*RESEARCH*
Department of Health and Human Services
National Institutes of Health
NATIONAL CANCER INSTITUTE

**Federal Award Date:** 03/25/2015

**Grant Number:** 1R01CA186730-01A1
**FAIN:** R01CA186730

**Principal Investigator(s):**
RICHARD HELLER, PHD

**Project Title:** Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression

Mrs. Harris, Stephanie
Sr. Grant & Contract Administrator
4111 Monarch Way
Suite 204
Norfolk, VA 235082561

**Award e-mailed to:** rfawards@odu.edu

**Period Of Performance:**
**Budget Period:** 04/01/2015 – 03/31/2016
**Project Period:** 04/01/2015 – 03/31/2020

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $371,451 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to Old Dominion University Research Foundation in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number R01CA186730. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Aida Vasquez
Grants Management Officer
NATIONAL CANCER INSTITUTE

Additional information follows

## SECTION I – AWARD DATA – 1R01CA186730-01A1

**Award Calculation (U.S. Dollars)**

| | |
|---|---|
| **Salaries and Wages** | $107,883 |
| **Fringe Benefits** | $48,753 |
| **Supplies** | $32,619 |
| **Travel Costs** | $3,320 |
| **Other Costs** | $7,885 |
| **Consortium/Contractual Cost** | $51,497 |
| **Federal Direct Costs** | $251,957 |
| **Federal F&A Costs** | $119,494 |
| **Approved Budget** | $371,451 |
| **Total Amount of Federal Funds Obligated (Federal Share)** | $371,451 |
| **TOTAL FEDERAL AWARD AMOUNT** | $371,451 |
| **AMOUNT OF THIS ACTION (FEDERAL SHARE)** | $371,451 |

| SUMMARY TOTALS FOR ALL YEARS | | |
|---|---|---|
| **YR** | **THIS AWARD** | **CUMULATIVE TOTALS** |
| 1 | $371,451 | $371,451 |
| 2 | Future Costs,Recommendations | |
| 3 | | |
| 4 | | |
| 5 | | |

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

**Fiscal Information:**

| | |
|---|---|
| **CFDA Name:** | Cancer Treatment Research |
| **CFDA Number:** | 93.395 |
| **EIN:** | 1546068198A1 |
| **Document Number:** | RCA186730A |
| **PMS Account Type:** | P (Subaccount) |
| **Fiscal Year:** | 2015 |

| IC | CAN | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|
| CA | 8479567 | $371,451 | Future Costs,Recommendations | | | |

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

**NIH Administrative Data:**
**PCC**: 6WBR / **OC**: 414A / **Released**: VASQUEZAI 03/19/2015
**Award Processed**: 01/15/2015 11:58:50 AM

## SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01CA186730-01A1

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

## SECTION III – TERMS AND CONDITIONS – 1R01CA186730-01A1

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.

d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

**Research and Development (R&D):** All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01CA186730. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

**Treatment of Program Income:**
Additional Costs

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**SECTION IV – CA Special Terms and Conditions – 1R01CA186730-01A1**

**INFORMATION**: In accordance with the National Cancer Institute's (NCI's) Fiscal Year (FY) 2015 funding policies, this award has been issued at 83% of the adjusted requested level*. Support recommended for future years has been adjusted accordingly.

*adjusted requested level: The requested level of support with adjustments made in accordance with the budget narrative in the summary statement and applicable grant policies.

*Spreadsheets used to calculate this award are available upon request.

**INFORMATION:** Future year total cost commitments appearing on the award notice under "Recommended Future Year Total Cost Support" have been calculated by applying the negotiated facilities and administrative cost rate(s) in effect at the time of this FY2015 award to the committed total direct cost level for each future year.

**INFORMATION:** This award includes funds awarded for consortium activity . Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH GPS is available at:http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch15.htm#_Toc271265264

**INFORMATION**: See "Assurance Requirements and Institutional Review Boards" under Part II, Subpart A, Human Subjects, in the NIH Grants Policy Statement (NIHGPS)(rev. 10/13), for specific requirements and grantee responsibilities related to the protection of human subjects, which are applicable to and are a term and condition of this award. The NIHGPS can be found at http://grants.nih.gov/grants/policy/nihgps_2013/index.htm. The referenced section of the NIHGPS is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch4.htm#human_subjects_federalwide_assurance.

This award reflects the National Cancer Institute's acceptance of the certification that all key personnel have completed education on the protection of human subjects, in accordance the NIH Grants Policy Statement (NIHGPS)(rev. 10/13), "Education in the Protection of Human Research Subjects." The NIHGPS can found at http://grants.nih.gov/grants/policy/nihgps_2013/index.htm. The referenced section of the NIHGPS is available at http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch4.htm#human_subjects_protection_education

Any individual involved in the design and conduct of the study that is not included in the certification must satisfy this requirement prior to participating in the project. Failure to comply can result in the suspension and/or termination of this award, withholding of support of the continuation award, audit disallowances, and/or other appropriate action.

**INFORMATION:** This award, including the budget and the budget period, has been discussed between Aida Vasquez of the National Cancer Institute and Stephanie Harris on 03/16/15.

**STAFF CONTACTS**

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

**Grants Management Specialist**: Aida Vasquez
**Email**: vasquez@mail.nih.gov **Phone**: (240) 276-6319

**Program Official:** Karen Muszynski
**Email**: muszynskik@mail.ncifcrf.gov **Phone**: 301-846-1101

**SPREADSHEET SUMMARY**
**GRANT NUMBER:** 1R01CA186730-01A1

**INSTITUTION:** Old Dominion University Research Foundation

| Budget | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|
| Salaries and Wages | $107,883 | Future Costs,Recommendations | | | |
| Fringe Benefits | $48,753 | | | | |
| Supplies | $32,619 | | | | |
| Travel Costs | $3,320 | | | | |

| | |
|---|---|
| Other Costs | $7,885 |
| Consortium/Contractual Cost | $51,497 |
| TOTAL FEDERAL DC | $251,957 |
| TOTAL FEDERAL F&A | $119,494 |
| TOTAL COST | $371,451 |

Future Costs, Recommendations

| Facilities and Administrative Costs | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|
| F&A Cost Rate 1 | 53% | | | | |
| F&A Cost Base 1 | $225,460 | | | | |
| F&A Costs 1 | $119,494 | | | | |

Future Costs, Recommendations

Aida K. Vasquez -S

Digitally signed by Aida K. Vasquez -S
DN: c=US, o=U.S. Government, ou=HHS, ou=NIH, ou=People, 0.9.2342.19200300.100.1.1=0010098603, cn=Aida K. Vasquez -S
Date: 2015.03.17 15:28:41 -04'00'

| | | |
|---|---|---|
| PI: **HELLER, RICHARD** | Title: Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression | |
| Received: 03/04/2014 | FOA: PA13-302 | Council: 10/2014 |
| Competition ID: FORMS-C | FOA Title: RESEARCH PROJECT GRANT (PARENT R01) | |
| **1 R01 CA186730-01A1** | Dual: EB | Accession Number: 3676311 |
| IPF: 6249601 | Organization: OLD DOMINION UNIVERSITY | |
| Former Number: | Department: Center for Bioelectrics | |
| IRG/SRG: GDD | AIDS: N | Expedited: N |
| Subtotal Direct Costs (excludes consortium F&A)<br>Year 1: 280,694<br>Year 2: Future Costs<br>Year 3:<br>Year 4:<br>Year 5: | Animals: Y<br>Humans: Y<br>Clinical Trial: N<br>Current HS Code: 30<br>HESC: N | New Investigator: N<br>Early Stage Investigator: N |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| Richard Heller Ph.D | Old Dominion University | PD/PI |
| Adil Daud M.B.B | University of California, San Francisco | Other (Specify)-Consortium Co-Investigator |
| Lawrence Fong M.D. | University of California, San Francisco | Other (Specify)-Consortium Co-Investigator |

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

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**
CA186730

**b. Agency Routing Number**

**2. DATE SUBMITTED** | **Application Identifier**

**c. Previous Grants.gov Tracking Number**
GRANT11598928

**5. APPLICANT INFORMATION** **Organizational DUNS*:** 041448465

Legal Name*: Old Dominion University
Department: Research Foundation
Division:
Street1*: 4111 Monarch Way
Street2: Suite 204
City*: Norfolk
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 23508-2561

Person to be contacted on matters involving this application
Prefix: Mrs. First Name*: Stephanie Middle Name: Last Name*: Harris Suffix:
Position/Title: Sr. Grant & Contract Administrator
Street1*: 4111 Monarch Way
Street2: Suite 204
City*: Norfolk
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 23508-2561
Phone Number*: 757-683-7225 Fax Number: Email: sl2harri@odu.edu

**6. EMPLOYER IDENTIFICATION NUMBER** *(EIN) or (TIN)** 546068198

**7. TYPE OF APPLICANT*** M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

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***
Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression

**12. PROPOSED PROJECT**
Start Date* 09/01/2014
Ending Date* 08/31/2019

**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
VA-003

# SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE



**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**

Prefix: Dr. First Name*: Richard Middle Name: Last Name*: Heller Suffix: Ph.D

Position/Title: Director and Professor
Organization Name*: Old Dominion University
Department: Center for Bioelectrics
Division:
Street1*: 4211 Monarch Way
Street2: Suite 300
City*: Norfolk
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 23508-2561
Phone Number*: 757-683-2690 Fax Number: Email*: rheller@odu.edu

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested* | $2,276,755.00 |
| b. Total Non-Federal Funds* | $0.00 |
| c. Total Federal & Non-Federal Funds* | $2,276,755.00 |
| 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*: Cynthia Middle Name: A. Last Name*: Matney Suffix:

Position/Title*: Director of Sponsored Programs
Organization Name*: Old Dominion University
Department: Research Foundation
Division:
Street1*: 4111 Monarch Way
Street2: Suite 204
City*: Norfolk
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 23508-2561
Phone Number*: 757-683-4293 Fax Number: Email*: rfawards@odu.edu

**Signature of Authorized Representative***
Julian Facenda

**Date Signed***
03/04/2014

**20. PRE-APPLICATION** File Name:

**21. COVER LETTER ATTACHMENT** File Name:1235-Cover Letter.pdf





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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OMB Number: 4040-0010
Expiration Date: 06/30/2016

# 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: | Old Dominion University |
| Duns Number: | 0414484650000 |
| Street1*: | 5115 Hampton Blvd. |
| Street2: | |
| City*: | Norfolk |
| County: | |
| State*: | VA: Virginia |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 23529-0001 |
| Project/Performance Site Congressional District*: | VA-002 |

**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 California, San Francisco |
| DUNS Number: | 0948783370000 |
| Street1*: | 3333 California St. |
| Street2: | Suite 435 |
| City*: | San Francisco |
| County: | |
| State*: | CA: California |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 94143-1241 |
| Project/Performance Site Congressional District*: | CA-012 |

File Name

**Additional Location(s)**

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

# 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 00000273

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3172-01

**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*** | 1236-Abstract.pdf |
| **8. Project Narrative*** | 1237-Narrative.pdf |
| **9. Bibliography & References Cited** | 1238-References.pdf |
| **10.Facilities & Other Resources** | 1239-Facilities.pdf |
| **11.Equipment** | 1240-EQUIPMENT.pdf |







Funding Opportunity Number: PA-13-302. Received Date: 2014-03-04T18:30:26.000-05:00

Delivery still remains as a barrier to achieving successful gene therapy. Administering gene delivery protocols in a manner that would allow better control over the expression pattern would enhance therapeutic outcomes. We have developed a delivery approach (gene electro transfer; GET) which utilizes pulsed electric fields that allows for controlled delivery. We have tested this approach as a means of delivering plasmids encoding immunostimulatory molecules. For immunotherapy, maintaining control over expression following plasmid delivery is critical to success as there is a fine balance between immunostimulation and immunosuppression. Manipulation of GET parameters can be used for controlled delivery of plasmid and will result in obtaining the appropriate transgene expression. The model system utilized to test this system is malignant melanoma which is a major health concern with no effective therapy for advanced disease. The incidence of melanoma continues to rise and it is estimated that there will be 76,690 new cases and 9,480 deaths in 2013. Melanoma is a good model for immunotherapy approaches as there is evidence demonstrating immune responsiveness including both innate and adaptive immunity. Recently, several new approaches have been tested as potential immunotherapies with some success. However, overall durable complete response rates (disease free survival) are low (<15%) and some of these therapies have significant adverse events documenting that there is still a need for more effective therapies. One potential new therapy is to deliver a plasmid encoding Interleukin-12 directly to the tumor to stimulate an immune response. The important criterion for success is administering IL-12 at the right dose and location. To address this, we have developed an effective means of delivering plasmid DNA utilizing GET. The hypothesis to be tested is: if appropriate delivery parameters are used to deliver plasmid IL-12 then a change in the tumor microenvironment will occur that will be associated with an appropriate therapeutic response. Therefore, it is critical to characterize the response and identify potential biomarkers that can signify proper delivery and expression. We also hypothesize that if an appropriate combination can be achieved then there will be an increased response at distant sites. The increased response rates together with boosting the immune response may lead to an effective therapy for metastatic melanoma due to a reduction of T-reg cells and enhanced activation of T-effector and memory cells. In this project, we will develop and test this approach in a mouse model and have the opportunity to determine how it correlates with samples obtained from an ongoing clinical trial. Thus, the work in this project is directly translatable. The following specific aims will be performed as part of this project. 1. Determine the influence expression profile has in inducing an effective anti-tumor response and determine if a specific pattern of response can be identified. 2. Evaluate expression patterns following delivery of plasmids encoding anti-PD1, anti-PD-L1 or anti-CTLA4. 3. Therapeutic efficacy of the approach in a mouse metastatic model.

