PI: **KRUPNICK, ALEXANDER S.**	Title: Novel Immuno-oncology Strategy for Targeted Cytotoxic Lymphocyte Activation	
Received: 01/04/2018	FOA: PA17-303	Council: 05/2018
Competition ID: FORMS-D	FOA Title: PHS 2017-02 Omnibus Solicitation of the NIH for Small Business Technology Transfer Grant Applications (Parent STTR [R41/R42])	
1 R41 CA224520-01A1	Dual: AI	Accession Number: 4122960
IPF: 10042030	Organization: COURIER THERAPEUTICS, INC.	
Former Number:	Department: Department of Surgery	
IRG/SRG: ZRG1 OTC-H (13)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 192,285	Animals: Y Humans: Y Clinical Trial: N Current HS Code: E4 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	[redacted]	PD/PI
[redacted]	[redacted]	Co-Investigator
[redacted]	[redacted]	Co-Investigator
[redacted]	[redacted]	Co-Investigator
[redacted]	[redacted]	Other (Specify)-Scientist
[redacted]	[redacted]	Consultant
[redacted]	[redacted]	Consultant

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
CA224520

b. Agency Routing Number
[redacted]

2. DATE SUBMITTED

Application Identifier

c. Previous Grants.gov Tracking Number
[redacted]

5. APPLICANT INFORMATION

Organizational DUNS* [redacted]

Legal Name*: COURIER THERAPEUTICS, INC.
Department:
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Sarah Middle Name: Last Name*: Hein Suffix: Ph.D
Position/Title: Director of Research
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

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

7. TYPE OF APPLICANT* R: Small Business

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

8. TYPE OF APPLICATION*
❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

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

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

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Novel Immuno-oncology Strategy for Targeted Cytotoxic Lymphocyte Activation

12. PROPOSED PROJECT
Start Date* Ending Date*
07/01/2018 06/30/2019

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TX-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: ALEXANDER Middle Name: SASHA Last Name*: KRUPNICK Suffix: M.D.

Position/Title: [redacted]
Organization Name*: [redacted]
Department: [redacted]
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$0.00
d. Estimated Program Income*	$0.00

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

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Sarah Middle Name: Last Name*: Hein Suffix: Ph.D

Position/Title*: Director of Research
Organization Name*: Courier Therapeutics
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative* [redacted]

Date Signed* [redacted]

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_letter.pdf

424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: Courier Therapeutics, Inc [redacted]
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Project/Performance Site Location 1

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

Organization Name: University of Virginia Medical Center
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303. Received Date: 2018-01-04T12:18:51.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

If YES, check appropriate exemption number: — 1 — 2 — 3 ✔ 4 — 5 — 6

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 10-25-2017

Animal Welfare Assurance Number [redacted]

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract.pdf
8. Project Narrative*	Narrative_IL2.pdf
9. Bibliography & References Cited	final_references_V9.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources.pdf
11.Equipment	
12. Other Attachments	SBC_001154063.pdf

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303. Received Date: 2018-01-04T12:18:51.000-05:00

PROJECT SUMMARY/ABSTRACT

Systemic administration of high-dose IL-2 has been used since the 1980's as an FDA-approved immunotherapy for metastatic cancer. Despite the fact that up to 9% of patients treated with high dose IL-2 achieve a durable, long term response, this therapy is rarely used today due to significant life-threatening complications. Such complications occur due to IL-2 activation of vascular endothelium, resulting in systemic capillary leak, as well as other adverse effects associated with "off target" signaling of IL-2. In addition, IL-2 preferentially activates CD4+Foxp3+ regulatory T cells that mitigate the tumor-specific response. We recently published an IL-2 fusion protein which targets and solely activates cytotoxic T lymphocytes in the absence of endothelial or regulatory T cell activation. In addition to a dramatically increased safety profile, our IL-2 fusion protein inhibits the growth of highly aggressive tumors normally resistant to other forms of immunotherapy. The purpose of the current proposal is to evaluate the immunogenicity of this protein, explore its clinical response when used in combination with checkpoint blockade immunotherapy, and evaluate its utility for expansion of tumor-reactive leukocytes for adoptive cell therapy. Successful completion of the proposed studies will support a Phase II STTR application and eventually advancement of this therapy to IND-enabling studies. Our ultimate goal is to submit this novel immunotherapy to the FDA for first-in-human clinical safety trials.

PROJECT NARRATIVE

Patients with advanced cancers, such as lung cancer, have few treatment options with limited long-term survival. FDA approved high-dose IL-2 therapy leads to a high rate of long-term durable remissions in several malignancies, but is infrequently utilized due to high rates of therapy-associated morbidity and mortality. Courier Therapeutics has developed a targeted form of IL-2 which dramatically reduces the adverse effects of IL-2 therapy while enhancing therapeutic efficacy. These studies will support future submission of this novel therapeutic agent to the FDA for a lung cancer human clinical trial.

Facilities and Other Resources

Laboratory – Houston

The Courier Therapeutics laboratory in Houston, Texas is located at the Johnson and Johnson JLabs facility. Courier Therapeutics was given the opportunity to license space at this facility through a competitive application process. This 17,000 square foot lab facility is BSL2 certified and fully equipped with all standard biotechnology lab equipment. Additionally, the JLabs staff maintain necessary laboratory permits, cleaning staff, waste removal, handle shipping and receiving, utilities, and coordinate facility use. In addition to the lab space, Courier personnel has access to a workstation space, general office equipment, conference rooms, and a kitchen.

Courier Therapeutics has a standard lab bench and associated facilities, including necessary certifications, hazardous and biohazardous waste removal, and 4 °C, -20 °C, -80 °C, and liquid nitrogen storage. Courier Therapeutics additionally has access to all necessary cell culture equipment including a laminar flow hood, tissue culture incubators, water baths, and other necessary equipment. The JLabs facility is fully equipped with a variety of necessary major equipment, including: Vectra Polaris Slide Imager and Analyzer, Sony Analyzer and Sony Cell Sorter, Analytik Jena Thermal Cycler and qPCR machine, PE Ensight Multimode Plate Reader, GE Cytell Cell Imaging System, DeltaVision Elite High Resolution Microscope, and a PE Liquid Handler. These equipment are supported by state-of-the art analytics machines including a machine learning platform for automated analysis of multiplexed images from the Vectra Polaris Slide Imager. Additionally, we have access to a variety of standard equipment such as cell culture and standard lab microscopes, centrifuges, balances, shakers, a pH meter, a RODI system, an autoclave, a lab-grade glass washer, and chemical fume hoods.

Laboratory – University of Virginia

The Krupnick laboratory in the University of Virginia is located in Medical Research Building 6 (MR6) which houses the Carter Center for Immunology. The Krupnick laboratory consists of 1600ft^2 of wet lab space with two tissue culture hoods, standard as well as -80° freezers and equipment for molecular biology diagnostics. General laboratory equipment includes: Analytical balance, top-loading balance, Beckman table-top centrifuge, two Eppendorf microcentrifuges, -20°C freezer, -70°C chest freezer, -80°C upright freezer, two hot plate/stirring plates, laboratory oven, pH meter, platform orbital shaker, two stirring plates, three vortex mixers, two 5L water baths, one 10L water bath, one 12L water bath, 18 MΩ water purification system, blotting apparatus, laminar flow hood with HEPA filtration clean bench, two multi-channel electrophoresis power supplies, horizontal and vertical gel electrophoresis apparatuses, dry bath incubator heat block, Polytron tissue homogenizer, hybridization oven/incubator, shaking incubator, ELISA microplate reader, Reacti-Vap nitrogen gas evaporator, autoradiography film cassettes, radiation survey meter, storage and shielding for Beta emitters, Shimadzu spectrophotometer, Eppendorf PCR thermal cycler, Leica cryostat, Nikon digital camera with microscope attachment, floatation water bath for paraffin sections, Reichert-Jung Biocut microtome, Nikon Labophot II with dual eyepiece microscope, Nikon Alphaphot II microscope, slide incubator, two class II biosafety cabinets, single and dual chamber CO2 incubators, Olympus CK2 inverted phase contrast microscope, aurum vacuum manifold and IKA MTS 2/4 96-well plate orbital shaker. However he has free and full access to all shared equipment in the Carter Center for Immunology.

Shared Research Facilities – University of Virginia

The University of Virginia Health System contributes an important aspect to the environment in the form of shared instrumentation service facilities that have evolved over a period of many years. These service centers complement the expertise of the laboratories of Immunology Faculty, and are supported by the School of

Medicine endowment and user fees. They provide extensive teaching and consultation through their faculty directors, and are a major part of the research environment at the School of Medicine.

The **Advanced Microscopy Facility** provides investigators at the University of Virginia access to electron and confocal microscopes, preparatory equipment, and technical assistance. Specific objectives include: (1) making state-of-the-art electron and confocal microscopes and related equipment available for research applications; (2) providing complete electron microscopy specimen preparation services; (3) providing training in the operation of electron and confocal microscopes; (4) furnishing technical assistance and consultation for routine and specialized microscopy applications; and (5) monitoring developments in the general field of microscopy and providing access to emerging technologies and related equipment. Facility equipment includes: ZEISS LSM 710 4-Channel Multi-Photon Confocal, upright; ZEISS LSM 700 4-Channel Confocal, inverted; ZEISS LSM 510 4-Channel Confocal, inverted, two microscopes; ZEISS LSM 510 META, 3-Channel, inverted; JEOL 1230 TEM (digital and film image capture); JEOL 6400 SEM (digital image capture); JEOL 100CX II TEM (digital and film capture); FE-SEM; OLYMPUS BX51 - upright compound microscope/digital camera: brightfield, fluorescence, phase, DIC; OLYMPUS SZX12 – dual objective stereomicroscope/digital camera: brightfield, darkfield, fluorescence; PREPARATION EQUIPMENT: Ultramicrotomes, Critical Point Dryer, Sputter Coater, High Resolution Metal Coater.

The **Keck Center for Cellular Imaging** includes a variety of state of the art microscopes for fluorescence and confocal microscopy, including conventional epifluorescence microscopy, laser scanning confocal microscopy, fluorescence resonance energy transfer, laser ablation and microinjection.

The **Research Histology Core** serves the histology needs of all UVA basic and clinical researchers. The director is Dr. Kenneth Tung, MD, a renal pathologist and ITP mentor. The core is staffed with a laboratory manager and two highly experienced laboratory technologists. The Histology core provides tissue processing, embedding, sectioning and staining with routine hematoxylin and eosin stain and other more specialized tissue stains. They process both animal and human tissues, and the laboratory is a BSL2 facility. The Core provides frozen sections for immunofluorescence studies, and other sections for in situ hybridization.

