test platform for SARS-2019-nCoV virus responsible for COVID-19.

W911NF-18-1-0169 (Multi-PI) 05/01/2018 – 04/30/2021 0.12 CAL
DOD – Army Research Office $343,809
Design of Protein Biomaterials Through Tailored Shape and Packing Strategies of Patchy Particles

Major Goals: Show the utility of supercharged protein assembly for a variety of militarily relevant applications, from printing structures to functionalizing assemblies with silicon and metals.

W911NF1610372 (Co-I with Jewett) 06/01/2016 – 05/31/2021 0.12 CAL
Army Research Office $1,142,291
Engineering the translation apparatus for synthesis of electronically active sequence-defined polymers
The goal of this project is to establish a framework to design and synthesize polymers of defined length and sequence that are comprised solely of non-natural monomers.

W911-NF-17-2-0091 (Ellington) 09/10/2019 – 06/29/2021 0.0 CAL
Army Research Labs $ 1,710,743.75
Synthetic assembly of bacterial communities
The goal of this project is the production of peptide and protein materials that contain non- standard amino acids that can improve the biophysical properties of the materials.

UTA18-000856 (Ellington) 07/15/2018 – 07/14/2021 0.12 CAL
ExxonMobil $797,547
Systems and Synthetic Biology Approaches to Plastic Degradation
Major Goals: To provide a better path towards engineering the degradation and biotransformation of plastics.

NSF1807269 08/01/2018 – 7/31/2021 0.24 CAL
National Science Foundation $450,243
Collaborative Research: SemiSynBio: YeastOns: Neural Networks Implemented in Communicating Yeast Cells
The goal of this project is to use synthetic biology to create small simple cells that will serve as the building blocks of cellular neural network computers.

FA9550-14-1-0089 (Alper) 08/15/2014 – 08/14/2021 0.6 CAL
DoD-Air Force Research Laboratory $666,483
Theory-based Construction of Synthetic Circuit Robustness through a Parts to Circuit Approach Focusing on Environmental and Evolutionary Robustness

RGP0015-2017 (PI) 11/01/2017 – 10/31/2021 0.36 CAL
Human Frontiers Science Program $300,000
Rebuilding and reimagining the last common ancestor, a ribo-organism
Major Goals: The goal of this project is to create a form of life that is a ribo-organism whose genetic code is read by ribozymes.

F-1654 (Ellington) 06/01/2019 – 05/31/2022 0.12 CAL
Welch Foundation $240,000
A Neural Network for Polymerase Engineering
Major Goals: Determine important residues and perform kinetic analyses for the error-correcting function of an evolved reverse transcriptase.

R01 GM124141 (Finkelstein) 09/15/2017 – 06/30/2022 0.36 CAL
NIH $603,872
Mechanism, specificity, and design of CRISPR RNA-mediated gene regulation
The goal of this project is to mechanistically dissect a newly discovered family of RNA-guided nucleases.

UTA18-000656 (Ellington) 09/02/2018 – 08/31/2022 0 CAL
Promega $600,000
Screening for reduced stutter polymerases
Major Goals: Develop a thermally-stable DNA amplification system with enhanced fidelity for amplifying STRs.

GT10481(Ellington;AnslynMultiple-PI) 01/01/2018 - 12/31/2022 0.6 CAL
HHMI $1,500,000
Accelerating Professional Development for Undergraduate Science Majors

Major Goals: Establish a unique training experience for undergraduate and graduate chemistry and biochemistry majors, aimed at creating the next generation of both scientist-leaders and scientist-entrepreneurs.

R01 EB026533 (Ellington) 05/04/2019 – 01/31/2023 0.6 CAL
NIH $1,541,640
Synthetic biology for controlled release
Major Goals: Develop gut bacteria that can produce the amino acid L-DOPA, which is a known treatment for Parkinson's disease, and determining if the bacterial factories can provide benefit to a Parkinson's mouse model.

17-NAI8_2-0026 (Johnson) 07/01/2018 – 06/30/2023 0.02 CAL
NASA $277,805
Agnostic biosignatures of extant life
Major Goals: Refine definitions of the fundamental characteristics of life and optimize measurement strategies in the search for agnostic biomarkers.

1R01EB027202-01A1 (Ellington) 3/15/2020 – 11/30/2023 0.6 CAL
NIH $1,519,460
Directed evolution of polymerases that can read and write extremely long sequences
Major Goals: To generate long read DNA polymerases that should prove capable of generating PCR amplicons > 100 kb in length, with few errors.