Public Health Relevance

A major obstacle for successful gene therapy is delivery of DNA in a manner that would result in better control of the expressed gene. We have developed a delivery approach that can deliver plasmid DNA in a manner that would result in a desired expression profile. This delivery approach will be tested by developing an immunotherapy protocol for melanoma which is a major health concern with an increasing incidence and death rate and no effective therapy for advanced disease.

## FACILITIES at Old Dominion University

The Frank Reidy Research Center for Bioelectrics is an interdisciplinary center located at Old Dominion University. The mission of the Center is to increase scientific knowledge and understanding of how intense, pulsed electromagnetic fields and cold ionized gases interact with biological systems and to apply this knowledge to the development of medical diagnostics and therapeutics as well as environmental decontamination. Over 40 researchers from more than 10 countries with expertise in engineering, physics, immunology, molecular biology and biology occupy newly constructed state-of-art-laboratories. Approximately 31,000 sq. feet is allocated to the Center which includes both laboratories and offices.

Laboratory: The principal investigator has access to two laboratories with approximately 1200 sq ft in the Center. The laboratories are equipped with a two biosafety cabinets, $CO_2$ incubators, Leica fluorescent inverted and upright microscopes with cameras, Leica fluorescent steroscope, Cellular Technology Immunospot Analyzer, Dynex MLX microplate luminometer, Licor Odyssey Infrared Imager, Thermo Multiskan MCC plate reader, a Bio-Rad CFX96 real time thermal cycler, and several centrifuges. The laboratories also havenanosecond pulsers, BTX ECM 820 and 830 pulse generators, an UltraVolt high power voltage system,digital storage oscilloscopes, and specially designed electrode arrays.

Shared equipment available includes: fume hoods, a variety of microscopes with cameras, ELISA readers, spectrofluorometer, BD Facs-Aria flow cytometer,Millipore Magpix Luminex multiplex analyzer, centrifuges and cryostat.

Animal:A new state-of-the-art 10,000 sq ft animal facility is located in the same building as the Center. The OLAW certified facility includes several procedure rooms as well as an imaging suite and surgical facilities. The facility is staffed by SoBran, Inc. (Fairfax, VA) per NIH guidelines. SoBran has 13 years of experience furnishing the onsite management and professional personnel necessary to perform the duties associated with animal research support services. They are the largest provider of onsite laboratory animal research support at the National Institutes of Health. SoBran partners with our customers supplying highly qualified research personnel, customer focused service and support, humane animal care, top-tier technical training and support, outstanding quality control, comprehensive AAALAC and USDA site visit preparation, and Institutional Animal Care and Use Committee coordination and administrative support.

Available equipment appropriate to rodent experiments includes a VisualSonicsVevo 770 High-Resolution In Vivo Imaging System, a Moor LDLS laser Doppler imager, a Caliper Life Sciences IVIS Spectrum, and a Olympus IV 100 Intravital Laser Scanning Microscope.

Computer/Office: The principal investigator has the use of a 200 sq ft office. A Dell Optiplex 960 computer with Microsoft Office, SPSS, GraphPad InStat, SlideWrite, SigmaPlot, Grapher, Endnote, Adobe Acrobat Pro software packages are available for use. This will be used to prepare reports and keep backup copies of all data.

Other:Additional available equipment at the Center includes biological safety cabinets, laminar flow hoods, fume hoods, $CO_2$ incubators, autoclaves, fluorescent inverted and upright microscopes,miscellaneous centrifuges (Eppendorf 5402 microfuge; Eppendorf 5415 microfuge, Eppendorf 5810 microfuge/centrifuge with A4-62 and FA 45-30-11 rotors, Beckman SW28/70TI/TI41 ultracentrifuge with swinging basket and fixed angle rotors, Beckman J2MI high speed centrifuge, Eppendorf 5810R microfuge/centrifuge with A4-62 / FA 45-30-11 rotors, Avanti J25 high speed centrifuge with JA17 / JA 25 / JA 20 / JA 10 fixed angle rotors), Gemini XPS Specrofluorometer (Molecular Devices), and cryostat. The Center also has various square wave pulse generators with pulse durations varying from milliseconds to 150 picoseconds with voltage varying from 1-550 kV, electroporation generators, ultravolt power supplies and oscilloscopes.

**FACILITIES at University California San Francisco**

Institutional:
UCSF Research Environment: University California, San Francisco is a dedicated health sciences university with Schools of Medicine, Dentistry, Nursing and Pharmacy. UCSF is one of the largest and most active research institutions in the country with approximately 1300 principal investigators and 3000 current research projects. Annual research funding is in excess of $400 million, with over $350 million from the National Institutes of Health (ranks 4th among all institutions). The School of Nursing ranks second and the School of Medicine ranks third among recipients of NIH funding. UCSF's two other health sciences schools – Pharmacy and Dentistry- both rank number one in the nation.

UCSF Helen Diller Family Comprehensive Cancer Center: This NCI-designated matrix center conducting a wide range of interdisciplinary research in the areas of laboratory, clinical, and population sciences. The Cancer Center integrates the activities of 331 members (247 program members, 23 members, 42 associate members, and 19 affiliate members) working at four major campus and hospital locations. The Center received NCI designation in August 1999 and Comprehensive status in January 2000.

UCSF Helen Diller Family Comprehensive Cancer Center Cutaneous Malignancies Program: Led by Dr. Adil Daud, this multi-disciplinary program encompasses both clinical and basic laboratory efforts focused on cutaneous malignancies including melanoma. Clinical care is provided by dermatologists, medical oncologists, surgical specialists, radiation oncologists and dermatopathologists at the Mt Zion Campus on the 4th floor. This community holds monthly scientific meetings as well weekly tumor boards. Laboratory based investigators include Drs. Martin McMahon, Lawrence Fong, James Cleaver, Ruby Ghadially, Farnk McCormack, Maria Wei and Alan Balmain. Clinical investigators include Drs. Adil Daud, Michael Alvarado, Siegrid Yu, Sarah Arron, Roy Ghrekin, Susanna Ortiz, Philip LeBoit, Timothy McCalmont and Naomi Schecter.

Dr Daud's office is approximately 260 sq feet, located at the Mt Zion campus on the 7th floor. Other members of the Hem/onc department sharing office space on this floor are Drs. Eric Small, Charles Ryan, Alan Venook, Margaret Tempero, Emily Bergsland and Pamela Munster. Administrative suppport for him is provided by Erin Oriolo and networked computers (both PC and Mac), fax, printer, scanner/copier and the Clinical Trial Office where lead CTC Emily Arimura are also in the same space.

UCSF Cancer Center Tissue Core: The tissue core <http://cancer.ucsf.edu/tissue/index.php> provides fresh, frozen and paraffin embedded tissue derived from surgical specimens from the 3 university hospitals: UCSF/Moffitt, UCSF/Mt. Zion, and San Francisco General Hospital. The core also provides tissue processing, sectioning and IHC services. An integrative database, which contains clinical parameters and outcomes, tumor tissue characteristics, and outcomes of testing on tissues is also managed by the Cancer Center tissue core.

## MAJOR EQUIPMENT at Old Dominion University

Major equipment in the laboratory includes two biosafety cabinets, $CO_2$ incubators, Leica fluorescent inverted and upright microscopes with cameras, Leica fluorescent steroscope, Cellular Technology Immunospot Analyzer, Dynex MLX microplate luminometer, Licor Odyssey Infrared Imager, Thermo Multiskan MCC plate reader, a Bio-Rad CFX96 real time thermal cycler, and several centrifuges. The laboratory also have BTX ECM 820 and 830 pulse generators, an UltraVolt high power voltage system, nanosecond pulsers, digital storage oscilloscopes, and specially designed electrode arrays.

Shared equipment available in the Center for Biolectrics include BD FACSAria flow cytometer and 4 way cell sorter with 3 air cooled lasers at 488nm, 633nm and 405nm and 9 detector color analysis with 11 parameters including forward and side scatter, 5 fluorescent detectors off 488nm, 2 fluorescent detectors off 633nm and 2 fluorescent detectors off 405nm and a Millipore Magpix with Luminex XMap Technology. Magnetic bead multiplex technology. Can be used with standard premade or made to order plates. Both the Flow cytometer and Magpix instruments are part of the Flow Cytometry core at the Center.

Other shared equipment within the Center for Bioelctrics that is available for use include: Molecular Devices Gemini XPS Specrofluorometer with dual monochromomters with wave length in 1nm increments that can scan 6-, 12-, 24-, 48-, 96- or 384- wells using to or bottom reading optics with a dynamic detection range from 10 -6 to 10 -11 molar fluoroscein at temperatures for 28-45 C; PTI Spectrofluorometer with a 75W xenon arc lamp and dual detectors, wavelength range from 200-650nm, and detection range from 185-680nm; 3 Olympus IX70 Microscopes; Confocal Microscope: Olympus Fluoview FV300 Confocal Microscope configured with IX71 Microscope and BRG lasers; Spinnning Disk Confocal Microscope: Perkin Elmer Ultra View RS Spinning Disk Confocal Imaging System, including a confocal scanning module, Argon/Krypton laser, piezo objective driver, imaging software and data analysis software; Ultrahigh (5ns) Time Resolution Epifluorescent Microscope, with relief contrast components, including relief/phase condenser, relief contrast 20X magnification fluorescent objective and relief contrast 40X magnification fluorescent objective; Leica fluorescent stereoscope.

Shared equipment in the vivarium that is part of the imaging core include visualSonics Vevo 770 High-Resolution In Vivo Imaging System, a Moor LDLS laser Doppler imager, a Caliper Life Sciences IVIS Spectrum, and a Olympus IV 100 Intravital Laser Scanning Microscope.