The **Biorepository & Tissue Research Facility** (BTRF) occupies 2260 sq. ft. of BSL-2 laboratories, freezer storage, and offices in the Carter-Harrison Building. The BTRF is staffed by 8 FTEs and by part-time MD pathology faculty and is the main core facility at UVA supporting procurement, processing, banking, and analysis of human biospecimens. Services include: tissue procurement from procedures performed at the UVA Hospital and Clinics, access to archival pathology specimens, biofluid processing and aliquoting, formalin fixation & paraffin embedding (FFPE), standard histology, cryo-histology, automated immunohistochemistry (IHC), tissue microarray (TMA) manufacture, digital slide scanning, robotic nucleic acid extraction, microcapillary electrophoresis, tissue digestion, peripheral blood leukocyte Ficoll isolation, and viable cell freezing. A panel of >40 IHC stains is available, as well as custom IHC workup for investigator-supplied antibodies. The BTRF maintains a tissue bank of annotated frozen and FFPE tissue samples quality-controlled by histologic examination by board-certified pathologists. TMAs that survey neoplastic and normal human tissues are available. The BTRF offers expert consultation in collection and utilization of human biospecimens, pathology consultation and interpretation, and training on the core's shared laser microdissection, digital photomicroscopy, and cryostat instruments.

The **Flow Cytometry Core Facility** (FCCF) provides all investigators at the University of Virginia access to high quality, cost effective flow cytometry services. In addition to providing access to a variety of instrumentation, the FCCF also provides scientific expertise necessary to effectively use this technology to enhance the scope and quality of scientific research performed at the University. The state of the art facility occupies approximately 1800 sq. ft. and houses a variety of basic to high-end flow cytometry instrumentation. This includes a three laser 8 color FACSVantage SE TurboSort DIVA, a six laser 18 color Reflection Cell Sorter, both which provide 4-way high-speed cell sorting and complex analytical services. In addition, the facility has two 2-laser 4-color FACSCalibur benchtop analyzers as well as 3-laser 5-color and a 4-laser 10-

color analyzers. Other instruments available include a three laser 9-color CyAn ADP LX bench top cytometer, a four laser LSRFortessa with 16 fluorescent detectors, an ImageStreamX imaging flow cytometer and a Luminex 100 IS bead-based multiplex analyzer. In the last year, FCCF has acquired a DVS Sciences CyTOF 2 mass cytometer, with over 100 detection channels and 30 isotopic metal probes, enabling state of the art analytical capabilities. For those requiring flow cytometry access in a BSL-3 environment, the FCCF has expanded services to the BSL3 suite in the Carter-Harrison Building with a five laser 17-color high speed Influx cell sorter. A multi-TB server is available for data storage as well as site licenses for data analysis software. Researchers have the option, once trained, of performing their own analysis or utilizing the expertise of the facility's staff to run their samples for them. Specialized training classes are offered for those researchers who wish to better understand the principles and techniques employed in this technology and prefer to directly acquire and/or analyze their own samples.

The **Lymphocyte Culture Center** (LCC) makes available to researchers the most current technology and expertise for the construction and selection of lymphocyte-myeloma hybridomas for the production of monoclonal antibodies. The LCC can also provide expertise in the use of these reagents in basic research programs. Services are customized to the specific requirements of individual investigators to optimize the recovery of appropriate antigen specific monoclonal antibodies. The LCC has also provided research support services to other academic institutions and companies, and to federal agencies such as the NIH, the FBI, and USAMRIID. The center occupies a 1270 ft^2 modern laboratory equipped with cell culture incubators, laminar flow hoods, centrifuges, inverted and standard microscopes, automated microplate washer and ELISA plate readers, automated HPLC systems, etc. They also have facilities for the cryopreservation and long-term storage of established hybridomas and myeloma cell lines in liquid nitrogen cell banks. The LCC will immunize animals, collect sera and develop ELISA assay strategies appropriate for screening large numbers of hybridoma culture supernatants and animal sera. The facility conducts all aspects of cell fusion, assaying by ELISA for specific antibody positive cultures, culturing, cloning, freezing, and recovery of specific antibody producing clones. The center also provides other extended services including bulk expansion of clones, isotyping and sub-isotyping of monoclonal antibodies, bulk monoclonal antibody production *in vitro* using single use bioreactors, and monoclonal and polyclonal antibody purification by affinity chromatography on recombinant Protein G columns. The LCC also serves as a repository, production facility and distribution point for intellectual property (hybridomas and monoclonal antibodies) licensed by the University of Virginia Patent Foundation.

The **DNA Sciences Core** provides expertise to investigators for experimental design, sample preparation, protocol optimization and execution and data analysis. Major services are DNA sequencing and genomic analyses carried out via deep sequencing (“next-gen”) and Sanger sequencing to suit a wide range of research projects requiring nucleic acid sequences. The core also provides gene expression and genotyping on microarray and real time PCR platforms. The DNA Sciences Core works synergistically with the Biorepository and Tissue Research Facility and Bioinformatics Core by sharing resources and complementary expertise in bioinformatics.

The **W. M. Keck Biomedical Mass Spectrometry Laboratory** analyses proteins, peptides and some other biologically interesting molecules for University of Virginia investigators. The laboratory is located centrally in a room with dedicated climate control and backup power supply, with nearby office space. There are three mass spectrometers for analyzing proteins, peptides, and other molecules. The Thermo Scientific Orbitrap Velos ETD, including an attached HPLC system, performs high accuracy and high sensitivity analyses of single proteins or complex mixtures of proteins, including post translational modifications, on samples either in solution or in gels. Analyses of complex mixtures, such as whole cell extracts, can give relative quantitation data. The Thermo Scientific TSQ Quantum Access, with dedicated HPLC and autosampler, provides absolute quantitation using selective reaction monitoring (SRM) for not only peptides but also for small molecules. The Bruker Microflex MALDI provides mass data of peptides, proteins and some other small molecules to verify synthesis, expression, etc. This instrument is open access so users can do QC on their own timeframe.

These instruments and a staff with over 20 years experience make a wide range of experiments feasible. The key mission is to provide UVA investigators and trainees with expertise in pre-experiment planning, data acquisition, and data interpretation for a wide variety of experimental questions ranging from basic science to clinical applications.

ITC-Academic Computing for Health Sciences, a division of Information Technology Services, is a 4,000 square foot facility of renovated space on the third floor of Hospital West. The division functions as not only user support for general information, account administration in Windows, Macintosh and Unix but it also provides specialized expertise in health informatics, biostatistics, data visualization, molecular modeling, molecular biology and image processing. It is co-directed by Dr. William Pearson, an international expert on protein and DNA analysis and the developer of the FASTA and FASTP software programs for sequence analyses. Current facilities comprise four computing laboratories: Health Informatics, Multimedia, Data Acquisition and Data Visualization.

The **Bioinformatics Core** is a centralized resource for providing expert and timely bioinformatics consulting and data analysis solutions. The core is staffed by bioinformaticians with the necessary expertise to identify opportunities and implement solutions for managing, visualizing, analyzing, and interpreting large-scale genomic studies using both array and next generation sequencing platforms. The core is also equipped with the necessary computational infrastructure for analyzing high-throughput genomic data, including access to 92 multi-core high-memory computing nodes (>1,500 cores and 3,000 GB RAM total), and licenses to commercial bioinformatics resources (including Ingenuity Pathway Analysis™ and Oncomine™ Premium) that will aid in data analysis and interpretation.

Animal Facilities – University of Virginia

The University of Virginia is an AAALAC, International accredited institution animal care and use program located in central Virginia. The Center for Comparative Medicine (CCM) oversees the operation of ten vivaria encompassing 102,000 sq ft of space housing an average daily population of approximately 75,000 animals predominantly barrier maintained mice. CCM operates under the direction of the University's attending veterinarian Sanford H. Feldman, DVM, PhD, DACLAM and is staffed by one other veterinarian (Dr. Shawn Rosensteel), four veterinary technicians, five administrative personnel and 85 animal care technicians. The Animal Care and Use Committee Office operates under the direction of Ms. Sarah Hudson. Mice are maintained in Allentown ventilated rack systems, fed Harlan Teklad diets and handled in animal transfer stations. Each vivarium contains procedural spaces for manipulation of mice inside the vivarium without the necessity to take animals to the laboratory spaces. All vivaria have single pass redundant air-handling systems that are on emergency power; they contain epoxy flooring and epoxy painted CMU block walls. All animal room temperatures are monitored centrally by the University's System Control and alarmed to notify personnel if any animal room is out of its specified temperature range. There is one or more animal rooms set aside in each vivarium for ABSL2 housing. Immune deficient mice receiving xenografts are housed in separate ABSL2 areas from mice receiving ABSL2 infectious agents. All ABSL2 spaces contain biological safety cabinets and soiled animal caging is steam sterilized prior to further cage processing.

The animal care staff observes each animal every day, year round for clinical signs of disease and adequate food, water and sufficiently clean caging. Animal caregivers report emergent cases immediately to the veterinary staff that perform a physical examination and treatment or euthanasia. The veterinary staff performs the initial physical examination and collection of diagnostic specimens.

There are two CCM surgical operating theaters for large animal species, a dedicated and fully equipped rodent surgery area and an OEC 8800 portable C-arm fluoroscopic unit with digital image processing and storage. There is a Shepard Mark IV rodent irradiator to facilitate mouse bone marrow transplantation. There are five veterinary technicians that perform anesthesia and biomethodology on a fee for service basis for the research

community. The CCM has anesthesia equipment, large and small animal intensive care chambers and a pharmacy to facilitate provision of care for the majority of veterinary emergencies. The veterinary staff oversees the large animal surgery areas, performs controlled substance acquisition and inventory control, provides training of research staff in biomethodology procedures, provide anesthesia and post-operative care support.

Computer and Office Equipment

All personnel have laptop computers and workstations, including standard office software, as well as access to printing facilities. All files are shared between the Courier Therapeutics sites via the cloud storage service Dropbox. Courier Therapeutics maintains a company web domain and website, and assigns associated dedicated email addresses to all personnel.