HDTRA1-20-1-0011 (Ellington) 06/17/2020 – 06/16/2024 0.6 CAL
DTRA $ 1,722,636
A structure-based machine learning framework to engineer antibody stability maturation and affinity
Major Goals: Use a neural network to predict potential mutations for the creation of more stable antibodies with greater affinity for their target.

PENDING

Manufacturing Innovation Institute (Ellington) 10/01/2020 – 03/31/2022 0.36 CAL
Department of Defense
Bio-manufacturing nanoparticles for antenna, transformers, supercapacitors, and stealth coatings
Major Goals: Further develop high-throughput screens and selections for organisms with enhanced magnetic particle formation.

NSF, Arlington, VA. (Ellington) 06/01/2020 – 5/30/2021 0.12 CAL
NSF $150,000
RAPID: Dual COVID-19 and Influenza Virus Detection via Target Antibody-Functionalized Graphene Field-Effect Sensing

UT Energy Institute (Ellington) 01/01/2020 – 12/31/2021 0.12 CAL
UT-Austin $300,000
Carbon transformation, from outgassing to rubber
Major Goals: We will develop new methods for transforming emitted methane and carbon dioxide into rubber – an invaluable polymer with uses from gloves to tires.

Cancer Informatics (Ellington - Multi-PI) 07/01/2020 – 06/30/2022 0.6 CAL
NIH $411,731
Integrating 3D CNNs into Cancer Precision Oncology
Major Goals: We will use a novel 3D convolutional neural net to identify individual protein mutations that may be functional contributing to a tumor's growth, and meld these with characterizations of molecular heterogeneity among patients.

Small Business Innovation Research (Ellington) 01/01/2021 – 12/31/2022 0.24 CAL
Department of Defense $94,637
Field portable LAMP-OSD environmental test kit and detection system
Major Goals: Develop more robust enzymes and assays that will work across a range of surfaces and environments and provide insights into how to carry out environmental testing.

EFRI E3P (Co-PI Alper) 09/01/2020 – 08/31/2024 0.37 CAL
National Science Foundation $1,981,000
Biodegrading Environmental Plastics Using Engineered and Bio-prospected Microbes and Enzymes
Major Goals: Establish new enzymatic and physio-chemical routes to both degrade and understand the kinetics of degradation for polystyrene as a major plastic material.

Dreyfus Foundation (Multi-PI) 09/01/2020 – 08/31/2024 0.6 CAL
Dreyfus Foundation $1,716,148
Neural Net Analysis of Synthetic Polymer Structure and Function
Major Goals: Apply previously developed convolutional neural nets to a new polymer class, oligourethanes, and to teach a new generation of students how to program CNNs for biology and chemistry.

DARPA – ADAPTOR (Multi-PI) 10/01/2020 – 03/31/2025 0.24 CAL
DARPA $1,693,561
A hybrid/cell material device that uses an all electrical strategy for fast detection of traveler's diarrhea and production of therapeutics
Major Goals: Developing biosensors and peptide antibiotics for the control of traveler's diarrhea.

NASA – ICAR (Multi-PI) 10/01/2020 – 09/30/2025 0.6 CAL
NASA $4,060,800 yearly
Synthetic Cellularization
Major Goals: Provide an experimental basis for addressing some of the key, unanswered questions surrounding both origins and a transition to cellular (and multicellular) life.

- **What other organizations were involved as partners?**
  - **Organization Name:** The University of Kansas Medical Center
  - **Location of Organization:** Kansas City, KS
  - **Partner's contribution to the projec**t
    - **Collaboration**: Discussion and analysis of workflows for anti-cancer TCR screening (with PI Andrew Godwin, Mentor); sharing of reagents, protocols, and clinical samples
    - **Personnel exchanges** Personnel travel between laboratories to discuss and facilitate collaboration.
    - **Other.**
  - **Organization Name:** The University of Texas at Austin
  - **Location of Organization:** Austin, TX
  - **Partner's contribution to the projec**t
    - **Collaboration**: Production and sharing of custom enzyme reagents, and advice/guidance connected with the use of those enzymes for single-cell genetic analysis.
    - **Personnel exchanges** N/A
    - **Other**

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

****

ADDITIONAL NOTES:

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**