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

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

## PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Richard Middle Name Last Name*: Heller Suffix: Ph.D

| | |
|---|---|
| Position/Title*: | Director and Professor |
| Organization Name*: | Old Dominion University |
| Department: | Center for Bioelectrics |
| Division: | |
| Street1*: | 4211 Monarch Way |
| Street2: | Suite 300 |
| City*: | Norfolk |
| County: | |
| State*: | VA: Virginia |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip/ Postal Code*: | 23508-2561 |

Phone Number*: 757-683-2690 Fax Number: E-Mail*: rheller@odu.edu

Credential, e.g., agency login: eRA Commons User Name(s)

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

Degree Type: PhD Degree Year: 1989

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1245-biosketch heller.pdf |
| **Attach Current & Pending Support:** | |

## PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Adil Middle Name Last Name*: Daud Suffix: M.B.B.S.

| | |
|---|---|
| Position/Title*: | HS Clinical Professor and Co-Director |
| Organization Name*: | University of California, San Francisco |
| Department: | Medicine & Melanoma Clin. Rsch |
| Division: | |
| Street1*: | 3333 California St. |
| Street2: | Suite 435 |
| City*: | San Francisco |
| County: | |
| State*: | CA: California |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip/ Postal Code*: | 94143-1241 |

Phone Number*: 415-502-0690 Fax Number: E-Mail*: adaud@medicine.ucsf.edu

Credential, e.g., agency login: eRA Commons User Name(s)

Project Role*: Other (Specify) Other Project Role Category: Consortium Co-Investigator

Degree Type: M.B.B.S. Degree Year: 1986

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1246-Daud Biosketch.pdf |
| **Attach Current & Pending Support:** | |





Tracking Number: GRANT11598944
Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

**PROFILE - Senior/Key Person**

Prefix: Dr. First Name*: Lawrence Middle Name Last Name*: Fong Suffix: M.D.

| | |
|---|---|
| Position/Title*: | Associate Professor |
| Organization Name*: | University of California, San Francisco |
| Department: | Medicine |
| Division: | Heme/Onc. |
| Street1*: | 3333 California St. |
| Street2: | Suite 435 |
| City*: | San Francisco |
| County: | |
| State*: | CA: California |
| Province: | |
| Country*: | USA: UNITED STATES |
| Zip / Postal Code*: | 94143-1241 |

Phone Number*: 415-514-3160 Fax Number: E-Mail*: lfong@medicine.ucsf.edu

Credential, e.g., agency login: eRA Commons User Name(s)

Project Role*: Other (Specify) Other Project Role Category: Consortium Co-Investigator

Degree Type: MD Degree Year: 1992

| | File Name |
|---|---|
| **Attach Biographical Sketch*:** | 1247-FONG biosketch.pdf |
| **Attach Current & Pending Support:** | |

Program Director/Principal Investigator (Last, First, Middle): Heller, Richard

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Richard Heller | Director and Professor |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>eRA Commons User Name(s) | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| Oregon State University (Corvallis, OR) | B.S. | 1975-79 | Microbiology |
| Long Island Univ., C.W. Post College (Greenvale, NY) | M.S. | 1982-84 | Health Sciences |
| University of South Florida (Tampa, FL) | Ph.D. | 1985-89 | Medical Sciences- Medical Microbiology and Immunology |
| University of South Florida (Tampa, FL) | Post-Doctoral | 1989-90 | Medical Microbiology and Immunology |

### A. PERSONAL STATEMENT

My laboratory has been focused on developing efficient delivery systems for plasmid DNA and chemotherapeutics. We have been instrumental in moving the use of electroporation (elelctrotransfer) from the laboratory benchtop to preclinical studies and eventually clinical studies. I have over 20 years experience in evaluating effects of electric pulses on biological systems. I am recognized as a pioneer in this field and have eighteen (22 total) issued US patents (additional 10 pending) related to the use of electric fields. A major focus of my research is to develop *in vivo* delivery procedures for non-viral gene transfer for enhancing immunotherapy for cancer treatment. I have previously utilized electrotransfer to deliver a variety of molecules including chemotherapeutic agents and plasmid DNA to a variety of tissue targets including tumors (melanoma, hepatocellular carcinoma, pancreatic, etc), skin, muscle, liver and heart. It is clear that the use of electric fields is now being used by many groups and the basic principles for delivery are relatively straight forward; however, there are many aspects of this approach that still need to be better understood and utilized to enhance the effectiveness. One major area that warrants additional study is the use of electrotransfer to deliver plasmids with better control over the expression characteristics. This is possible because there are many variables that can be manipulated when performing elelctrotransfer that can directly affect delivery and the resulting expression profile. While it may seem complicated to manipulate a larger number of variables, it is these variables that allow us to achieve better control over the expression profile and distribution leading to better therapeutic outcomes. This is an important breakthrough and is instrumental in facilitating the use of gene transfer for immunotherapy applications. It should also be noted, that a critical understanding of these variables is important to achieve the right expression profile. My laboratory is well suited for this task. One of the major research areas my laboratory has been focusing on is developing immune therapy approaches by delivering plasmids encoding cytokines directly to tumors. This experience is directly related to this project. I also have experience in translating these technologies and was involved in the first-in-human study utilizing *in vivo* electroporation mediated delivery of a plasmid. My expertise in the use and development of puse electric field protocols and experience in translational research makes me well suited to serve as Prinicple investigator on this project.

### B. POSITIONS

1990-1996 **Assistant Professor**, Department of Surgery, Joint Appointments - Department of Medical Microbiology and Immunology; Department of Chemical Engineering; University of South Florida, Tampa, Florida.

1997-1999 **Director, Division of Surgical Research**, Department of Surgery, University of South Florida, Tampa, Florida.
1996-2002 **Associate Professor**, Department of Surgery, Joint Appointments - Department of Medical Microbiology and Immunology; Department of Chemical Engineering; University of South Florida, Tampa, Florida.
1999-2008 **Co-Director**, Center for Molecular Delivery, Tampa, FL
2002-2004 **Professor**, Department of Surgery, Joint Appointments - Department of Medical Microbiology and Immunology; Department of Chemical Engineering; University of South Florida, Tampa, Florida.
2004-2008 **Professor**, Department of Medical Microbiology and Immunology (2006 name change to Molecular Medicine), Joint Appointment - Department of Chemical Engineering; University of South Florida, Tampa, Florida.
2008-Present **Professor**, Department of Medical Diagnostics and Translational Sciences, College of Health Sciences; Old Dominion University, Norfolk, VA
2008-Present **Director,** Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA.
2008-Present **Adjunct Professor,** Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, VA

**AWARDS AND HONORS**

2003 Biotechnology Achievement Award, Univ. of South Florida, Health Sciences Center
2004 Iwao Yasuda Award from Society for Physical Regulation in Biology and Medicine
2004 Society for In Vitro Biology Fellow Award
2005 Society for In Vitro Biology Distinguished Service Award
2006 Biotechnology Achievement Award, Univ. of South Florida, Health Sciences Center
2007 Tampa Bay Business Journal Health Heroes Finalist
2013 Frank Reidy Award for Outstanding Achievements in Bioelectrics

**C. SELECTED PEER-REVIEWED PUBLICATIONS** (selected from over 90 peer reviewed manuscripts)
**Most relevant to the current application (in chronological order)**

Gilbert, R., Jaroszeski, M. J., **Heller, R**. Novel electrode designs for electrochemotherapy. Biochemica Biophysica Acta 1334:9-14, 1997.

Niu, G., **Heller, R**., Catlett-Falcone, R., Coppola, D., Jaroszeski, M., Dalton, W., Jove, R. and Yu, H. Gene therapy with dominant-negative Stat3 suppresses growth of the murine melanoma B16 Tumor in vivo. Cancer Research 59:5059-5063, 1999.

Lucas, ML, Heller, L., Coppola, D. and **Heller, R.** IL-12 Electrogene-therapy for the successful treatment of established subcutaneous B16F10 melanoma. Mol. Therapy, 5(6):668-675, 2002.

Lucas, M.L. and **Heller, R**. IL-12 gene therapy using an electrically mediated non-viral approach reduces metastatic growth of melanoma. DNA and Cell Biology 22(12):755-763., 2003.

Heller, L.C., Merkler, K., Westover, J., Cruz, Y., Coppola, D., Benson, K. Daud, A. and **Heller, R**. Evaluation of toxicity following electrically mediated interleukin-12 gene delivery in a B16 mouse melanoma model. Clinical Cancer Research, 12(10):3177-83, 2006.

Heller, L.C. and **Heller, R**. In vivo electroporation for gene therapy. Human Gene Therapy, 17(9):890-897, 2006.

Daud, A.I., DeConti, R.C. Andrews, S., Urbas, P., Riker, A.L., Sondak, V.K., Munster, P.**N.**, Sullivan, D.M., Ugen, K.E., Messina, J.L., and **Heller, R**. First human trial of *in vivo* electroporation mediated gene transfer: safety profile and tumor regression after IL-12 plasmid delivery to metastatic melanoma patients. Journal Clinical Oncology, 26(36): 5896–5903, 2008.

Heller, L.C., **Heller, R.** Electroporation Gene Therapy Preclinical and Clinical Trials for Melanoma, Curr Gene Ther, 10:312-317, 2010.

Heller, L.C., Cruz, Y.L., Ferraro, B., Yang, H. and **Heller, R.** Plasmid injection and application of electric pulses alter endogenous mRNA and protein expression in B16.F10 mouse melanomas. Cancer Gene Therapy, 17:864-871, 2010.

Marrero, B and **Heller, R.** The use of an in vitro 3D melanoma model to predict in vivo plasmid transfection using electroporation. Biomaterials, 33:3036-3046, 2012

**Additional related publications**

Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, **Heller R**, Coppola D, Dalton W, Jove R, Pardoll D, Yu H. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med. 10(1):48-54, 2004.

Heller, L.C., Jaroszeski, M.J., Coppola, D., McCrae, A.N., Hickey, J., and **Heller, R**. Optimization of Cutaneous Electrically Mediated Plasmid DNA Delivery Using a Novel Electrode. Gene Therapy 14(3):275-80, 2007.

**Heller, R**., Cruz, Y., Heller, L.C., Gilbert, R. and Jaroszeski, M.J. Electrically Mediated Delivery of Plasmid DNA to the Skin Using a Multi Electrode Array. Human Gene Therapy, 21:357-362, 2010.

Ferraro, B., Cruz, Y.L., Baldwin, M., Coppola, D. and **Heller, R.** Increased perfusion and angiogenesis in a hindlimb ischemia model with plasmid FGF-2 delivered by non-invasive electroporation. Gene Therapy, 17:763-769, 2010.

Hargrave, B., Downey, H., Strange, R. Jr., Murray, L., Cinnamond, C., Lundberg, C., Israel, A., Chen, Y-J., Marshall, W. Jr. and **Heller R.** Electroporation-mediated gene transfer directly to the swine heart. Gene Therapy, 20:151-157, 2012.

**Patents** (Related to application out of 22)

U.S. Patent 8,026,223, 9/27/11, Treating malignant tumors with high field strength electroporation of plasmids encoding IL-12. Inventors: **Heller R**, Lucas, ML

U.S. Patent 7,879,610, 2/1/11, Electroporation system and method for facilitating entry of molecules into cells in vivo. Inventors: **Heller R**, Gilbert R, Jaroszeski

U.S. Patent 7,781,195, 8/24/10, Electroporation device. Inventors: **Heller R**, Jaroszeski, MJ, Gilbert R.

U.S. Patent 7,769,440, 8/2/10, Electromanipulation device and method. Inventors: Hoff, AM, Gilbert, R, **Heller, R** and Jaroszeski, MJ

U.S. Patent 7,668,592 2/23/10: Electroporation and electrophoresis system and method for achieving molecular penetration into cells *in vivo*. Inventors: **Heller R**, Gilbert R, Jaroszeski MJ, Heller LC, Lucas ML.

U.S. Patent 6,314,316 B1, 11/06/01, Nonpenetrating Electroporation Device and Method. Inventors: Gilbert, R, **Heller, R**, Jaroszeski, MJ,

U.S. Patent 5,702,359,12/30/97, Needle Electrodes for Mediated Delivery of drugs and Genes. Inventors: Dev, SB, Hofmann, GH, **Heller, R.,** Gilbert, R., Jaroszeski, M.

**D. RESEARCH SUPPORT**

1 R01 CA115955-01 (Role on Project - PI) 4/25/08-2/28/15 (NCE)
National Institutes of Health
Therapeutic Potential of IL-15 Plasmid Delivery to Tumors Using Electroporation
The major goal of this project is to examine the use of non-viral gene transfer approach that can be used for immunotherapy protocols and its potential as a treatment of solid tumors. The specific research proposed in this application is intended to determine if the using in vivo electroporation to deliver a plasmid DNA coding for the cytokine IL-15 can be effective in treating metastatic melanoma.

1 R33 HL089017-01 ((Role on Project - PI) 8/1/08-11/30/14 (NCE)
National Institutes of Health
Electro Gene Transfer for Coronary Artery Disease
The major goal of this project is to evaluate a non-viral deliver system to perform gene therapy directly to the heart. The work involves developing electroporation protocols that could be used to efficiently deliver plasmid DNA to the heart for potentially treating ischemia.

Completed in last three years

11059006 (Role on Project - PI) 9/06/11-10/05/13
U.S. Army Medical Research Acquisition Activity (TATRC)

Program Director/Principal Investigator (Last, First, Middle): Heller, Richard

Bioelectrics Research for Casualty Care and Management
The major goal of this project is to develop methods to accelerate wound healing and/or reduce or eliminate the potential for wound infections. The approach will utilize a combination of electrically mediated gene delivery, platelets activated by nanosecond pulse electric fields and air plasma. This is a continuation of a previous grant funded by TATRC

(Role on Project - PI) 7/1/11-12/30/13
Private Support

Flu Vaccine Technology Program
The major goal of this project is to test deliver of linear DNA cassettes with electroporation as a potential DNA vaccine for influenza.