SBIR·STTR
America's Seed Fund™
POWERED BY SBA

SBIR.gov SBC Registration

SBC Control ID:	SBC_001154063		
Company Name:	Courier Therapeutics Inc		
Address:	[redacted]		
City:	[redacted]		
State:	[redacted]	**Zip:**	[redacted]
EIN (TIN):	[redacted]	**DUNS:**	[redacted]
Company URL:	http://www.couriertherapeutics.com		
Number of Employees:			15
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			[redacted]

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: ALEXANDER Middle Name SASHA Last Name*: KRUPNICK Suffix: M.D.

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: MD Degree Year: 1996

Attach Biographical Sketch*: File Name: Biosketch_-_SK.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*: Courier Therapeutics, Inc
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail* [redacted]

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

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PHD Degree Year: 1984

Attach Biographical Sketch*: File Name: Biosketch_-_IK.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: [redacted] Middle Name Lee Last Name*: [redacted] Suffix: [redacted]

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: (434) 982-3276

E-Mail*: [redacted]

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

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD Degree Year:

Attach Biographical Sketch*: File Name: Biosketch_-_CS.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: [redacted] Middle Name Last Name*: [redacted] Suffix: M.D.

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login:

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD Degree Year: 2008

Attach Biographical Sketch*: File Name: Biosketch_-_RG.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: [redacted] Middle Name Marie Last Name* [redacted] Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login:

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

Degree Type: PhD Degree Year: 2016

Attach Biographical Sketch*: File Name: Biosketch_-_SH.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: [redacted] Middle Name [redacted] Last Name*: [redacted] Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: PHD Degree Year: 1993

Attach Biographical Sketch*: File Name: Biosketch_-_DF.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: [redacted] Middle Name [redacted] Last Name*: [redacted] Suffix: Ph.D

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Consultant Other Project Role Category:

Degree Type: PHD Degree Year: 2009

Attach Biographical Sketch*: File Name: Biosketch_-_EL.pdf

Attach Current & Pending Support: File Name:

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: COURIER THERAPEUTICS, INC.

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		[redacted]		[redacted]	Ph.D	Co-Investigator	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .		[redacted]		[redacted]	Ph.D	Consultant		[redacted]			[redacted]	[redacted]	[redacted]
3 .		[redacted]		[redacted]	Ph.D	Consultant		[redacted]			[redacted]	[redacted]	[redacted]
4 .		Sarah		[redacted]	Ph.D	Scientist	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name: **Total Senior/Key Person** [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: COURIER THERAPEUTICS, INC.

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

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

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

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

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

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: COURIER THERAPEUTICS, INC.

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . F&A	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

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

J. Fee	Funds Requested ($)*
	[redacted]

K. Budget Justification*	File Name: Budget_Justification_Courier_IL2.pdf (Only attach one file.)

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

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

BUDGET JUSTIFICATION

BUDGET JUSTIFICATION for Courier, Inc., Houston, Texas

Justification for Phase I budget excess over the $150K statutory cap: Our Phase I budget total (direct costs, indirect costs and fee) is $288K total cost; which exceeds the cap by $138K. However, our proposal fits the list of approved topics found in Appendix A at the end of the PHS 2016-2 SBIR/STTR Program Descriptions and Research Topics for NIH, CDC, FDA, and ACF, which extends the cap to $300k. Despite prudent budgeting and efficient task assignment, this amount represents minimum funding required to accomplish the Proof-of-Concept and Feasibility metrics we set for meaningful Phase I success and transition to Phase II work.

The delineation of coordinated efforts between Courier Therapeutics and [redacted] lab in UVA is generally as follows. All animal work and flow cytometry analysis will take advantage of the state of the art facilities at the University of Virginia, in order to leverage these existing resources. Subsequent assays on serum, tumor, and other samples will be performed by Courier Therapeutics at the JLABS@TMC campus in Houston. All non-animal work related to Ai, including linker design, expression and production of proteins, and subsequent evaluation of the immunogenic response, will be performed by Courier aided by the expert knowledge of [redacted] Similarly, histological, serum, and other analysis of tumor and other murine samples from Aim 2 will be performed by Courier, leveraging the extensive resources available at JLABS including machine-learning guided automated histological imaging and analysis.

A Senior/Key Persons ($17,500)

[redacted] is a highly experienced researcher and developer of biological drug candidates. He designed manufacturing cell lines to complement vector production for GenVec's product candidates. He directed preclinical manufacturing of biological based product candidates and assay development. In the past he has been responsible for the CMC section of several INDs, including product candidates that are in Phase II and Phase III clinical development. He also directed cGMP manufacturing through CROs and product release. For five years he was sign-off executive for regulatory and FDA interactions. [redacted] had line management responsibility for 40 scientists with budgetary responsibility of more than $4 million/yr. He is holder of over 100 US and Foreign issued patents and will be responsible for evaluating all intellectual property issues.

He will be responsible for developing and managing the project as well as maintaining collaborative links with [redacted], laboratory personnel, and consultants. He will be involved in the planning and analysis of all experiments and in all data analysis. He will also make all strategic and product development decisions including formulation, purification, and production issues.

[redacted] is Professor of Pathology and Immunology, Biochemistry and Molecular Biophysics at the Washington University in St.Louis. [redacted] is focused on studying molecular and cellular mechanisms of immunological processes, including issues related to pathogen immune evasion. He studies the mechanisms used by viruses to disrupt cytokine inflammatory signaling, antigen processing and presentation, and humoral immunity. The systems investigated include both large DNA viruses (i.e., pox and herpes) as well as enveloped RNA viruses, especially flaviviruses. His lab also has developing projects in cancer immunotherapy and anti-inflammatory drug development. [redacted] is an expert on a broad spectrum of approaches including protein engineering, x-ray crystallography, surface plasmon resonance, flow cytometry, and immunological signaling assays. His expertise on this project will be particularly valuable for evaluating the physical properties of the biological molecules. [redacted] is a founder and has a continued interest in Courier's success, and so will be contributing his time and expertise gratis on an as-needed basis.

[redacted] is a virologist and protein chemist. He has studied multiple aspects of host-pathogen interactions of herpesviruses and poxviruses. His research goals are to study how these viruses interact with humans to better understand our own biology, and as a result translate their novel strategies of cell manipulation into new therapeutics. [redacted] had the idea of utilizing a high-affinity, virally encoded NKG2D ligand known as orthopoxvirus major histocompatibility complex class I like protein or OMCP for the targeting of NK cells. He designed the OMCP-mutIL-2 molecule that was the basis of

research that ultimately led to the founding of Courier Therapeutics and is the subject of this grant application. [redacted] knowledge of virology and protein biochemistry will be important to improve and increase our understanding this protein-based therapeutic. [redacted] is a founder and has a continued interest in Courier's success, and so will be contributing his time and expertise gratis on an as-needed basis.

[redacted] is Director of Research at Courier. She has gained business development experience as a venture fellow at Mercury Fund since completion of her PhD training with [redacted] at the Baylor College of Medicine on breast cancer. She is a well-trained biologist with extensive experience in cancer and immunology. She has a strong background in cancer research, statistical analysis, and has coordinated both internal and CRO research work executed since joining Courier Therapeutics. [redacted] is responsible for managing product development in Houston, Texas in the Courier laboratory facilities and managing outsourced efforts (aim #1, Epivax). She will be responsible for flow cytometric data analysis (data will be generated at the University of Virginia and shared via Courier Therapeutics DropBox) for aims 2 and 3. For aim #2 she will be in charge of processing and analyzing histology for autoimmune features, serum analysis for liver enzymes. For aim #3 she will analyze and lot the flow cytometry data generated on human ex vivo samples.

F. Other Direct Costs

1. Materials and Supplies [redacted]
Histology supplies ($7500) – This includes costs of cutting and preparing slides, antibodies, and specialized reagents required for immunohistochemistry and immunofluorescence profiling. The major costs associated with these studies will be the purchase of specialized antibodies and secondary reagents compatible with the Vectra Polaris imaging system at JLABS@TMC, which enables whole tissue imaging and machine learning profiling of complex histological specimens. Flow-cytometry-like data is generated by this machine with additional spacial information of immune cell penetration and phenotype, and we feel the value of this data justifies the additional costs.

ELISA reagents and antibodies [redacted] – These assays will cover the serum profiling required for our safety studies in Aim 2, particular assays measuring alanine and aspartate aminotransferase (ALT and AST) as a measure of liver damage.

General laboratory chemicals and supplies [redacted] – This covers gloves, pipettes, centrifuge tubes, and other small equipment and consumables as is necessary to complete the described experiments.

2. Equipment or Facility [redacted] – This is the cost to rent space at JLABS@TMC, which includes 6 linear feet of bench space and office space. All standard lab equipment is included such as centrifuges, plate readers, microscopes and other imaging systems, cell culture hoods, etc, as well as state-of-the art equipment such as the Vectra Polaris imaging system. These licensing fees additionally cover equipment maintenance, compliance training and recordkeeping, and waste management.

3. Consultants ([redacted]
[redacted] EpiVax will provide in silico analysis of our lead 30AA and backup 20AA constructs in aid of the completion of Aim 1. Epivax has extensive expertise and technological development in this area, and their analysis will provide both an immunogenicity metric as well as a measure against clinically-utilized therapeutics. Please see letter of support.

5. Subaward/Consortium Costs [redacted]
[redacted] his laboratory at University of Virginia will be the subaward in this application. The costs of the subaward include salary and benefits for [redacted] and collaborators [redacted] their staff, laboratory supplies (including plastic ware and immunological assays), the costs of production, the costs of animals and their maintenance and core recharges. See the Subcontract budget and justification for details. The research entity's component of the total budget (Direct plus Indirect) is [redacted]

H. Indirect Costs [redacted]
F&A costs of 25% of the modified total direct cost base are requested.

J. Fee ([redacted]
A standard fixed fee of 7% of total costs (direct and indirect) is requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	[redacted]	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees	[redacted]	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		[redacted]

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: University of Virginia, School of Medicine

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	[redacted]	[redacted]	[redacted]		PD/PI	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
2 .		[redacted]		[redacted]	Ph.D	Co-Investigator	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]
3 .	Dr.	[redacted]		[redacted]		Co-Investigator	[redacted]	[redacted]			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	[redacted]			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	[redacted]			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

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

Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of Virginia, School of Medicine

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

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

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

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

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

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

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

ORGANIZATIONAL DUNS* [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: University of Virginia, School of Medicine

Start Date*: 07-01-2018 **End Date*:** 06-30-2019 **Budget Period:** 1

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

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

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . F&A	61.5	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: Budget_Justification_UVA_IL2.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

BUDGET JUSTIFICATION

BUDGET JUSTIFICATION for Dr Krupnick Laboratory, University of Virginia.