(Role on Project - PI) 1/01/12-9/30/13
Private Support

Development and Commercialization of Pulse Power Technology
Major goal of this project is to develop pulse generators and electrode systems for efficiently applying nanosecond pulse electric fields.

(Role on Project - PI) 1/01/12-3/31/13
Private Support

A Nonchemical Approach For Creating Platelet Gel
The major goal of this project is to develop an efficient system for creating platelet gels to be utilized in accelerating wound healing.

09036003 (Role on Project - PI) 8/02/10-9/01/11
U.S. Army Medical Research Acquisition Activity (TATRC)
Bioelectrics Research for Casualty Care and Management
The major goal of this project is to develop methods to accelerate wound healing and/or reduce or eliminate the potential for wound infections. The approach will utilize a combination of electrically mediated gene delivery, platelets activated by nanosecond pulse electric fields and air plasma.

(Role on Project - PI) 9/01/09-12/31/11
Private Support

Long Term Inducible Expression of Therapeutic Genes Following Electroporation into Rat Muscle.
The major goal of this study is to evaluate appropriate electroproation parameters and devices to obtain long term expression following delivery of plasmid DNA to muscle in a rat model.

1 R01 EB005441 (Role on Project - PI) 9/01/06-6/31/11
National Institutes of Health
Electroporation System for Cutaneous Gene Transfer
The major goal of this study is to evaluate a new electrode system for efficient delivery of plasmid DNA to the skin.

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| **Adil I. Daud, M.B.B.S** | Clinical Professor of Medicine and Dermatology |
| eRA COMMONS USER NAME<br>eRA Commons User Name(s) | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| St. Francis De Sales College, Nagpur, India | HSSC | 1980 | General Science |
| Government Medical College, Nagpur, India | M.B.B.S. | 1986 | Medicine |

### A. Personal Statement

I have extensive experience in clinical trial design and drug development especially in melanoma. At Memorial Sloan Kettering Cancer Center, I trained with Drs David Spriggs, Carol Aghajanian and Paul Chapman gaining a broad understanding of experimental therapeutics and melanoma clinical research. Over the next 7 years, at the Moffitt Cancer Center, I designed and conducted numerous Phase I and II clinical trials collaborating with my colleagues Drs Richard Heller, Richard Jove, Hua Yu, Dmitry Gabrilovich, Timothy Yeatman, Pamela Munster and Daniel Sullivan, many with first in man or novel compounds and mostly in patients with melanoma. At Moffitt Cancer Center, I set up a Florida-wide network of collaborators to treat patients and biopsy liver tumors and collect and analyze RNA from these to develop a signature for response. One of my most productive collaborations has been with Dr Heller who had observed dramatic activity of plasmid IL-12 EGT in the B16 mouse melanoma model. At UCSF, I lead the melanoma program and several early phase and melanoma specific clinical trials as well as collaborating with my colleagues Lawrence Fong, Martin McMahon, Boris Bastian and others within and outside UCSF to develop new agents for this disease.

### B. Positions and Honors

**Positions and Employment**

| | |
|---|---|
| 1987-1990 | Postdoctoral Fellow, Laboratory of F. Merlin Bumpus, PhD and Ahsan Husain, PhD, Cleveland Clinic Foundation, Cleveland, OH |
| 1990-1994 | Research Assistant Staff, Laboratory of Loren Field, PhD, Krannert Institute of Cardiology. Indiana University Medical Center, Indianapolis, IN |
| 1994-1997 | Internship and Residency in Internal Medicine, Indiana University Medical Center, Indianapolis, IN |
| 1997-2000 | Fellowship in Hematology/Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY. |
| 2000-2001 | Clinical Attending Physician, Developmental Chemotherapy, Memorial Sloan-Kettering Cancer Center, New York, NY |
| 2001-2005 | Assistant Professor, Cutaneous Oncology and GI Comprehensive Program, H. Lee Moffitt Cancer Center, Tampa, FL |
| 2006-2008 | Associate Professor, Cutaneous Oncology, GI and Experimental Therapeutics Program, H Lee Moffitt Cancer Center, Tampa, FL |
| 2006-2008 | Director, Moffitt Clinical Research Network. |
| 2008-2010 | Clinical Associate Professor, Director, Melanoma Clinical Research, University of California, San Francisco, CA |
| 2010-Present | HS Clinical Professor of Medicine, Co-Director Melanoma Program, University of California, San Francisco, San Francisco CA |

**Honors and Awards**

| | |
|---|---|
| 1986 | Honors in Pediatrics, Government Medical College, Nagpur, India |

| | |
|---|---|
| 2000 | Young Investigator Award, American Society for Clinical Oncology for project "Regulation of mRNA stability by Elongation factor I. |
| 2005-7 | Clinical Trial Award: Highest Number of Patients Enrolled in clinical trials at Moffitt Cancer Center |
| 2007-11 | Best Doctors in America Award, Castle Connolly |

**Committees**

| | |
|---|---|
| 2002-present | Member of the NCCN Melanoma Panel |
| 2003-2008 | Scientific Review Committee, National Gene Vector Laboratory |
| 2003-2007 | Protocol Review and Monitoring Committee, Moffitt Cancer Center |
| 2004-2008 | Institutional Review Board, University of South Florida |
| 2008-Present | Protocol Review Committee, University of California, San Francisco |
| 2008-Present | Data Safety and Monitoring Committee, University of California, San Francisco |
| 2009-Present | Chair, Skin Endocrine Gyn-Onc Cancer Center Site Committee |
| 2008-Present | Scientific Advisory Board, Gene Therapy Resource Program, NHLBI |

**Journal Editorial Boards**

Journal of Clinical Oncology, Human Vaccines and Immunotherapy. Ad Hoc Reviewer for numerous journal including Clinical Cancer Research, Investigational New Drugs, British Journal of Cancer, Molecular Cancer Therapeutics, Cancer, Cancer Investigations American Journal of Pathology and Journal of Oncology.

**C. Selected Peer-Reviewed Publications (Selected from over 70 publications)**


1. **Daud AI**, Bumpus FM and Husain A. Selective expression of Angiotensin II receptors on atretic follicles in the rat ovary. *Endocrinology. 1988 Jun;122(6):2727-34.*

2. **Daud AI**, Bumpus FM and Husain A. Characterization of ACE containing follicles during the rat estrus cycle and effect of ACE inhibitors on Ovulation. *Endocrinology. 1990 June; 126(6):2927-35.*

3. **Daud AI**, Bumpus FM and Husain A. Angiotensin II: Does it have a direct obligate role in ovulation? *Science 1991 vol 245:870.*

4. **Daud AI**, Lanson N, Claycomb W and Field LJ. Identification of SV-40 T-antigen-associated proteins in cardiomyocytes from transgenic mice. *Am J Physiol 1993 May; 264 (5 Pt 2):H1693-700.*

5. **Kim KK***, **Daud AI***, Wong SC, Pajak L, Tsai SC, Wang HW, Henzel W and Field LJ. Mouse RAD 50 Has limited epitopic homology to P53 and is expressed in the mouse myocardium. *J Biol Chem. 1996 15;271(46):29255-64. (**co-first authors**)*

6. Aghajanian C, Soignet S, Dizon, DS, Pien CS, Adams J, Elliott PJ, Sabbatini P, Miller V, Hensley ML, Pezzulli S, Canales C, **Daud AI**, and Spriggs DR. Phase I Trial of the Novel Proteasome Inhibitor PS341 in Advanced Solid Tumor Malignancies. *Clin Cancer Res 2002 8: 2505-2511.*

7. **Daud AI**, Valkov N, Centeno B, Derderian J, Sullivan P, Munster P, Urbas P, Deconti RC, Berghorn E, Liu Z, Hausheer F, Sullivan D, Phase II trial of Karenitecin in patients with malignant melanoma: Clinical and translational study. *Clin Cancer Res. 2005 Apr 15;11(8):3009-16.*

8. Munster PN, Marchion D, Bicaku E, Schmitt M, Padilla B, Stauffer P, Garrett C, Chiappori A, Lush R, Sullivan D, and **Daud AI.** Phase I trial of histone deacetylase inhibition by valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors: A clinical and translational study. *J Clin Oncol 2007 May 20;25(15):1979-85*

9. **Daud AI,** Mirza N, Lenox B, Andrews S, Urbas P, Gao GX, Lee JH, Sondak VK, Riker AI, Deconti RC, Gabrilovich D. Phenotypic and functional analysis of DC and clinical outcome in patients with high risk melanoma treated with adjuvant GM-CSF. *J Clin Oncol. 2008 Jul 1;26(19):3235-41*

10. **Daud AI,** DeConti R, Andrews SM, Sondak VK, Riker AI and Heller R. First in Man Phase I trial of IL-12 plasmid Electroporation in patients With Malignant Melanoma. *J Clin Oncol* November 24, 2008, 15.6794

11. **Daud AI,** Dawson J, DeConti RC, Bicaku E, Marchion D, Bastien S, Neuger A, Hausheer FA, Lush R, Sullivan DM and Munster PN. Potentiation of a Topoisomerase I inhibitor, Karenitecin, by the Histone Deacetylase Inhibitor Valproic Acid in Melanoma: Translational and Phase I/II Clinical Trial. *Clin Cancer Res. 2009 Apr 1;15(7):2479-87.*

12. **Daud AI**, Xu C, Hwu WJ, Urbas P, Andrews S, Papadopoulos NE, Floren LC, Yver A, Deconti RC, Sondak VK Pharmacokinetic/pharmacodynamic analysis of adjuvant pegylated interferon alpha-2b in patients with resected high-risk melanoma. *Cancer Chemother Pharmacol. 2010 May 28.*

13. **Daud AI**, Krishnamurthy S, Saleh M et al. Phase I Study of Bosutinib, a Src/Abl Tyrosine Kinase Inhibitor, Administered to Patients With Advanced Solid Tumors. *Clin Cancer Res. 2012 Feb 15;18(4):1092-100.*

14. Flaherty KT, Infante JR, **Daud A**, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med. 2012 Nov;367(18):1694-703.*

15. **Hamid O*, Robert C*, Daud A*,** Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss- Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A. **(Co-first Authors)** *N Engl J Med. 2013 Jun 2.*

ACTIVE

Private Support (Daud) 12/15/2011 – 12/15/2015

This is a Phase II Trial of intratumoral pIL-12 electroporation in advanced stage cutaneous and in transit malignant melanoma

Private Support (Daud) 7/01/2011 – 6/30/2016

This is a Phase I Study of Single Agent MK-3475 in Patients with Progressive Locally Advanced or Metastatic Carcinomas and Melanoma

Private Support 01/01/09 – 12/31/14

This is a Phase 1 Dose-Escalation Study of SCH 900776 as Monotherapy and in Combination with Gemcitabine in Subjects with Advanced Solid Tumors or Lymphoma

Private Support (Daud) 07/01/2013 – 06/30/2014

*A Phase I/II trial of the MEK inhibitor GSK 1120212 + PI3Kinase Inhibitor GSK 2126458 in BRAF wild type melanoma (with or without NRAS mutation)*

COMPLETED

Private Support (Daud) 02/26/10 – 02/25/13

This clinical trial is A Randomized Discontinuation Study of XL184 in Subjects with Advanced Solid Tumors

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Fong, Lawrence | Associate Professor of Medicine |
| eRA COMMONS USER NAME<br>eRA Commons User Name(s) | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Columbia University, New York, NY | BA | 1984-1988 | Economics |
| Stanford University, Stanford, CA | MD | 1988-1992 | Medicine |
| University of Washington, Seattle, WA | Residency | 1992-1994 | Internal Medicine |
| Stanford University, Stanford, CA | Fellowship | 1994-1997 | Oncology |

### A. Personal Statement

I have focused on modulating immune responses to tumors for cancer immunotherapy throughout my career. After completing Oncology Fellowship at Stanford, I complete post-doctoral training with Drs. Ed Engleman and Mark Davis focused on tumor immunology. I then began my independent research program at UCSF and have continued to focus on how the immune system interacts with cancer as well as developing tumor immunotherapies in both humans and mouse models. We described the immunogenicity of prostate acid phosphatase (PAP), which is the target antigen for sipuleucel-T, now an FDA-approved immunotherapy for prostate cancer. We were also involved with the first-in-man clinical trials with ipilimumab, an anti-CTLA4 antibody that is now FDA approved for melanoma. We continue to investigate how immunotherapies such as CTLA-4 blockade and vaccines can impact anti-tumor immunity in patients. We also utilize autoimmune prone mouse models to define tumor-associated antigens, which may represent novel vaccine candidates. I have also served on multiple NIH study sections and on the NCI Steering Committees for Genitourinary Cancers and Investigational Drugs-Immunotherapy. I have also served on the education program committee of ASCO, including serving as track chair (Developmental Therapeutics) and as current faculty for the annual AACR/ASCO Vail Methods in Clinical Research Workshop. I am also the site (UCSF) PI for the NCI Cancer Immunotherapy Trials Network (CITN).

### B. Positions and Honors

**Positions and Employment**

| | |
|---|---|
| 1996-2001 | Post-Doctoral Fellow, Department of Pathology, Stanford University School of Medicine |
| 2001-2002 | Acting Assistant Professor, Department of Pathology, Stanford University School of Medicine |
| 2002-2008 | Assistant Professor, Department of Medicine, Division of Heme/Onc, University of California, San Francisco |
| 2008- | Associate Professor, Department of Medicine, Division of Heme/Onc, University of California, San Francisco |

**Other Experience and Professional Memberships**

| | | |
|---|---|---|
| 1996 | American Board of Internal Medicine | Diplomat |
| 1997,2007 | American Board of Internal Medicine, Medical Oncology | Diplomat |
| 2004-2005 | NIH, NCI P01 Review Panel | Ad Hoc Member |
| 2007-2011 | Journal of Clinical Oncology | Editorial Board |
| 2009-2011 | NIH, NCI CII Study Section | Ad Hoc Member |
| 2006-present | NIH, NCI Special Emphasis Panels - Clinical P01 | Ad Hoc Member |
| 2007-present | NIH, NCI CONC Study Section | Member |

| | | |
|---|---|---|
| 2011-present | Cancer Vaccine Collaborative (CVC) | Coordinating and Review Committee |
| 2012-present | NCI Investigational Drug Steering Committee (IDSC) | Immunotherapy Task Force |
| 2012-present | NCI Genitourinary Cancers Steering Committee (GUSC) | Elected Member |
| 2012-present | Journal of Immunotherapy of Cancer | Associate Editor |
| 2012-present | Cancer Immunology Research | Senior Editor |

**Honors**

| | |
|---|---|
| 1990 | Stanford Alumni Medical Scholar |
| 1993 | Alpha Omega Alpha |
| 1997 | American Society of Clinical Oncology Young Investigator Award |
| 1997 | American Cancer Society Post-Doctoral Fellowship Award |
| 1997 | American Association for Cancer Research AFLAC Award |
| 2002 | American Association for Cancer Research Scholar-in-Training Award |
| 2003 | V Foundation Scholar |

**C. Selected peer-reviewed publications (from over 40 publications)**


1. **Fong L**, Ruegg C, Brockstedt DG, Engleman EG, Laus R. Cutting Edge: Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization: Implications for prostate cancer immunotherapy. J Immunol 159:3113-3118, 1997. [http://www.ncbi.nlm.nih.gov/pubmed/9317107]
2. **Fong L**, Hao Y, Rivas A, Benike C, Yuen A, Fisher G, Davis MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci 98(15):8809-14, 2001. [http://www.ncbi.nlm.nih.gov/pubmed/11427731]
3. Small EJ, Tchekmedyian NS, Rini BI, **Fong L**, Lowy I, Allison JP. A Pilot Trial of CTLA-4 Blockade with Human Anti-CTLA-4 in Patients with Hormone-Refractory Prostate Cancer. Clin Cancer Res 13(6):1810-5, 2007. [http://www.ncbi.nlm.nih.gov/pubmed/17363537]
4. Fasso M, Waitz R, Rim T, Hou Y, Greenberg NM, Shastri N, **Fong L**, Allison JP. SPAS-1 (stimulator of prostatic adenocarcinoma specific T cells-1)/SH3GLB2: A prostate tumor antigen identified by CTLA-4 blockade. Proc Natl Acad Sci 105(9):3509-14, 2008. [http://www.ncbi.nlm.nih.gov/pubmed/18303116]
5. Hou Y, Kavanagh B, **Fong L**. Distinct CD8+ T cell repertoires primed with agonist and native peptides derived from a tumor-associated antigen. J Immunol 180(3):1526-34, 2008. [http://www.ncbi.nlm.nih.gov/pubmed/18209048]
6. Kavanagh B, O'Brien S, Hou Y, Weinberg V, Rini B, Allison JP, Small EJ, **Fong L**. CTLA4 blockade expands FoxP3+ regulatory and activated effector CD4+ T cells in a dose-dependant fashion. Blood 112(4):1175-83, 2008. [http://www.ncbi.nlm.nih.gov/pubmed/18523152]
7. **Fong L**, Kwek SS, O'Brien S, Kavanagh B, Weinberg V, Lin A, Rosenberg J, Ryan CJ, McNeel D, Rini B, Small EJ. Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. Cancer Res 69, 609-615, 2009. [http://www.ncbi.nlm.nih.gov/pubmed/19147575]
8. Hou Y, Devoss J, Dao V, Kwek SS, Simko J, McNeel D, Anderson MS, **Fong L**. An aberrant prostate antigen-specific immune response causes prostatitis in mice and is associated with chronic prostatitis in humans. J Clin Invest 119: 2031-2041, 2009. [http://www.ncbi.nlm.nih.gov/pubmed/19603556]
9. Shum AK, DeVoss J, Tan CL, Hou Y, Johannes K, O'Gorman CS, Jones KD, Sochett EB, **Fong L**, Anderson MS. Identification of an autoantigen demonstrates a link between interstitial lung disease and a defect in central tolerance. Science- Transl Med, 1:9ra20, 2009. [http://www.ncbi.nlm.nih.gov/pubmed/20368189]
10. Ryan CJ, Smith MR, **Fong L**, Rosenberg JE, Kantoff P, Raynaud F, Martins V, Lee G, Kheoh T, Kim J, Molina A, Small EJ. Phase I clinical trial of the CYP 17 inhibitor abiraterone acetate (CB7630), Demonstrating clinical activity in castration-resistant prostate cancer patients with prior ketoconazole therapy. J Clin Oncol, 28:1481-1488, 2010. [http://www.ncbi.nlm.nih.gov/pubmed/20159824]
11. Chung K, Gore I, **Fong L**, Vennok A, Beck SB, Dorazio P, Crisciteiello PJ, Healey DI, Huang B, Gomez-Navarro J, Saltz LB. A Phase II Study of the Anti-CTLA4 Monoclonal Antibody, Tremelimumab, in Patients

With Refractory Metastatic Colorectal Cancer. J Clin Oncol, 28(21):3485-90, 2010. [http://www.ncbi.nlm.nih.gov/pubmed/20498386]
12. Moltzahn F, Olshen A, Haehner L, Peek A, **Fong L**, Stoppler H, Simko J, Hilton, J, Carroll P, Blelloch R. Microfluidic based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in sera of prostate cancer patients. Can Res, 15;71(2):550-60, 2011.[http://www.ncbi.nlm.nih.gov/pubmed/21098088]
13. Cha E, **Fong L.** Immunotherapy for Prostate Cancer: Biology and Therapeutic Approaches. J Clin Oncol, 29(27):3677-85, 2011. [http://www.ncbi.nlm.nih.gov/pubmed/21825260].
14. Kwek S, Cha E, **Fong L**. Unmasking the immune recognition of prostate cancer with CTLA-4 blockade. Nat Rev Cancer, 12(4):289-97, 2012. [http://www.ncbi.nlm.nih.gov/pubmed/22378189].
15. Kwek S, Dao V, Roy R, Hou Y, Alajajian D, Simko JP, Small EJ, **Fong L**. Diversity of antigen-specific responses induced in vivo with CTLA-4 blockade in prostate cancer patients. J Immunol, 189(7):3759-66, 2012. [PMID:22956585]

**D. Research Support**

**Ongoing Research Support**

1R01 CA136753-A01 Fong (PI) 3/1/09-2/28/14
NIH/NCI
Immunotherapy of Prostate Cancer
The goal of this project is to develop immunomodulatory treatment for prostate cancer by performing a clinical trial with CTLA4 blockade.
Role: Principal Investigator

1U01CA154967-01 Cheever (PI) 9/2/10-8/30/15
NIH/NCI
Cancer Immunotherapy Trials Network
The goal of this project is to develop a network of centers to perform immunotherapy trials in cancer.
Role: Site Principle Investigator

1R01 CA163012-01A1 Fong (PI) 9/1/12-6/30/17
Prostatitis and Prostate Cancer Development
This proposal will determine whether chronic inflammation in the prostate promotes the development of prostate cancer.
Role: Principal Investigator

**Completed Research Support**

Fong (PI) 6/1/09-5/30/13
Private Support
Neoadjuvant sipuleucel-T for Localized Prostate Cancer
The goal of this project is examine the capacity of sipuleucel-T to induce an antigen-specific immune response in the primary prostate tumor and gland.
Role: Principal Investigator

Private Support Small (PI) 1/1/10-12/31/12
The goal of this grant is to provide infrastructure and scientific support for clinical research in prostate cancer.
Role: Co-Investigator

Private Support McNeel (PI) 6/1/11-5/30/13
This is a clinical trial investigating the immunogenicity of a DNA vaccine in patients with biochemically relapsed prostate cancer following definitive therapy performed at the University of Wisconsin and UCSF.
Role: Site Principle Investigator, Co-Investigator

R01 HL080074 Cabana (PI) 8/27/04-6/30/16

NIH/Inst for Health Policy Studies
Trial of Infant Probiotic Supplementation to Prevent Asthma
The goal of the project is to evaluate the efficacy of daily infant probiotic supplementation in preventing the development of early markers for asthma.
Role: Co-Investigator

Private Support — Stock (PI) — 4/1/04-1/31/10

Pancreatic Islet Cell Transplantation
The purpose of this project is to develop and apply novel methods for detecting auto- and allo-reactive T cells in the setting of islet cell transplantation.
Role: Co-Investigator (Immune Monitoring Core Leader)

R01 CA102303 Fong (PI) — 4/1/04-3/31/09
NIH/NCI
Dendritic Cell Immunotherapy for Colorectal Cancer
The objective of this project is to evaluate novel approaches to generating antigen-pulsed dendritic cells (DC) for the treatment of colorectal cancer.
Role: Principal Investigator

U19 AI056388 Wofsy (PI) — 9/30/03-3/31/10
NIH/NIAID
UCSF Autoimmune Center of Excellence (ACE)
Core A- Immune Monitoring Core
The role of this core is to develop novel immune monitoring assays to characterize patients with autoimmune disease. The core will also perform immune monitoring assays in the context of clinical trials supported by ACE awards.
Role: Co-Investigator (Immune Monitoring Core Leader)

P50 CA89520 Shuman (PI) — 9/25/00-8/31/06
NIH/NCI
UCSF Prostate Cancer SPORE
Project 6
The objective of this project is to develop novel immunotherapies for prostate cancer, and to characterize immune responses in prostate cancer patients receiving these treatments.
Role: Co-Investigator (Project Co-Leader, Project 3)

K23 CA82584 Fong (PI) — 7/1/99 – 6/30/04
NIH/NCI
Dendritic Cell Immunotherapy for Lung and Colon Cancer
The objective of this project is to evaluate the potential utility of utilizing antigen-pulsed dendritic cells in the treatment of lung and colorectal cancer.
Role: Principal Investigator

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

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

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Enter name of Organization:** Old Dominion University

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **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. | Richard | | Heller | Ph.D | PD/PI | Inst. Base Salary | Calendar Months | | | 36,301.00 | 9,801.00 | 46,102.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **46,102.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| 1 | Post Doctoral Associates | 12.00 | | | 47,431.00 | 24,809.00 | 72,240.00 |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 2 | Technicians | 13.00 | | | 46,248.00 | 24,128.00 | 70,376.00 |
| **3** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **142,616.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **188,718.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **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** | |
| **Additional Equipment:** File Name: | |