Salaries and positions in the budget correspond to established guidelines of University of Virginia. Fringe benefits are calculated [redacted].

A. Senior/Key Persons ($33,563)

[redacted]) is an Associate Professor of surgery and Vice Chairman for research at the University of Virginia. He is a clinician-scientist with both a clinical surgical practice and a research lab studying the interplay between the lungs and immune system. He has a strong publication record in the field of immunology and lung cancer immunotherapy, and is a founder to Courier Therapeutics. Dr. Krupnick will oversee studies taking place at the University of Virginia, in addition to dedicating time to the oversight of the project as a whole. He will be involved in planning all experiments and in data analysis in collaboration with Drs. Kovesdi and Hein.

[redacted] is a Professor of Surgery and the Director of the Human Immune Therapy Center at the University of Virginia. He has been an integral part of immunotherapy research since the 1990's, and along with Victor Englehard led some of the first immunotherapy trials in melanoma. TILs from such trials will now be used to test the quantitative and qualitative utility of OMCP-mutIL-2 in TIL expansion.

[redacted] is an Assistant Professor of Medicine in the Division of Oncology. He is an expert in check point blockade immunotherapy and will lend his expertise to preclinical translational studies outlined in aim #2.

B. Other Personnel ($21,538)

[redacted] a staff scientist working with Dr. Sasha Krupnick, in the Department of Thoracic and Cardiovascular Surgery at the University of Virginia. She is a well-trained immunologist with extensive experience in tumor immunotherapy, CAR-T cells, and animal models. She will have primary oversight for aim #2 and will execute the combination studies of checkpoint blockade and cytokine therapy.

[redacted]is a senior research technician who has extensive expertise in expansion of TILs from human melanoma samples. She will perform the experiments outlined in aim #3 along with guidance from Drs. Krupnick and Slingluff.

F. Other Direct Costs

[redacted] This includes all culture media, fetal bovine serum, antibiotics, trypsin, etc. The major cost in this category is fetal bovine serum, which is based on our current rate of consumption. Reagents, serum, antibiotics, supplements, and other tissue culture supplies including sterile tissue culture flasks and dishes, and glassware will be needed for the expansion of cells in vitro.

[redacted] This is a major expense and includes gloves, pipette tips, microfuge tubes, pipettes, centrifuge tubes, etc. This category also includes general chemicals such as buffers, salts, acids, bases, etc.

[redacted] - This includes chemicals, primary antibodies, chromogen, signal detection kit reagents, secondary antibodies, cytokine detection kits, flow cytometry reagents, and antibodies utilized for the flow cytometry studies.

8. Animals and housing ($9,426)

Animal purchase **–** Mice are needed to perform the necessary experiments in this proposal. C57BL/6 wild-type and transgenic mice will be purchased from Jackson Labs at 6-8 weeks of age.

Animal maintenance funds are requested for care of the experimental animals. Animals for all studies will be housed in the MR5 animal facility Building, University of Virginia, School of Medicine.

9. [redacted]
Requested to defray the expense of shipping costs to Courier, Inc. This includes production and transfer of OMCP-mutIL-2 between collaborators.

H. [redacted]
A [redacted] previously negotiated by the University of Virginia, of the modified total direct cost base are requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	[redacted]	
9. Other 2	[redacted]	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		0.00

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Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	0	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

❍ SBIR ● STTR ❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 3

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ❍ No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ❍ Yes ❍ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ❍ No

Funding Opportunity Number: PA-17-303. Received Date: 2018-01-04T12:18:51.000-05:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ❍ No

If "Yes" to euthanasia

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

● Yes ❍ No

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

3. *Program Income Section

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

❍ Yes ● No

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

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

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

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

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12540871

Funding Opportunity Number: PA-17-303 . Received Date: 2018-01-04T12:18:51.000-05:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

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Tracking Number: GRANT12540871
Funding Opportunity Number: PA-17-303. Received Date: 2018-01-04T12:18:51.000-05:00

INTRODUCTION TO APPLICATION

We want to thank the ZRG1 OTC-H study section for the fair and accurate review of our R41 entitled "Novel Immuno-oncology Strategy for Targeted Cytotoxic Lymphocyte Activation". We are excited the reviewers found "investigative team and environment very strong" and "supportive preliminary studies, well-designed and straightforward research strategy with clearly outlined milestones." We also realize the concerns and have made significant efforts to address them.

Immunogenicity: We have made significant progress on aim #1 (immunogenicity). By eliminating the His and Flag tags from the construct we were able to completely eliminate the anti-OMCPmutIL-2 antibody response in the murine model, now shown as preliminary data in the proposal. Notably, the immune response was generated to the linker region and not IL-2 or OMCP targeting vector. Thus, we now have partnered with EpiVax to perform in silico analysis of human epitopes that could generate an immune response. We will use this modeling to refine our lead product (if needed) and to compare in silico immunogenicity to recombinant proteins in clinical use.

Adjuvant for immunization (former aim #2): We have significantly revised this part of the grant to take advantage of new data generated since the last submission. Specifically, we now demonstrate that OMCP-mutIL-2 offers an advantage for expansion of T cells in vitro (specifically CD8+ T cells, NKT cells, and $\gamma\delta$ T cells). Based on murine preliminary data we now explore the possibility for ex vivo expansion of human tumor-reactive lymphocytes using OMCP-mutIL-2 (new aim #3). This possibility would not only expand the use of our product **but could also provide a more rapid pathway to the market as a reagent rather than a biologic**.

Modification of the team: We agree that the team would benefit from a medical oncologist, and have thus recruited Dr. Ryan Gentzler, involved in multiple clinical trials of checkpoint blockade immunotherapy, to oversee aim #2 on combination immunotherapy. We also recruited Dr. Craig Slingluff who will lend his expertise to aim #3.

Justify the concurrent use of checkpoint blockade and IL-2 therapy: Multiple clinical and preclinical trials have demonstrated an improvement in efficacy with minimal side effects in combining check point blockade with targeted, chemo, or radiotherapy (Ledford, H, Nature (2016) 532:162). Such advantages have been attributed to the targeting of different and non-overlapping pathways of tumor control. OMCP-mutIL-2 stimulates the immune system, potentially increasing efficacy in "cold" tumors without significant immune activation and thus represents a non-overlapping and supporting combination therapy.

IL-2 therapy in lung cancer: IL-2 is approved for melanoma and renal cell carcinoma. Early clinical studies in man and murine studies have demonstrated exciting data for lung cancer responses to IL2. Since there are >250,000 new cases of lung cancer yearly in the US alone (vs 80,000 melanoma), we decided to focus on this cancer type due to clinical need. We hope the reviewers agree.

Competing strategies: As IL-2 is the ONLY form of immunotherapy leading to a true long-term cure for metastatic cancer, there are competing strategies and companies in this space. This includes pegylation to increase serum half-life (Nektar), as well as non-IL2Rα binding mutants by several other start-ups. However, the direct targeted delivery to cytotoxic lymphocytes proposed by Courier Therapeutics is completely unique in its approach. We suspect our targeted approach will offer superior immunotherapy by maintaining efficacy while increasing safety.

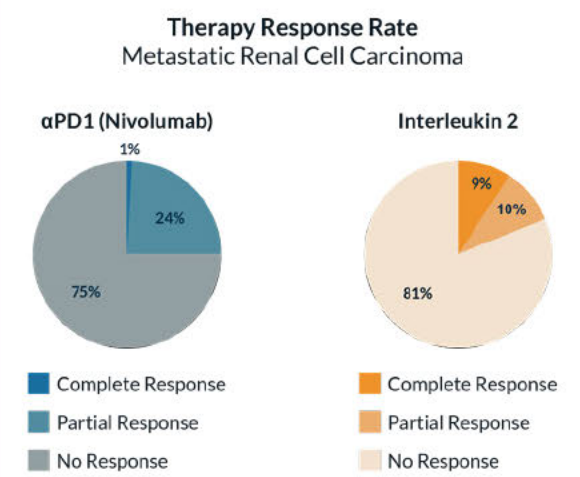
Future clinical trial plans: We are now moving forward with plans for clinical trial which is being planned by Dr. Ryan Gentzler now. We are also applying for NCI NexT Program and VA MERIT for drug development to help with risk mitigation and GMP production.

Unknown whether increased CD8 and NK function would lead to toxic effects: We have published the safety of OMCP-mutIL-2 and toxicity as monotherapy (Ghasemi et al. Nature Communications 2016). The toxicity profile of OMCP-mutIL-2 and checkpoint blockade is addressed in the new Aim #2.

Overly ambitious: We have made great progress with limited funding and have generated substantial new data since the A0 submission. Funding from the STTR mechanism would help us move even quicker. As we have expanded the team and have obtained new expertise we have expanded our budget to fit our needs. The three aims will be performed concurrently by different teams (Aim #1-EpiVax; Aim #2-Krupnick laboratory/Courier; Aim #3 Slingluff laboratory/Courier).

SPECIFIC AIMS

The cytokine interleukin-2 (IL-2) is considered a master regulator of the immune system(1). Its anti-tumor effects are utilized for expansion of tumor reactive lymphocytes ex vivo(2) and its in vivo administration was the first immunotherapy approved by the FDA(3-7). In renal cell carcinoma high dose IL-2 (HD IL-2) induces long-term cure of 5-9% of patients, while the anti-PD-1 therapy Nivolumab, which recently received FDA fast-track approval, has a <1% complete remission rate(8) (**Summary Figure**). Despite these promising results HD IL-2 has fallen out of favor due to adverse side effects including fever, malaise, and life-threatening systemic capillary leak(9). These side effects result in a 50% rate of therapy discontinuation and a mortality of 2-5% (10). If a safe and efficacious form of IL-2 were available it would have wide-spread utility for multiple malignancies.