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | 4,000.00 |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | **4,000.00** |

| **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** | |

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **Budget Period:** 1

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 39,300.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | 61,931.00 |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8 . Animals | 6,500.00 |
| 9 . Cost Center Charges | 3,000.00 |
| **Total Other Direct Costs** | **110,731.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **303,449.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 53.00 | 266,518.00 | 141,254.00 |
| | | **Total Indirect Costs** | **141,254.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): ONR, Deborah K. Rafi, 703-696-5641

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **444,703.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Enter name of Organization:** Old Dominion University

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period: 2**

**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. | Richard | | Heller | Ph.D | PD/PI | Inst. Base Salary | Calendar Months | | | 36,301.00 | 9,801.00 | 46,102.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **46,102.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| 1 | Post Doctoral Associates | 12.00 | | | 48,854.00 | 26,714.00 | 75,568.00 |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 2 | Technicians | 13.00 | | | 47,635.00 | 25,574.00 | 73,209.00 |
| **3** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **148,777.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **194,879.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period:** 2

**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** | |

**Additional Equipment:** File Name:

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | 4,000.00 |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | **4,000.00** |

| **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** | |

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period:** 2

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 33,900.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | 63,050.00 |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8 . Animals | 19,500.00 |
| 9 . Cost Center Charges | 3,000.00 |
| **Total Other Direct Costs** | **119,450.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **318,329.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 53.00 | 255,279.00 | 135,298.00 |
| | | **Total Indirect Costs** | **135,298.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): ONR, Deborah K. Rafi, 703-696-5641

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **453,627.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Enter name of Organization:** Old Dominion University

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 3

**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. | Richard | | Heller | Ph.D | PD/PI | Inst. Base Salary | Calendar Months | | | 36,301.00 | 9,801.00 | 46,102.00 |
| **Total Funds Requested for all Senior Key Persons in the attached file** | | | | | | | | | | | | |
| **Additional Senior Key Persons:** | | | File Name: | | | | | | | | **Total Senior/Key Person** | **46,102.00** |

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| 1 | Post Doctoral Associates | 12.00 | | | 50,320.00 | 28,793.00 | 79,113.00 |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 2 | Technicians | 13.00 | | | 49,064.00 | 27,132.00 | 76,196.00 |
| **3** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **155,309.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **201,411.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 3**

**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** | |

**Additional Equipment:** File Name:

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | 4,000.00 |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | **4,000.00** |

| **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** | |

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 3

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 33,900.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | 64,020.00 |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8 . Animals | 8,475.00 |
| 9 . Cost Center Charges | 3,000.00 |
| **Total Other Direct Costs** | **109,395.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **314,806.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 53.00 | 250,786.00 | 132,916.00 |
| | | **Total Indirect Costs** | **132,916.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): ONR, Deborah K. Rafi, 703-696-5641

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **447,722.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Enter name of Organization:** Old Dominion University

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

**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. | Richard | | Heller | Ph.D | PD/PI | Inst. Base Salary | Calendar Months | | | 36,301.00 | 9,801.00 | 46,102.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **46,102.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| 1 | Post Doctoral Associates | 12.00 | | | 51,829.00 | 20,710.00 | 72,539.00 |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 2 | Technicians | 13.00 | | | 50,536.00 | 28,821.00 | 79,357.00 |
| **3** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **151,896.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **197,998.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

**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** | |

**Additional Equipment:** File Name:

| D. Travel | Funds Requested ($)* |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | 4,000.00 |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | **4,000.00** |

| 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** | |

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 39,300.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | 64,853.00 |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8 . Animals | 7,454.00 |
| 9 . Cost Center Charges | 3,000.00 |
| **Total Other Direct Costs** | **114,607.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **316,605.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 53.00 | 251,752.00 | 133,428.00 |
| | | **Total Indirect Costs** | **133,428.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): ONR, Deborah K. Rafi, 703-696-5641

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **450,033.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Enter name of Organization:** Old Dominion University

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

**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. | Richard | | Heller | Ph.D | PD/PI | Inst. Base Salary | Calendar Months | | | 36,301.00 | 9,801.00 | 46,102.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **46,102.00**

**B. Other Personnel**

| Number of Personnel* | Project Role* | Calendar Months | Academic Months | Summer Months | Requested Salary ($)* | Fringe Benefits* | Funds Requested ($)* |
|---|---|---|---|---|---|---|---|
| 1 | Post Doctoral Associates | 12.00 | | | 53,384.00 | 33,545.00 | 86,929.00 |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 2 | Technicians | 13.00 | | | 52,052.00 | 30,644.00 | 82,696.00 |
| **3** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **169,625.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **215,727.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

**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** | |

**Additional Equipment:** File Name:

| **D. Travel** | **Funds Requested ($)*** |
|---|---|
| 1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions) | 4,000.00 |
| 2. Foreign Travel Costs | |
| **Total Travel Cost** | **4,000.00** |

| **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** | |

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0414484650000
**Budget Type*:** ● Project ○ Subaward/Consortium
**Organization:** Old Dominion University

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 37,300.00 |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | 65,022.00 |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8 . Animals | 11,639.00 |
| 9 . Cost Center Charges | 3,000.00 |
| **Total Other Direct Costs** | **116,961.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **336,688.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . MTDC | 53.00 | 271,666.00 | 143,982.00 |
| | | **Total Indirect Costs** | **143,982.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): ONR, Deborah K. Rafi, 703-696-5641

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **480,670.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1234-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

**Old Dominion University Research Foundation**
**BUDGET JUSTIFICATION OF COST DETAIL**

SALARIES & WAGES

Principal Investigator

Faculty salary for the Principal Investigator, Dr. Richard Heller, is based on a 12-month performance period. Amounts charged are calculated as follows: salary/12 = rate per month. Rate per month x number of months in semester x percent effort in semester = charge per period. Dr. Heller's allowable salary at the start of this project will be $181,500 in accordance with NIH's salary limitation to Executive Level II of the Federal Pay Scale, and he will devote approximately [ ] months of calendar year effort to this project each year.

Calendar Months

Post Doctoral Associate

Calendar Months

[ ] of funding per year are requested to support a research assistant, Shawna Shirley, Ph.D., who will assist in all facets of the project. Dr. Shirley will oversee the animal experiments and the mouse immunological studies. She will also work with Dr. Heller in the data processing and analysis. The wages for this effort are $47,431 in year 1, and a 3% salary increase has been budgeted for each project year.

Technicians

Calendar Months

We are requesting funding for [ ] months of effort each project year for the Technician, Cathryn Lundberg, based on a 12-month performance period. Amounts charged per project period were calculated as follows: academic year salary/12 = rate per month. Rate per month x number of months in period x percent effort in period = charge per period. The technician's salary is budgeted at $43,497 in year 1, and a 3% salary increase has been budgeted as for each project year. Ms. Lundberg will work closely with Drs. Shirley and Heller and will assist in all *in vivo* experiments and subsequent assays.

Calendar Months

We are requesting funding for [ ] months of effort each project year for the Technician, Niculina Burcus, based on a 12-month performance period. Amounts charged per project period were calculated as follows: academic year salary/12 = rate per month. Rate per month x number of months in period x percent effort in period = charge per period. The technician's salary is budgeted at $40,000, and a 3% salary increase has been budgeted for each project year. Ms. Burcus will work closely with Drs. Shirley and Heller and will assist in running the laboratory assays collected during the in vivo experiments.

FRINGE BENEFITS (ONR negotiated rate dated November 1, 2013)

Principal Investigator

The fringe benefit rate applicable to university faculty salaries is 27% of the salary attributable to this project. This rate includes the university's contribution to the Virginia Supplemental Retirement System, FICA, health, life and disability insurance premiums, worker's compensation, unemployment insurance premiums, annual leave, and sick leave.

Post Doctoral Associate

FICA, unemployment insurance, worker's compensation, health, dental, life and disability insurance premiums, and annual and sick leave premiums have been budgeted for this position in accordance with current Old Dominion University Research Foundation policies.

Technicians

FICA, worker's compensation, unemployment insurance, health, dental, life, and disability insurance premiums, annual and sick leave earnings, tuition reimbursement, and a fringe benefit contribution in lieu of retirement have been budgeted for these positions in accordance with current Old Dominion University Research Foundation policies.

TRAVEL

The amount of $4,000 per year in domestic travel is requested to attend professional and research conferences to discuss and present results of this research. Part of these funds is also requested for the PI to travel to UCSF to meet with Co-Investigator.

OTHER DIRECT COSTS

Material and Supply Costs

Supplies essential to performing the laboratory and animal work include: **consumables, $8,300 (years 1-5)** is requested for the purchase of pipets, syringes, plasticware, needles, etc. **culture supplies, $7,500 (years 1-5)** is requested for the purchase of media, antibiotics, serum, etc. **chemicals,** $4,**500 (years 1-5);** is requested for the purchase of plasmids, anesthesia, scavenger, therapeutic antibodies, etc. **Immunological analysis**, $**9,500 (year 1); $10,400 (year 2); $10,400 (year 3); $9,500 (year 4) and $7,500 (year 5)** is requested for the purchase of Magpix plates, ELISA plates, ELISPOT reagents including peptides, additional antibodies for flow cytometry analysis. The magpix plates average around $2,200 and the variation in cost is based on number of plates needed each year and number of other assays needed. **Histology supplies and processing**, 9,**500 (year 1); $3,200 (year 2); $3,200 (year 3); $9,500 (year 4) and $9,500 (year 5)** is requested for processing samples and analysis. Costs include processing as well as antibodies for immunohistochemistry. Processing and staining will be approximately $10/sample.

Animals

Funds are requested each year for research related animals. Funds are requested each year for research related animals. Two strains of mice will be used during this project. C57Bl/6 mice and C57 albino. The C57Bl/6 mice cost approximately $26.00. The C57 albino cost $29.60 (6 week). Per diem charges are $1.05 per day per cage of 5 mice. In year 1 we anticipate utilizing 180 C57Bl/6 mice for work pertaining to Section C.2.1.3 and initiate the work in Section C.3.2. Mice will be housed for the following amount of time: 140 mice for 70 days, 24 mice for 30 days and 16 for 14 days. The cost for **year 1** including per diem and delivery charges will be **$6,500.** In year 2 we anticipate utilizing 600 C57Bl/6 mice for work pertaining to continuing Section C3.2. Mice will be housed for the following amount of time: 360 mice for 30 days and 176 mice for 70 days and 54 for 14 days. The cost for **year 2** including per diem and delivery charges will be **$19,500.** In year 3 we anticipate utilizing 245 C57Bl/6 mice for work pertaining to completing Section C.3.2 and initiating 4.2.2. Mice will be housed for the following amount of time: 222 mice for 70 days and 23 for 14 days. The cost for **year 3** including per diem and delivery charges will be **$8,475.** In year 4 we anticipate utilizing 158 C57Bl/6 mice and 80 C57 albino mice for work pertaining to completing Section C.4.2.2 and initiating Section C.4.3.2. Mice will be housed for the following amount of time: 218 mice for 50 days and 20 mice for 14 days. The cost for **year 4** including per diem and delivery charges will be **$7,454.** In year 5 we anticipate utilizing 113 C57 Albino mice and 110 aged C57Bl/6 mice for work pertaining to completing Section C.4.3.2. Mice will be housed for the following amount of time: 193 mice for 50 days and

20 mice for 14 days. The cost for **year 5** including per diem and delivery charges will be **$11,639** (price for aged mice is $65/mouse).

Cost Center Charges

The amount of $3,000 is requested each year for use of for use of core facilities. This will include the imaging core in the vivarium and the flow cytometry core.

Subcontract

We have requested funding in the amount of $318,876 to enter into a contractual agreement with the University of California, San Francisco for Co-Investigator Drs. Daud and Fong to collect and ship tumor and blood clinical samples to ODU and to aid Dr. Heller in the analysis of the correlation as well as the immunological analysis.

INDIRECT COSTS

Our ONR negotiated agreement dated April 12, 2011, authorizes an on-campus indirect cost rate of 53% of modified total direct costs effective July 1, 2011, through June 30, 2014.

# RESEARCH & RELATED BUDGET - Cumulative Budget

| | Totals ($) | |
|---|---|---|
| **Section A, Senior/Key Person** | | **230,510.00** |
| **Section B, Other Personnel** | | **768,223.00** |
| Total Number Other Personnel | 15 | |
| **Total Salary, Wages and Fringe Benefits (A+B)** | | **998,733.00** |
| **Section C, Equipment** | | |
| **Section D, Travel** | | **20,000.00** |
| 1. Domestic | 20,000.00 | |
| 2. Foreign | | |
| **Section E, Participant/Trainee Support Costs** | | |
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | |
| 4. Subsistence | | |
| 5. Other | | |
| 6. Number of Participants/Trainees | | |
| **Section F, Other Direct Costs** | | **571,144.00** |
| 1. Materials and Supplies | 183,700.00 | |
| 2. Publication Costs | | |
| 3. Consultant Services | | |
| 4. ADP/Computer Services | | |
| 5. Subawards/Consortium/Contractual Costs | 318,876.00 | |
| 6. Equipment or Facility Rental/User Fees | | |
| 7. Alterations and Renovations | | |
| 8. Other 1 | 53,568.00 | |
| 9. Other 2 | 15,000.00 | |
| 10. Other 3 | | |
| **Section G, Direct Costs (A thru F)** | | **1,589,877.00** |
| **Section H, Indirect Costs** | | **686,878.00** |
| **Section I, Total Direct and Indirect Costs (G + H)** | | **2,276,755.00** |
| **Section J, Fee** | | |

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

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

**ORGANIZATIONAL DUNS*:** 0948783370000

**Budget Type*:** ○ Project ● Subaward/Consortium

**Enter name of Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **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. | | Adil | | Daud | | PD/PI | Inst. Base Salary | Calendar Months | | | 9,075.00 | 3,025.00 | 12,100.00 |
| 2 | | Lawrence | | Fong | | Co-Investigator | | | | | 9,075.00 | 3,025.00 | 12,100.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **24,200.00**

**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 | | | | | | |
| 1 | Clinical Research Coordinator | 1.20 | | | 6,862.00 | 2,836.00 | 9,698.00 |
| **1** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **9,698.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **33,898.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **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**

**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**

**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**

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





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2014 **End Date*:** 08-31-2015 **Budget Period:** 1

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 5,000.00 |
| 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. Data Network/Device Charge | 278.00 |
| **Total Other Direct Costs** | **5,278.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **39,176.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1. Research - On Campus | 58.08 | 39,176.00 | 22,755.00 |
| | | **Total Indirect Costs** | **22,755.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **61,931.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1241-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0948783370000

**Budget Type*:** ○ Project ● Subaward/Consortium

**Enter name of Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period:** 2

**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. | | Adil | | Daud | | PD/PI | Inst. Base Salary | Calendar Months | | | 9,075.00 | 3,207.00 | 12,282.00 |
| 2. | | Lawrence | | Fong | | Co-Investigator | | | | | 9,075.00 | 3,207.00 | 12,282.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **24,564.00**

**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 | | | | | | |
| 1 | Clinical Research Coordinator | 1.20 | | | 6,933.00 | 3,004.00 | 9,937.00 |
| **1** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **9,937.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **34,501.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period:** 2

**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**

**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**

**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**

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





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2015 **End Date*:** 08-31-2016 **Budget Period:** 2

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 5,000.00 |
| 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 . Data Network/Device Charge | 278.00 |
| **Total Other Direct Costs** | **5,278.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **39,779.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . Research - On Campus | 58.50 | 39,779.00 | 23,271.00 |
| | | **Total Indirect Costs** | **23,271.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **63,050.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1241-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0948783370000

**Budget Type*:** ○ Project ● Subaward/Consortium

**Enter name of Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 3

**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. | Adil | | Daud | | PD/PI | Inst. Base Salary | Calendar Months | | | 9,075.00 | 3,388.00 | 12,463.00 |
| 2. | Lawrence | | Fong | | Co-Investigator | | | | | 9,075.00 | 3,388.00 | 12,463.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **24,926.00**

**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 | | | | | | |
| 1 | Clinical Research Coordinator | 1.20 | | | 7,004.00 | 3,175.00 | 10,179.00 |
| **1** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **10,179.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **35,105.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 3

**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**

**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**

**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**

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





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 3

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 5,000.00 |
| 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 . Data Network/Device Charge | 286.00 |
| **Total Other Direct Costs** | **5,286.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **40,391.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . Research - On Campus | 58.50 | 40,391.00 | 23,629.00 |
| | | **Total Indirect Costs** | **23,629.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **64,020.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1241-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0948783370000

**Budget Type*:** ○ Project ● Subaward/Consortium

**Enter name of Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

**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. | | Adil | | Daud | | PD/PI | Inst. Base Salary | Calendar Months | | | 9,075.00 | 3,539.00 | 12,614.00 |
| 2. | | Lawrence | | Fong | | Co-Investigator | | | | | 9,075.00 | 3,539.00 | 12,614.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **25,228.00**

**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 | | | | | | |
| 1 | Clinical Research Coordinator | 1.20 | | | 7,077.00 | 3,326.00 | 10,403.00 |
| **1** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **10,403.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **35,631.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

**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**

**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**

**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**

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





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 4

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 5,000.00 |
| 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 . Data Network/Device Charge | 286.00 |
| **Total Other Direct Costs** | **5,286.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **40,917.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1 . Research - On Campus | 58.50 | 40,917.00 | 23,936.00 |
| | | **Total Indirect Costs** | **23,936.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **64,853.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1241-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

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

# RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0948783370000

**Budget Type*:** ○ Project ● Subaward/Consortium

**Enter name of Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

**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 | | Adil | | Daud | | PD/PI | Inst. Base Salary | Calendar Months | | | 9,075.00 | 3,539.00 | 12,614.00 |
| 2 | | Lawrence | | Fong | | Co-Investigator | | | | | 9,075.00 | 3,539.00 | 12,614.00 |

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

**Additional Senior Key Persons:** File Name:

**Total Senior/Key Person** **25,228.00**

**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 | | | | | | |
| 1 | Clinical Research Coordinator | 1.20 | | | 7,149.00 | 3,360.00 | 10,509.00 |
| **1** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | **10,509.00** |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | **35,737.00** |

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





Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

**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**

**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**

**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**

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





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

# RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

**ORGANIZATIONAL DUNS*:** 0948783370000
**Budget Type*:** ○ Project ● Subaward/Consortium
**Organization:** Regents of the University of California, San Francisco

**Start Date*:** 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 5

| F. Other Direct Costs | Funds Requested ($)* |
|---|---|
| 1. Materials and Supplies | 5,000.00 |
| 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. Data Network/Device Charge | 286.00 |
| **Total Other Direct Costs** | **5,286.00** |

| G. Direct Costs | Funds Requested ($)* |
|---|---|
| **Total Direct Costs (A thru F)** | **41,023.00** |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | Funds Requested ($)* |
|---|---|---|---|
| 1. Research - On Campus | 58.50 | 41,023.00 | 23,999.00 |
| | | **Total Indirect Costs** | **23,999.00** |

**Cognizant Federal Agency** (Agency Name, POC Name, and POC Phone Number): DHHS, Jeanette Lu, 415-437-7820

| I. Total Direct and Indirect Costs | Funds Requested ($)* |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | **65,022.00** |

| J. Fee | Funds Requested ($)* |
|---|---|
| | |

**K. Budget Justification*** File Name: 1241-Budget Justification.pdf
(Only attach one file.)

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




Tracking Number: GRANT11598944
Funding Opportunity Number: PA-13-302 . Received Date: 2014-03-04T18:30:26.000-05:00

Budget Justification:

Percentage of Effort

Adil Daud, MD Principal Investigator: [redacted]% effort years 01-05, salary support requested, is budgeted for Dr. Daud to serve as PI of clinical trial of IL-12 electroporation in melanoma. He will recruit patients, monitor adverse events, file regulatory documents with the FDA and local IRB. Dr. Daud will also recruit patients and supervise their treatment and will biopsy patients on designated days and bisect and store samples and ship to RH Lab for analysis as required. A total of 25 patients will be enrolled on this trial which is already activated.

Percentage of Effort

Lawrence Fong MD Co-Investigator: [redacted]% effort years 01-05, salary support requested, will carry out immune analysis on peripheral blood including the circulating T cell functional analysis, NK cell analysis, ELISPOT and antibody responses.

Percentage of Effort

Jade Yen, CRC: [redacted]% effort years 01-05, salary support requested, is budgeted for a Clinical Research Coordinator. S/he will monitor patients for AE, schedule patient visits including setting up biopsy procedures fill reports and enter patient information and biopsy specimens in study CRF

Supplies: $5000 is budgeted in each year to obtain biopsies (include 2 mm and 4 mm punch biopsy tool and Bard Monopty 18 gauge needle, lidocaine and suture, suture holder and suture removal kits, sterile gauze and chloraseptic clean swabs), store biopsy specimens (Dry ice, sterile cryovials) and insulated styrofoam shippers as well as FedEx account for shipping specimens.

UCSF Data Network and Device Recharges: The Chancellor's Executive Committee has approved UCSF data network services and device recharges. The recharges provide funding for critical equipment in support of the campus network and are budgeted UCSF-wide on a per capita basis. Per review and agreement by our cognizant federal agency, these recharges are an allowable direct expense and budgeted on a per FTE basis in accordance with the set recharge rates. We request $278 in years 1-2 and $286 in years 3-5 for a total of $1,414 over five years.

# RESEARCH & RELATED BUDGET - Cumulative Budget

| | Totals ($) | |
|---|---|---|
| **Section A, Senior/Key Person** | | **124,146.00** |
| **Section B, Other Personnel** | | **50,726.00** |
| Total Number Other Personnel | 5 | |
| **Total Salary, Wages and Fringe Benefits (A+B)** | | **174,872.00** |
| **Section C, Equipment** | | |
| **Section D, Travel** | | |
| 1. Domestic | | |
| 2. Foreign | | |
| **Section E, Participant/Trainee Support Costs** | | |
| 1. Tuition/Fees/Health Insurance | | |
| 2. Stipends | | |
| 3. Travel | | |
| 4. Subsistence | | |
| 5. Other | | |
| 6. Number of Participants/Trainees | | |
| **Section F, Other Direct Costs** | | **26,414.00** |
| 1. Materials and Supplies | 25,000.00 | |
| 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. Other 1 | 1,414.00 | |
| 9. Other 2 | | |
| 10. Other 3 | | |
| **Section G, Direct Costs (A thru F)** | | **201,286.00** |
| **Section H, Indirect Costs** | | **117,590.00** |
| **Section I, Total Direct and Indirect Costs (G + H)** | | **318,876.00** |
| **Section J, Fee** | | |

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

## 1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*: Richard
Middle Name:
Last Name*: Heller
Suffix: Ph.D

## 2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

## 3. 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

## 4. Program Income*

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)* |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |





Tracking Number: GRANT11598944 Funding Opportunity Number: PA-13-302. Received Date: 2014-03-04T18:30:26.000-05:00

# PHS 398 Cover Page Supplement

## 5. Human Embryonic Stem Cells

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

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:

**Cell Line(s):** Specific stem cell line cannot be referenced at this time. One from the registry will be used.

## 6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ● No

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

Previously Reported*: ○ Yes ○ No

## 7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

# PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

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## INTRODUCTION

Response to Critique

## SPECIFIC AIMS

Gene delivery still remains a significant obstacle in achieving effective gene therapy. During the past decade significant technological advances have been developed that has enhanced overall gene delivery. However, successful gene therapy is dependent on achieving the appropriate expression for the desired therapeutic response. Being able to control delivery in such a way as to achieve effective dosing of the expressed transgene is a critical element that most of these delivery approaches fall short of. We have pioneered the use of electric fields to facilitate delivery of plasmid DNA. Gene electrotransfer (GET) is an effective method to deliver plasmids in a controlled manner to a variety of tissues. Parameters such as pulse duration, number, magnitude, direction, frequency and applicator can be manipulated to achieve a desired expression pattern and enhance overall effectiveness of the therapeutic approach[1-3].

During the past two decades we have worked on developing an effective delivery protocol using GET to delivery plasmids encoding cytokines as a potential therapy for melanoma. Malignant melanoma continues to be a significant health concern with rising incidence and no effective therapy for advanced disease[4]. Our previous studies evaluated delivery of IL-12 and IL-15 and the therapeutic potential of both were demonstrated[5-6]. In addition, we developed a GET protocol that was translated to the clinic resulting in positive outcomes in a Phase I study. A 40% objective response rate was achieved and 10% of patients were observed to have a durable complete response for multiple years as all lesions (treated and untreated) totally regressed[7]. This approach is currently being tested in a Phase II trial and interim results suggest confirmation of the Phase I results. These results are extremely encouraging and it is critically important to further advance this approach. First, our preclinical studies (see Preliminary Results section) have demonstrated the importance of achieving the appropriate expression in order to achieve the correct immunostimulation. Second, it is apparent from the clinical studies that while there is documented effectiveness, the therapeutic effect takes months to be achieved. Patients with visceral metastases or a short life expectancy are unlikely to obtain a complete response due to this delayed effect. In this project, we propose to address both of these issues to enhance the effectiveness of this therapeutic approach.