Summary Figure of Complete Response (Cure) Achieved with IL-2 vs. PD-1 Blockade

The structure of the IL-2 receptor (IL-2R) provides opportunities to modify IL-2 to reduce toxicity. The signaling portion of IL-2R consists of the β and γ chains, while the non-signaling high affinity α-chain functions to efficiently capture IL-2 at the cell surface. Together these chains form the high affinity trimeric IL-2αβγ receptor (IL-2Rαβγ) which is broadly expressed across many hematopoietic and non-hematopoietic cells. For these reasons HD IL-2 broadly activates multiple cells and tissues resulting in many "off target" side effects. For example HD IL-2 promotes the proliferation of cytotoxic lymphocytes (NK cells and CD8+ T cells) but it also promotes the expansion of regulatory T cells (T_{regs}) that inhibit the immune responses. Activation of vascular endothelium, which also expresses IL-2Rαβγ, induces devastating and life-threatening complications(11). An improved form of IL-2, that solely activates cytotoxic lymphocytes (CTLs) without activating T_{regs} or vascular endothelium, may offer the opportunity to expand IL-2 use in the clinic.

NKG2D is an activating receptor that is expressed solely on CTLs, such as NK cells and activated/memory $CD8^+$ T cells. It is not present on T_{regs}, vascular endothelium or other lymphocytes(12). We have recently described the construction of a fusion protein that delivers IL-2 directly to NKG2D-expressing cells through a ligand known as orthopoxvirus major histocompatibility complex class I-like protein (OMCP). Thus NKG2D substitutes for the high affinity α-chain of the IL-2 receptor, resulting in specific and precise activation of cytotoxic lymphocytes. Such a strategy avoids activation of most other cells, including vascular endothelium and T_{regs} (13). Our fusion protein, called OMCP-mutIL-2, offers a dramatic reduction in adverse side effects and superior tumor control over wild-type IL-2(13). In this application we seek to further develop OMCP-mutIL-2 into a viable drug candidate and broaden its application for stimulation of CTLs both in vitro and in vivo.

Specific Aim #1: To utilize computational modeling to define immunogenicity of OMCP-mutIL-2. Since the A0 application we have eliminated the murine humoral immune response to OMCP-mutIL-2 by modifying the peptide linker region. We now plan to utilize in silico analysis to test human immunogenicity to these constructs in direct comparison to proteins in clinical use already. Such data will allow us to refine our final lead product for clinical work.

Specific Aim 2: **To evaluate OMCP-mutIL-2 as an adjuvant for checkpoint blockade immunotherapy.** We hypothesize that the complementary and non-overlapping mechanisms of action of OMCP-mutIL-2 will substantially decrease the growth of lung cancer without an increase in adverse events when combined with checkpoint blockade.

Specific Aim 3: **To evaluate the utility of OMCP-mutIL-2 for expansion of human tumor-reactive lymphocytes in vitro.** Preliminary data generated in murine models demonstrates that OMCP-mutIL-2 offers a quantitative and qualitative advantage for T cell expansion. Here we plan to compare OMCP-mutIL-2 to conventional cytokine stimulation for expansion of tumor-reactive lymphocytes from patients vaccinated against malignant melanoma.

RESEARCH STRATEGY

A. SIGNIFICANCE

1. Expansion of HD IL-2 is hindered by off target side effects. HD IL-2 can cure some patients with advanced melanoma and renal cell cancer; however, its use is limited by complications and adverse effects such as blood pressure changes, pulmonary edema, renal failure, as well as a 2-5% rate of treatment-related mortality(14). These side effects result from direct binding of IL-2 to the high-affinity IL-2 receptor (IL-2Rα) on vascular endothelium(11). Furthermore, the clinical efficacy of IL-2 is limited by activation of $CD4^+Foxp3^+$ regulatory T cells (T_{regs})(15), as well as competition from multiple other hematopoietic and non-hematopoietic cells which also express the IL-2 receptor but do not directly participate in the anti-tumor immune response (**Figure 1a**). A form of IL-2 that could specifically activate CTLs, such as NK cells and $CD8^+$ T cells, offers to improve on this FDA-approved form of immunotherapy.

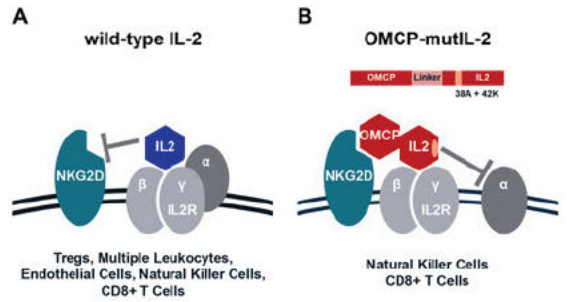


Figure 1: (A) Wild-type IL-2 binds with high affinity to the α chain and signals through the βγ chains of the trimeric IL-2 receptor. The trimeric IL-2 receptor is expressed on multiple cells leading to global activation. Wild-type IL-2 does not bind to NKG2D. (B) OMCP-mutIL-2 binds to NKG2D with high affinity and signals through the βγ chains of the IL-2 receptor. NKG2D is expressed on a limited cell repertoire of NK cells and activated CD8+ T cells.

2. There are currently no IL-2 variant therapeutics which decrease toxicity while maintaining full efficacy. Strategies to overcome IL-2 toxicity generally revolve around limiting binding to the high-affinity IL-2Rα chain, which is expressed at a higher density on T_{regs} than on effector NK and CD8+ T cells. Such a strategy leads to "indirect" specificity of IL-2 for cytotoxic lymphocytes. One such IL-2 mutant product in development is ALKS4230 (Alkermes Inc.)(16). Other strategies, such as IL-2 PEGylation have been promoted by Nektar Therapeutics and function by both blocking IL-2Rα chain binding and increasing the IL-2 half-life in the serum(17). This strategy makes IL-2 more bioavailable to NK and CD8+ T cells, which have lower affinity for IL-2 than T_{regs}. While these strategies improve safety over HD IL-2 administration, such an approach does not directly target cytotoxic lymphocytes, has a reduced overall efficacy, and still results in toxicity(18). Others have attempted to target IL-2 to the tumor bed directly using a tumor-specific antibody conjugated to IL-2(19). As solid tumors actively exclude NK and CD8+ T cells, this approach may not be as efficacious as IL-2 delivery to cytotoxic lymphocytes. Furthermore, as tumors tend to rapidly mutate and can downregulate or shed tumor associated antigens the concept of tumor-targeting using a defined antibody may have limited durability. Clinical trials using this approach in neuroblastoma have demonstrated some efficacy, but such an approach is limited to tumors with a well-defined surface antigen for targeting(20). Thus we plan to evaluate a unique and previously untested approach of directly targeting systemic CTLs, located both within and outside the tumor bed, by delivering IL-2 to NKG2D-expressing cells(13).

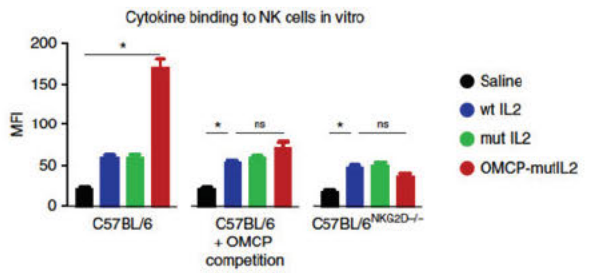


Figure 2: Binding of IL-2 constructs to C57BL/6 NK cells without (left) or with (middle) OMCP competition. Binding of IL-2 constructs to $NKG2D^{-/-}$ NK cells (right). from Ghasemi et. al. Ref 13

3. Construction of an IL-2 fusion protein targeted to NKG2D-expressing CTLs was recently described by our group. NKG2D is an activating receptor that is expressed on both human and murine NK and $CD8^+$ T cells but is virtually absent from other lymphocytes or non-hematopoietic cells(12). We thus thought that targeting IL-2 to NKG2D may offer a method of specific and precise cytokine delivery to CTLs without "off target" side effects. To accomplish this we decreased IL-2 binding to the IL-2α chain through substitutions of alanine for arginine at the 38 position (R38A) and lysine for phenylalanine at the 42 position (F42K)(21). This IL-2Rα non-binding mutant form of IL-2 (mutIL-2 from here) was then linked to a high affinity virally encoded NKG2D ligand called orthopoxvirus major histocompatibility complex class I-like protein (OMCP). OMCP binds both human and murine NKG2D with an affinity equal to, or greater than, all other known NKG2D ligands(22, 23). As OMCP binds NKG2D

with higher affinity than IL-2 binds IL2Rα (K_d of 0.2 vs. 14 nM respectively) (23) it serves as an ideal targeting vector to deliver and "redirect" IL-2 specifically to NKG2D-expressing CTLs (**Figure 1b**)(**Figure 2).** We thus believe our construct "retargets" IL-2 signaling from IL-2Rα-expressing cells to NKG2D-expressing cells resulting in precise and specific activation of CTLs without "off targets" effects.

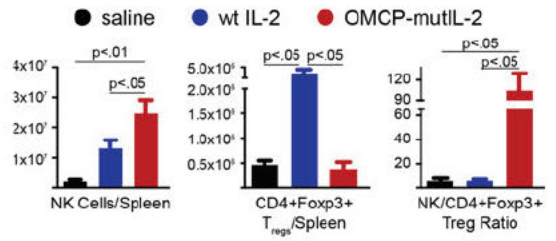


Figure 3: Treatment of C57Bl/6 wild-type mice with 750,000IU of OMCP-mutIL-2 (red bar) leads to superior expansion of NK cells (left), little expansion of Tregs (middle) and a higher NK/T_{reg} ratio (right) over wild-type IL-2 (blue bar). from Ghasemi et. al. Ref 13

B. INNOVATION

1. Precise targeting of mutant IL-2 through the NKG2D receptor increases efficacy. The key innovation of the Courier Therapeutics approach comes from the direct targeting of IL-2 specifically to CTLs using NKG2D as a "targeting receptor". This approach represents significant deviation from competitive strategies(17). First and foremost, we now effectively substitute NKG2D for the α-chain of the IL-2 receptor. We thus create a new form of IL-2 receptor that is only expressed on NK cells and some CD8+ T cells. Such precise targeting cannot be achieved through competing or alternative strategies described above, which generally result in ligands with lower overall affinity and activity. By utilizing a virally encoded "targeting-ligand" we take advantage of evolutionary refinement to engineer a vector that is non-toxic, non-immunogenic, and binds to NKG2D with high affinity of multiple species(22, 23). OMCP-mutIL-2 results in superior activation of NK cells and no expansion or activation of regulatory T cells (T_{regs}) (**Figure 3**)(13).

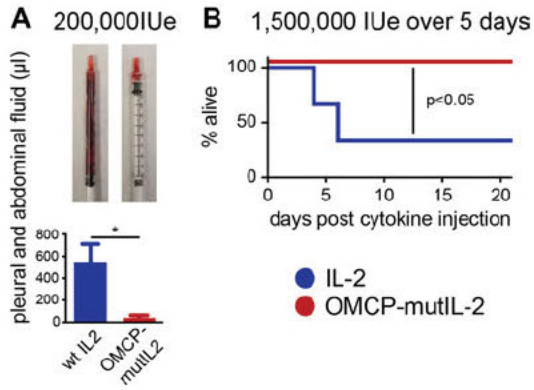


Figure 4: (A) Wild-type IL-2 results in a systemic capillary leak even in low doses while OMCP-mutIL-2 does not. Graphic representation of one animal ascites on top and composite volume from 5 animals at the bottom. (B) At higher doses wild-type IL-2 leads to significant animal mortality while OMCP-mutIL-2 does not. from Ghasemi et. al. Ref 13

2. OMCP-mutIL-2 has an improved safety profile over wild-type IL-2 despite an increase in efficacy. Unlike wild-type IL-2, therapeutic doses of OMCP-mutIL-2 do not result in vascular leak (**Figure 4**) or deleterious organ inflammation(13, 24). Even in supratherapeutic doses, OMCP-mutIL-2 is well tolerated and does not lead to animal demise, unlike the significant mortality induced by wild-type IL-2 (**Figure 4**). Additionally OMCP-mutIL-2 has a short serum half-life, identical to wild-type IL-2(13). Rapid cytokine clearance offers a safety margin where dosing can be stopped if side effects occur. Decreased growth and improved survival of lung cancer-bearing mice was evident in OMCP-mutIL-2 over wild-type IL-2 treated animals (**Figure 5**). Similar results were evident for other NK-sensitive tumors as well(13). Taken together we can conclude that NKG2D-targeted delivery of IL-2 has potential as a superior strategy for immunotherapy of cancer.

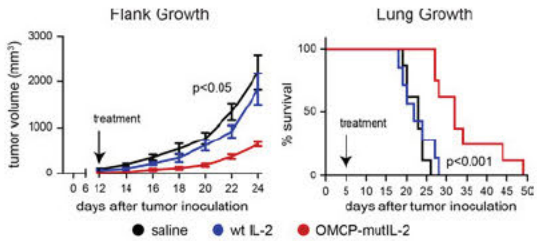


Figure 5: Decreased growth of Lewis Lung Carcinoma (LLC) injected into the flank (left) and improved survival of LLC injected into the lung (right) is evident in OMCP-mutIL-2 treated mice over IL-2 or saline-treated controls. from Ghasemi et. al. Ref 13

SUMMARY: To our knowledge, no other therapeutic candidate in development has overcome the challenges of direct targeting of CTLs in the absence of "off target" activation of other cells. We have achieved this by creating a new high-affinity form of the IL-2 receptor where NKG2D is substituted for the α chain for IL-2 delivery. While this strategy has already shown efficacy in a variety of tumors several aspects need to be refined prior to advancement toward clinical applications. ***Successful completion of this proposal will support the promotion of OMCP-mutIL-2 to IND-enabling studies and GMP manufacturing. Our ultimate goal is to submit this novel immunotherapy to the FDA for a first-in-human clinical safety trial and eventual approval for clinical use.***

C. APPROACH

Specific Aim #1: To utilize computational modeling to define immunogenicity of OMCP-mutIL-2.

1. Preliminary Data: Our fusion protein consists of OMCP linked to mutIL-2 through the well-established glycine/serine linker with polyhistidine tag in the linker (His-tag). The His-tag eased purification by affinity chromatography and was based on previously described linker optimization studies(25, 26). In the A0 submission we demonstrated the development of an antibody response against the His-tag expressing linker. To explore this further we next generated OMCP-mutIL-2 constructs **without** the His-tag and purified our construct using ion exchange followed by size exclusion chromotography. Based on preliminary data that the linker was the site of the antibody-directed immune response, we also varied the linker length from 30 to 20 amino acids, to reduce possible immunogenic epitopes that may increase with longer linker length(26). Of note similar CTL immunoactivation in vivo was evident with 30 and 20 amino acid linkers (data not shown). We next evaluated the antibody response 50 days after administration of OMCP-mutIL-2. We again confirmed that antibodies developed against the 30 amino acid linker construct containing a His-tag. No antibodies developed against either OMCP or mutIL-2 confirming our assumption that the linker region is the immunogenic portion of our construct (**Figure 6**). By eliminating the His-tag from the linker, however, no antibody response was evident against OMCP-mutIL-2 with either the 20 or 30 amino acid linker **(Figure 6).** Taken together we can conclude that OMCP-mutIL-2 can be designed to offer a non-immunogenic platform for immunoactivation and the linker, which was the immunogenic portion of the molecule, can be modified to ameliorate immunogenicity. Nevertheless, human modeling is necessary prior to IND-enabling studies.

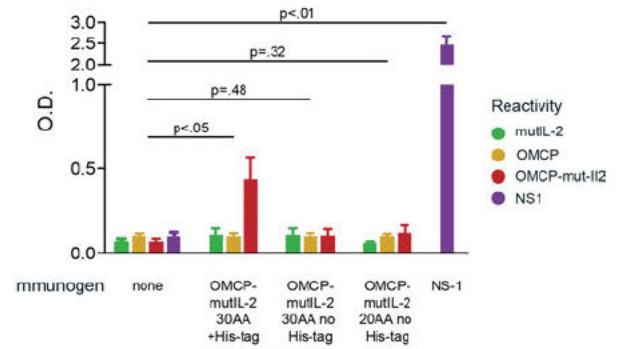


Figure 6: Mice treated with OMCP-mutIL-2 develop an antibody response directed to His-tag containing construct as measured by serum ELISA on day 50 post-immunization. No antibody response develops toward constructs without His-tag in the linker and no OMCP or mutIL-2-directed antibody response developed in any of the conditions. NS-1 is a highly immunogenic influenza protein that acted as the positive control.

2. Experimental design and methods: To accomplish this aim we will work with a partner, EpiVax, to perform *in silico* analysis of OMCP-mutIL-2 without His-tags (please see letter of support). Epitope prediction modeling will be performed as previously described (27, 28). Briefly the 305 and 315 amino acid constructs (20 and 30 amino acid linkers respectively) will be computationally parsed into overlapping 9-mer frames where each frame overlaps the last by eight amino acids. Each frame will then be scored for predicted binding to each of eight common Class II HLA alleles (DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, DRB1*1301, and DRB1*1501) which cover ≈97% of the human population(29). A raw binding score for each 9-mer sequence will be normalized with respect to a distribution of scores derived from a very large set (>10,000) of randomly generated 9-mer sequences to generate a "Z" score. Any peptide scoring above 1.64 (approximately the top 5% of the random peptide set) is predicted to have a significant chance of binding to the MHC molecule for which it was predicted. Peptides scoring above 2.32 on the scale (the top 1%) are extremely likely to bind and published T-cell epitopes fall within this range. Therefore, the higher the Z score, the higher is the probability that a peptide will be presented to T-cells by the antigen presenting cells. Based on compound Z-scores generated for every 9-mer peptide derived from OMCP-mutIL-2, EpiVax will rank the input sequences against other well-studied proteins, including those in clinical use, and these sequences will be ranked on an immunogenicity scale. This extensive list of over 1000 compounds includes Interferon beta, which is known to be immunogenic and high on EpiVax's prediction scale for immunogenicity, as well as poorly immunogenic proteins such as human serum albumin or immunoglobulin Fc (30).

3. Anticipated results, interpretation, potential difficulties and alternative approaches: At the completion of this aim we will have an estimate of human immunogenicity of our primary 30 and secondary 20 amino acid linker OMCP-mutIL-2 constructs. For this aim we plan to focus on MHC Class II-restricted responses due to the critical role of this pathway in both cellular and humoral immunity. Previous studies have demonstrated that the algorithm described above accurately predicts published MHC ligands and T-cell epitopes across many types of proteins with human clinical trial validation(31, 32). Furthermore, using established databases, this platform

would allow us to compare predicted immunogenicity of our constructs to recombinant proteins in medical use today. While in silico modeling does have limitations, obtaining this data would allow us to further refine our lead product (20 vs 30 amino acid linker), identify specific immunogenic epitopes within our protein and possibly re-engineer them. Such data is also critical for a pre-IND meeting with the FDA.

Specific Aim 2: To evaluate OMCP-mutIL-2 as an adjuvant for checkpoint blockade immunotherapy.

1. Preliminary Data: As lung cancer represents the number one cancer related cause of death in the western world a significant effort has focused on checkpoint blockade immunotherapy for its treatment. However, checkpoint blockade alone leads to a prolongation of survival in ~20% of patients (33), and this moderate effect, has spurred efforts to provide additional immunostimulation. Such efforts have included the addition of IL-2 to lung cancer immunotherapy (34) (35). This is specifically relevant since checkpoint blockade immunotherapy, which "takes the foot off the brake" could in theory complement immunostimulation with IL-2 which "puts the foot on the gas". In addition these two mechanisms of immunotherapy have complementary and non-overlapping mechanisms of action and toxicity that may benefit patient outcome. IL-2 and checkpoint blockade have also been shown to synergize in clearance of virus (36).

The C57BL/6 syngeneic Lewis Lung Carcinoma cell line (LLC) offers a highly clinically relevant model for human lung cancer immunotherapy. It expresses PD-L1 on its surface (**Figure 7a**), a characteristic that has been used to define responsiveness to PD-1 checkpoint blockade(37, 38). Despite PD-L1 expression, LLC is resistant to single agent PD-1 checkpoint blockade without adjuvant therapy(39), and thus models the poor response evident in human clinical trials of aggressive lung cancer. Preclinical data suggests that LLC may respond, albeit at low to moderate rates, to CTLA-4 checkpoint blockade when combined with other adjuvant therapy(40). LLC also offers a model of orthotopic lung cancer as intravenous injection of $1x10^5$ cells leads to rapid tumor growth in the lungs. The quantifiable increase in pulmonary weight (**Figure 7b**) has been used by us and others to assess tumor growth and response to therapy(41, 42). Finally, LLC is responsive to immunotherapy via stimulation of the IL-2 pathway of immunoactivation(13). For these reasons the model of intravenous injection of LLC with combination checkpoint blockade and IL-2 immunotherapy offers a relevant preclinical small animal model to test efficacy, safety, and toxicity. ***We hypothesize that OMCP-mutIL-2 immunotherapy, in combination with checkpoint blockade, will increase therapeutic efficacy without increasing side effects or toxicity of checkpoint blockade immunotherapy.*** Thus, our primary objective is to evaluate the efficacy of such combination therapy on tumor growth with a secondary objective of safety evaluation.

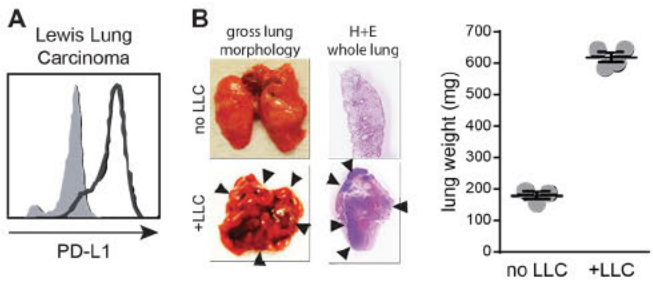


Figure 7 (A) LLC expresses PD-L1 in vivo. (B) I.V. injection of LLC results in rapid growth of pulmonary tumor with nodules visible grossly and histologically (arrows) and an increase in pulmonary weight.

Table 1

Group	Checkpoint Blockade	Cytokine
1	IgG control	none
2	anti-PD-1	none
3	anti-CTLA-4	none
4	anti-PD-1+ anti-CTLA-4	none
5	IgG control	OMCP-mutIL-2
6	anti-PD-1	OMCP-mutIL-2
7	anti-CTLA-4	OMCP-mutIL-2
8	anti-PD-1+ anti-CTLA-4	OMCP-mutIL-2
9	IgG control	wild-type IL-2
10	anti-PD-1	wild-type IL-2
11	anti-CTLA-4	wild-type IL-2
12	anti-PD-1+ anti-CTLA-4	wild-type IL-2

2. Experimental design and methods. In order to study such combination therapy, we will inject C57BL/6 mice with $1x10^5$ LLC intravenously and start immunotherapy 5 days later, once tumors have established within the lung. Mice will be randomized into 12 separate groups (**Table 1**) and treated with either anti-PD-1(clone RMP1-14), anti-CTLA-4 (clone 9H10; BioXCell) or both in combination with wild type IL-2 or OMCP-mutIL-2 or nothing. Since the 20 and 30 amino acid linker-containing constructs demonstrate similar potency for immunoactivation and neither are immunogenic in mice **(Figure 6)**, for this aim as well as aim #3 we plan to utilize OMCP-mutIL-2 with the 30 amino acid linker(13). Immunogenicity studies defined in

specific aim #1 will guide human clinical trials but not animal experiments in this aim. Cytokine dosing will be based on previous reports of 750,000 IU (calculated as molar equivalent) given over five days in 10 separate doses(13) and 200 µg of blocking antibody given intraperitoneally every three days for five doses(43, 44). For the experiments outlined here combination immunotherapy will be performed concurrently starting five days after tumor injection. Twenty-one days after tumor injection animals in all groups will be sacrificed and our primary end point of tumor growth will be evaluated by total lung weight as well as histologic assessment of tumor burden. The secondary end point, of adverse immunologic side effects will be evaluated throughout the study and upon sacrifice as well. Animals will be weighed daily, and immunotherapy will be stopped in animals in any of the groups that demonstrate weight loss of over 20% of starting body weight. Such animals will be considered to suffer from immunotherapy-related adverse events. At the time of sacrifice, either prematurely due to adverse events or twenty-one days after tumor injection, blood serum will be collected and evaluated for elevation of alanine and aspartate aminotransferase (ALT and AST) as a measure of liver damage. Upon necropsy gross and histologic evaluation of the liver, colon, and small bowel will be performed for signs of autoimmune disease.

3. Anticipated results, interpretation, potential difficulties and alternative approaches: We suspect that the combination of PD-1 and CTLA-4 checkpoint blockade will significantly decrease the growth of LLC in combination with OMCP-mutIL-2 without an increase in adverse events over PD-1 and CTLA-4 alone. We suspect that mice treated with combination of checkpoint blockade immunotherapy and high dose of wild-type IL-2 will suffer a higher rate of adverse events without significant decrease in tumor growth over checkpoint blockade alone. Based on our previous experience with the LLC i.v. injection tumor model, we performed a 1-way ANOVA pairwise, 2-sided equality power analysis in R with 10 pairwise comparison, a power of 0.80, and an alpha error value of 0.05, we find that we will require 7 mice per group for a total of 84 mice to test all checkpoint therapy combinations. If necessary we would detect discordance between lung weight and histologic tumor volume assessment by other means such as small animal MRI(45), histologic segmentation of slides, or direct flow cytometric counting of LLC cells expressing GFP(46). If we identify a regimen that results in optimal immunotherapy, we would then dissect the immunologic factors contributing to the control of tumor growth, specifically the role of CD8+ T cells, NK cells and T_{regs} in the immune response. Such mechanistic investigations are routinely performed in our laboratory and will consist of antibody-mediated depletion of either NK or CD8+ T cells or T_{reg} depletion through the use of Foxp3-dyptheria toxin receptor expressing mice (Jackson Labs). If we do not detect a benefit for combination of PD-1 and/or CTLA-4 checkpoint blockade and OMCP-mutIL-2 we would try different adjuvant agents such as chemo/immunotherapy or alternative checkpoint blockade agents such as anti-Tim-3 or Lag-3.

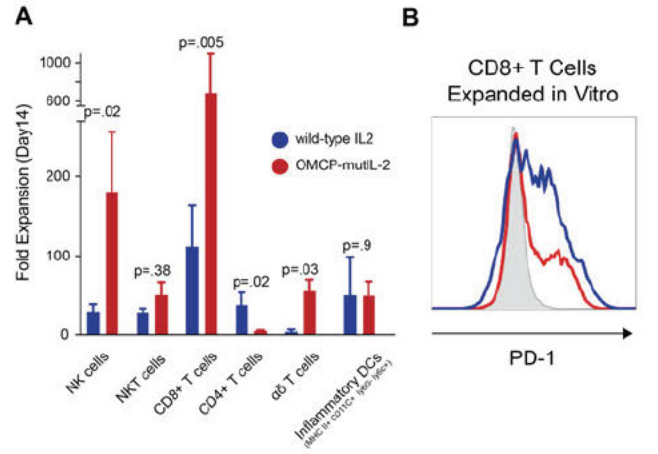


Figure 8. Lymphocytes infiltrating B16 melanoma tumors were expanded in high-dose wild-type IL-2 (blue) or OMCP-mutIL-2 (red) for 2 weeks. (A) More CTL expansion was evident in OMCP-mutIL-2 treated cultures (B) with lower levels of exhaustion as measured by PD-1 expression.

Specific Aim 3: **To evaluate the utility of OMCP-mutIL-2 for expansion of human tumor-reactive lymphocytes in vitro.**

1. Preliminary Data: Ex vivo expansion and adoptive transfer of lymphocytes enriched for tumor reactive cells is a viable therapeutic strategy for controlling malignancies. Such lymphocytes represent either tumor-infiltrating cells, such as antigen specific CD8+ T cells and γδ T cells that reside in the tumor bed, antigen-specific CD8+ T cells generated by vaccination strategies, or engineered lymphocytes such as CAR-T cells(2). Protocols for expansion of such cells, which traditionally rely on IL-2 or other common gamma chain cytokines for cytokines(47), could be improved to enhance efficacy, streamline production and to reduce the substantial cost of generating this class of therapeutics. In order to investigate the utility of NKG2D-redirected cytokine stimulation for lymphocyte expansion we isolated tumor infiltrating lymphocytes from murine B16 melanoma and expanded them for 2 weeks in either IL-2 or OMCP-mutIL-2 utilizing transient CD3/CD28 stimulation and continuous high-dose (5000 IU/ml) cytokine stimulation based on clinically utilized protocols(47). Two weeks later we evaluated the relative expansion of various leukocyte subsets and noted that OMCP-mutIL-2 led to

preferential expansion of CD8+ T cells, NK cells as well as γδ T cells (all of which have the capacity to express NKG2D), while wild-type IL-2 expanded CD4+ T cells as well as subsets of myeloid cells (**Figure 8a**). We also observed that CD8+ T cells expanded in OMCP-mutIL-2 demonstrate lower levels of surface exhaustion markers such as PD-1 (**Figure 8b**). Thus in a murine model NKG2D-redirected stimulation of the IL-2 receptor results in preferential expansion of lymphocytes with known anti-tumor activity. Nevertheless the ability of OMCP-mutIL-2 to expand human antigen-specific tumor reactive CD8+ T cells is unknown. Such data would expand our understanding of OMCP-mutIL2 biology and would potentially open up an avenue for marketing our product as a reagent for T cell expansion in addition to its potential use as a biologic for in vivo administration.

2. Experimental design and methods. Recently executed clinical trials by our collaborator (please see letter of support from Craig Slingluff) have demonstrated that immunization with a 12 peptide vaccine results in a robust immune response as well as an improvement in melanoma survival(48) (**Table 2**). As part of this multi-peptide vaccination trial the lymph node draining the replicate immunization site was harvested and a single cell suspension of lymphocytes was cryopreserved as described(48). This lymphocyte population is enriched for antigen-specific, melanoma-reactive CD8+ T cells and can be used to test the utility of NKG2D-immunotargeting for cytotoxic lymphocyte expansion.

Based on previously published protocols as well as our murine data described above lymphocytes will be thawed and relative number of $CD8^+$ T cells, melanoma antigen-specific $CD8^+$ T cell (as defined by tetramer staining)(**Table 2**), γδ T cells, CD4+ T cells, CD4+ T_{regs} and myeloid cells will be quantitated by flow cytometry. Lymphocyte expansion will then be carried out by plating $1x10^6$ lymphocytes in 5000 IU of either IL-2 or OMCP-mutIL-2 in the presence of 72 hour CD3/CD28 stimulation similar to previously described methods(47). After two weeks the cultures will be analyzed flow cytometrically and the relative expansion of various lymphocyte subsets, as well as antigen specific CD8+ T cells as defined by tetramer staining.

Table 2

HLA	Peptide	Source Protein (residues)
A1	DAEKSDICTDEY	Tyrosinase (240-251)
	SSDYVIPIGTY	Tyrosinase (146-156)
	EADPTGHSY	MAGE-A1 (161-169)
	EVDPIGHLY	MAGE-A3 (168-176)
A2	YMDGTMSQV	Tyrosinase (369-377)
	IMDQVPFSV	gp100 (209-217)
	YLEPGPVTA	gp100 (280-288)
	GLYDGMEHL	MAGE-A10 (254-262)
A3	ALLAVGATK	gp100 (17-25)
	LIYRRRLMK	gp100 (614-622)
	SLFRAVITK	MAGE-A1 (96-104)
	ASGPGGGAPR	NY-ESO-1 (53-62)

Functional evaluation of antigen-specific CD8+ T cells, after in vitro expansion, will be performed using IFN-γ ELIspot analysis as previously described by our group(48). Based on HLA phenotype lymphocytes expanded in IL-2 or OMCP-mutIL-2 will be restimulated in vitro with C1R-A1, C1R-A2 or C1R-A3 EBV-transformed B-cell lines pulsed with the appropriate vaccination peptides and an irrelevant negative control peptide as previously described(48). Assessment of immunologic response will be based on a fold-increase measure as well as on the number of spots counted per 100,000 cells plated as described(48).Based on specimen availability we expect to evaluate leukocytes from 15 patients across all 3 HLAs. Each patient will serve as his or her own control and IL-2 and OMCP-mutIL-2 expanded cultures will be compared by t-test.

3. Anticipated results, interpretation, potential difficulties and alternative approaches: At the completion of this aim we will know whether: 1)OMCP-mutIL-2 mediates preferential expansion of cytotoxic lymphocytes and; 2) whether antigen specific T cells retain ability to secrete IFN-γ. By focusing on both quantitative analysis by tetramer staining and IFN-γrecall responses we will get a sense of whether CD8+ T cells retain function or develop "exhaustion". For this aim we plan to utilize global T cell receptor stimulation using agonistic CD3/CD28 antibodies. Based on the data generated here we plan to focus on antigen-specific expansion of CD8+ T cells using specific tumor-associated peptide stimulation of lymphocytes. If human data mirrors that of murine lymphocytes described above (**Figure 8**) we would seek to partner with companies focused on ex-vivo expansion of tumor reactive lymphocytes or CAR-T cells. We would also then explore mechanistic aspects of OMCP-mutIL-2 that mediate its superior effect. Specifically we would look at IL-2 receptor signal transduction, kinetics of T cell proliferation, as well as T cell exhaustion, death and terminal differentiation. Such mechanistic studies, however, rely on obtaining functional and physiologic data described above. If our drug underperforms in human cells in vitro, we will continue to pursue its well-supported potential as an in vivo therapeutic(13).

PROTECTION OF HUMAN SUBJECTS

Description of proposed use of human cells and tissues

We are investigating whether our drug candidate, OMCP-mutIL-2, is able to expand tumor-reactive lymphocytes. Such data will allow us to identify whether our drug candidate may have applicability as a reagent for possible use as a reagent for adoptive immunotherapy of cancer. We plan to use single cell suspension of de-identified primary human lymphocytes of melanoma patients entered into an immunization trial as provided by Dr. Slingluff, University of Virginia. The lymphocytes have already been collected as part of a clinical trial authorized under institutional review board approval (HIC 8878) and Food and Drug Administration approval (BB-IND 9847) and have been maintained frozen in liquid nitrogen. Thus no further intervention will be necessary for the patients. The human biological specimens to be used for this study (primary lymphocytes) are completely de-identified as defined by the OHRP Guidance on Research Involving Coded Private Information or Biological Specimens. Therefore research is exempt under Exemption 45 CFR 46.101(b)(4) from all 45 CFR part 46 requirements.

VERTEBRATE ANIMALS

1. DESCRIPTION OF THE PROPOSED USE OF ANIMALS

All portions of the studies requiring the manipulation of live animals will be conducted by Dr. Krupnick at the University of Virginia, under IACUC protocol #4149.

Specific Aim 2: To evaluate OMCP-mutIL-2 as an adjuvant for checkpoint blockade immunotherapy.

The purpose of this study is to evaluate the potential for OMCP-mutIL-2 to function as an adjuvant to the increasingly important T-cell checkpoint inhibitor therapies.

Justification of cohort sizes: Based on our previous experience with the LLC i.v. injection tumor model, we expect to find that normal, non-tumor bearing lungs weight approximately 179 ± 21.2 mg, whereas LLC tumor bearing lungs weigh approximately 618 ± 31.2 mg. We estimate that a therapeutically meaningful treatment will reduce the tumor load by approximately 50%, or to 398.5 mg. As we have more than two groups, we performed a 1-way ANOVA pairwise, 2-sided equality power analysis in R with 10 pairwise comparisons, equal group sizes, a power of 0.80, and an alpha error value of 0.05. Using the parameters, we find that we will require 7 mice per group, for a total of 84 C57Bl/6 mice to test all checkpoint therapy combinations.

Table 3

Group	Checkpoint Blockade	Cytokine	N
1	IgG control	none	7
2	anti-PD-1	none	7
3	anti-CTLA-4	none	7
4	anti-PD-1 and anti-CTLA-4	none	7
5	IgG control	OMCP-mutIL-2	7
6	anti-PD-1	OMCP-mutIL-2	7
7	anti-CTLA-4	OMCP-mutIL-2	7
8	anti-PD-1 and anti-CTLA-4	OMCP-mutIL-2	7
9	IgG control	Wild-type IL-2	7
10	anti-PD-1	Wild-type IL-2	7
11	anti-CTLA-4	Wild-type IL-2	7
12	anti-PD-1 and anti-CTLA-4	Wild-type IL-2	7
		Total mice	84

Throughout the tumor initiation, therapeutic dosing, and tumor growth period mice will be monitored for pain and distress, according to details under “Minimization of Pain and Distress,” below. Mice will be additionally weighted daily after the initiation of therapy. Immunotherapy will be stopped in animals in any of the groups that demonstrate stress of weight loss of over 20% of starting body weight. Such animals will be considered to suffer from adverse immunotherapy-related adverse events and sacrificed. Should any of the mice show severe distress they will be prematurely and humanely euthanized using C02 inhalation followed by cervical dislocation. All remaining animals will be humanely euthanized twenty-one days after tumor injection and the lungs will be collected in order to evaluate the primary end point of tumor growth as measured by total lung weight. Lungs will be subsequently fixed and embedded for histological assessment of tumor burden. Mice will be further necropsied and evaluated for adverse immunologic side effects, with particular focus on the liver, colon, and small bowel. A portion of each of these tissues will be fixed for histological analysis and evaluated for autoimmune pathology. Finally, blood serum will be collected for later measurement of ALT and AST levels, which indicate liver damage.

2. VETERINARY CARE

Animals for all studies will be housed in the Medical Research Building #5 at the University of Virginia Animal Facility, and all procedures will be carried out under Dr. Krupnick’s protocol approved by the University of Virginia Institutional Animal Care and Use Committee (IACUC; protocol #4149). These facilities employ two veterinarians and four veterinary technicians, which supervise veterinary care, protocol review, surgical services, pathology services, diagnostic laboratory services, training of investigators and investigator staff, and compliance. All tumor-bearing animals will be monitored daily by both Dr. Krupnick’s staff as well as the facility veterinary staff for signs of distress, pain, side effects, or other signs of distress. Should the veterinary staff notice an animal in need of urgent veterinary care prior to Dr. Krupnick’s personnel, they will immediately attempt to contact Dr. Krupnick and his personnel for appropriate action. Should they exhaust all reasonable attempts at communication they will act as appropriate in order to ensure that animals do not undergo undue distress or pain. Additionally, Dr. Krupnick and his laboratory staff will monitor animals daily for signs of distress and pain, and euthanize mice where appropriate.

3. MINIMIZATION OF PAIN AND DISTRESS

All efforts will be made to limit distress of the animals, however there are some studies which may cause unavoidable stress, including the described tumor studies. All researchers coming in contact with the animals will be fully trained in the proper handling and care of those animals. The University of Virginia animal facilities has expert veterinary staff on hand who are available for consultation should concerns on animal safety or care arise.

After throughout injection of therapeutic cytokine or antibody agents, mice will be monitored daily for signs of distress. For the tumor flank injections and tail-vein injections, mice will be given an analgesic prior to injection such as a standard ketamine-xylazine combination drug. Additionally, after flank injection of tumor cell lines, mice will be monitored daily and any visible tumor will be measured with calipers until it reaches a size of 1.5 cm in diameter or interferes with normal activity. Mice injected either intravenously or via the flank with tumor cell lines will be monitored daily for signs of distress, particularly as the intravenous injection of tumor cells is not expected to generate a visible tumor. Animals showing signs of severe distress, outlined in the American Veterinary Medical Association (AVMA) guidelines, will be humanely euthanized. Signs of distress include:

1. Ruffled fur
2. Lethargy, depression, or reluctance to move
3. Hunched back
4. Labored breathing
5. Weight loss (up to 20% of the animal's original weight)

Any mice displaying a level of these symptoms that leads to a body condition of BC2 will be immediately euthanized.

4. EUTHANASIA

Animals will be euthanized consistently with the recommendation of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia of Animals. For these studies, mice will first be euthanized with CO2 delivered to a chamber at a 20% fill rate, monitored by a flow meter, and will remain in these conditions for an additional 3 minutes after visible movement has ceased. Subsequently, cervical dislocation will be utilized as a secondary method of euthanasia to confirm death. These methods are considered humane and painless.

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Authentication of Key Biological and Chemical Resources

The key resources in this proposal will be purchased from reputable commercial vendors. Below are the measures taken by the Krupnick and Courier laboratories to confirm identity, validity, and reproducibility of key biological and or chemical resources. Cell lines are not used in this grant proposal and therefore will not require validation or quality control assessments.

Mice: This grant proposal in all aims relies heavily on mouse strains generated by the Jacobsen laboratory. The mice are all C57BL/6J (Jackson Laboratories) and maintained by the vendor with genetic testing to prevent genetic drift.

Chemicals: OMCP-mutIL-2 is produced in mammalian cell lines at the protein production core at UNC (https://www.med.unc.edu/csb/pep) on a contractual basis. Purity and LPS contamination is routinely tested by standard QC protocols prior to release for use. Wild-type IL-2 is obtained from the NCI Biological Research Branch (BRB).

Antibodies: All antibodies used for flow cytometric analysis and stimulation of T cells are purchased from commercial sources including BD Bioscience, eBioscience (ThermoFischer) as well as Fischer Scientific. All reagents are thus standardized based on lot. No in house of proprietary antibodies will be used in this project.