When utilizing GET it is important to achieve the appropriate balance between transgene expression and tissue damage in order to appropriately stimulate the host immune response to reach the clinically desired outcome. We hypothesize that if appropriate delivery parameters are used to deliver plasmid IL-12 then a change in the tumor microenvironment will occur that will be associated with an *effective* therapeutic response. Therefore, it is critical to characterize the response and identify potential biomarkers that can signify proper delivery and expression. To address the second issue, appropriate delivery of plasmid IL-12 will be combined with plasmids encoding antibodies that block T-cell inhibitory signals such as anti-CTLA-4, anti PD-1 and anti-PD-L1. These *checkpoint blockade* agents have been shown to *generate systemic immune response* and achieve objective response rates (*approximately 10-53%) and sometimes produce long term clinical benefits*[8-11]. Both approaches (IL-12 and antibodies) are potent T-cell stimulators and reduce levels of T-regulatory cells (T-regs) and should act in synergy to enhance the therapeutic outcome. Therefore, we also hypothesize that if an appropriate combination can be achieved then a more robust immune response will be achieved leading to an increased response at distant sites. The increased response observed will be due to a reduction of T-reg cells and enhanced activation of T effector and memory cells. In this project, we will fully develop and test this therapeutic approach in a mouse model and have the opportunity to determine how it correlates with samples obtained from an ongoing clinical trial.

To test the hypotheses the following Specific Aims will be performed:

1. *Determine the influence expression profile has in inducing an effective anti-tumor response and determine if a specific pattern of response can be identified.* Better characterize the events that occur following delivery in order to determine potential mechanism and if a pattern can be identified that documents appropriate delivery. In addition, tumor and blood samples from patients enrolled in clinical trial receiving gene electrotransfer with plasmid encoding IL-12 will be evaluated to determine cytokine and cellular *activation* patterns.
2. *Evaluate expression patterns following delivery of plasmids encoding anti-PD1, anti-PD-L1 or anti-CTLA4.* Determine the correct GET parameters for delivering these plasmids to skin and muscle.
3. *Therapeutic efficacy of the approach in a metastatic mouse model.* Will evaluate delivery of plasmid Il-12 combined with the plasmids from Aim 2 to determine if a more robust systemic response can be generated. *Test the combination therapy to effectively treat and/or block metastatic spread.*

## A. Significance

Successful completion of the work proposed in this application will result in significant advances in two major areas. The first is demonstration of controlled delivery of plasmid DNA in a manner which allows specific expression patterns that can achieve desired therapeutic outcomes. The second major significant outcome will be an enhanced therapy for melanoma.

### Gene Electro Transfer

An important criterion for successful gene transfer is effective delivery. In recent years, breakthroughs in non-viral delivery have been made typically utilizing physical methods including hydrodynamic delivery, ultrasound and electroporation[12-18]. Electroporation of plasmid DNA (gene electrotransfer or GET) was initially tested in skin, liver, muscle, and tumors[19-22]. Since then, this technique has been applied to many other tissues and animal models[1,23]. Currently, there are sixty-one clinical trials listed at clinicaltrials.gov with electroporation as part of the protocol. Forty-two utilize electrotransfer to deliver plasmid DNA all are either in Phase I or II. While the work over the past two decades have documented the potential for GET, as with most gene therapy approaches there is still a great deal of work to be done to move it to standard use and approved therapies.

A major hurdle for achieving this goal is better control of the dose of the transgene. While controlling the administered dose of the gene is achievable, controlling the levels of the expressed protein is more elusive. Delivery can play a vital role in controlling the expression of the transgene. Inducible promoters can be used to impart some control; however, for applications that require short term or strict control of protein expression levels, control through the delivery method would be more desirable. This is particularly important for immunotherapy, delivery of a transgene encoding an immunostimulating molecule requires a particular expression pattern to achieve an effective therapeutic response with minimal toxicity. Utilizing GET has the potential to achieve this goal. **Manipulation of GET parameters including electric field strength, pulse duration, pulse number, electrode geometry and configuration can be selected to control the onset, level, and duration of protein expression of the transgene.**

### *Melanoma Therapy*

Malignant melanoma is the deadliest form of skin cancer. The incidence of melanoma continues to rise and it is estimated that there will be 76,690 new cases in the U.S. in 2013[4]. Advanced melanoma has a poor prognosis and approximately 20% of primary melanomas will have metastatic spread[24-26]. There are few good options for patients with advanced disease and survival is < 1 year for patients with distant metastases[26-27]. Options for these patients include Dacarbazine with an overall response rate of < 20% and only rare instances of complete responses with no impact on overall survival[28-30]. BRAF inhibitors can prolong survival in BRAF mutant melanoma, but most patients progress in 6-8 months[31] and durable complete remissions are rare. Systemic High-Dose IL-2 has resulted in durable complete responses in ~5% of patients and no improvement in overall survival[10, 32-33]. Interferon-α has had response rates of 6-12% as single agent and ~20% when combined with DTIC or temozolomide[34-38]. Adoptive cell transfer with IL-2 and lymphodepletion produced a high response rate of 51% (out of 35 patients) with 3 exhibiting a durable long-term complete response[10, 39-41]. While encouraging, the therapy is associated with significant adverse events[10]. Recently, ipilimumab (anti-CTLA-4) received FDA approval after demonstrating improved overall survival[8]. Removing T-cell blocking signals can augment immune responsiveness and lead to tumor regression[42-44]. While a step forward, the objective response rate is still low (10-15%)[8-10, 45-48]. In addition, mean survival improved only about 4 months and serious adverse effects were reported[9, 49-50]. Results from a clinical trial utilizing an antibody against PD-1 reported objective response rates of *30-53%* with 10% complete responses with low levels of toxicity when administering the drug is continued for long periods[11]. While these results are encouraging there is still a need to establish improved therapies that can increase the number of complete durable responses and increase disease free survival.

The success of anti-CTLA-4 and anti-PD-1 has opened up opportunities for combination therapies that can improve overall clinical responses. One challenge will be to destroy cancer cells without damaging healthy tissue. Immunotherapeutic approaches require control to achieve a balance between effective therapy and minimizing adverse reactions. The use of GET to deliver one or more plasmids has the potential to achieve this goal with minimal toxicity. Our initial clinical results delivering plasmid encoding IL-12 has demonstrated this potential (see preliminary studies). The next critical steps are to increase the responsiveness of this approach and to add a component to the therapy to facilitate responses at distant and visceral locations.

IL-12 is a potent stimulator of both innate and adaptive immunity[51]. IL-12 induces immune stimulation through activation of T-cells and NK cells[52-53] and promotes the production of cytokines including interferon-γ. Initially, IL-12 was shown to have significant anticancer properties in mouse models and human clinical trials[54-57]. The presence of IL-12 within the tumor was shown to increase the presentation of tumor antigens to the immune system to generate a specific response[58]. Production of IL-12 at the tumor site induced tumor rejection by CD8+ T cells[54, 59]. In human clinical trials, delivery of recombinant IL-12 protein resulted in approximately 10% efficacy in melanoma patients. However, significant toxicity was noted in many of these studies[54] and some trials were stopped early. It is probable that dosing of IL-12 was responsible for the adverse events. Utilizing a gene-based approach to administer IL-12 has reduced associated toxicity and has the potential to enhance antitumor activity of this cytokine[7, 60-64] by directly altering the tumor immune microenvironment. In our approach, pulsed electric fields deliver plasmid encoding IL-12 directly to cutaneous or subcutaneous melanoma. This localized delivery has led to responses in untreated lesions. Since it is localized, this approach reduces toxicities that typically occur when systemic doses of cytokines are administered.

### *B.* Innovation

The work proposed in this study has several innovative components.

1. Developing a delivery method that allows for better control over the expression profile
2. Gene-based approach for enhancing TH1 responses through blocking T-cell inhibitory signals
3. Establish a protocol for the effective treatment of melanoma
4. Demonstrating that IL-12 can be utilized safely and effectively when delivered in the appropriate manner

Gene transfer protocols are typically developed to achieve the highest and longest expression. This is in contrast to conventional drug therapy which is based on the effective dose and kinetics of the drug that can be administered safely. Our novel approach is to determine the correct dose of the expressed transgene and establish the delivery protocol to achieve it. As shown in our preliminary data, *optimal expression is not necessarily the highest level or longest duration.* We are well suited to accomplish this as my laboratory was one of the first to demonstrate that electrotransfer could be utilized to deliver plasmid DNA to tissues in vivo[20]. In 1999, we published the first study utilizing *in vivo* GET for a therapeutic application in tumors[65]. The core principles of GET have been established by us and are utilized by many other groups. We were also involved in the first studies to translate GET into the clinic[7]. **The next major advance is demonstrating that by careful manipulation of GET parameters, optimal expression patterns can be induced to achieve a desired therapeutic effect.**

Currently, antibodies are utilized to block T-cell inhibitory signals to enhance immune responsiveness. For some of these molecules there are associated toxicities and for others there is a need for multiple injections or continuous treatments. Switching to a plasmid based treatment, would reduce the number of treatments, could reduce *systemic* toxicity and potentially improve the therapeutic outcome. A plasmid based approach has not been fully tested as of yet and combination with IL-12 is also novel. Combining pIL-12 delivery with plasmids encoding anti-CTLA4, anti-PD-1 or anti-PD-L1 has the potential to complement each other and induce a much more robust antitumor response. In addition, by delivering all the reagents in gene transfer protocols allows for greater flexibility particularly utilizing GET to establish specific expression profiles for each plasmid.

The approach developed for this application contains multiple components that required several years of development. The innovation is inherent in the GET parameters as well as electrode design and in the use of the protocol and devices to achieve specific expression patterns. It should be noted that we are proposing major advances to this approach building on a solid foundation of previous successes. The new innovative components being incorporated into this project could enhance the therapeutic potential and translatability.

### C. Approach

C.1.1 Introduction

Gene therapy is a maturing field with increasing successes in clinical applications. Delivery and particularly controlled expression of the transgene still remains as one of the major hurdles to overcome in order to achieve the full potential of this modality. GET is a powerful and reliable physical method of transferring plasmid DNA into tissues[39]. This approach has been shown to be an efficient method for gene therapy providing a safer alternative to other methods used such as viral vectors[1,66]. To achieve an appropriate therapeutic outcome it is important to select GET parameters that can minimize damage but achieve the appropriate transgene expression[67]. Careful selection of GET parameters allows for a more controlled delivery

of the plasmid in order to achieve the desired expression pattern. For example, while Lucas, et al[5, 68] elicited complete responses and long-term disease free survival, Lohr, et al[69] obtained regression but not long lasting complete responses. These studies each used different GET parameters, which probably resulted in different expression profiles illustrating the importance of obtaining the appropriate expression characteristics. While this proposed study is focused on melanoma it is possible that this modality could also be applied to other tumor types where local recurrence or invasiveness is a major issue.

C.1.2 Initial pre-clinical studies GET was evaluated in vivo to deliver plasmid DNA to melanoma tumors. C57Bl/6 mice with established subcutaneous B16-F10 melanoma tumors were treated by injecting 50 µg of plasmid DNA encoding IL-12 into the tumor (IT) or the gastrocnemius muscle (IM) followed by electrotransfer. Then a second treatment was administered seven days later or two treatments were given 4 and 7 days following the initial treatment. IT GET conditions were 6 pulses 100 µs long and 1300 V/cm. The only mice that showed a response were in the groups which received treatment with pIL-12 injected IT followed by electrotransfer. Mice that received two treatments resulted in a 60% (9 out of 15) complete response rate (CR) and those receiving three treatments had an 80% (12 out of 15) CR[5, 68] (Figure 1). When challenged with B16.F10 cells, all 12 (100%) of the disease free mice in the three treatment groups were resistant, and eight out of nine mice (88.9%) in the two-treatment group were resistant suggesting development of an immune memory response.

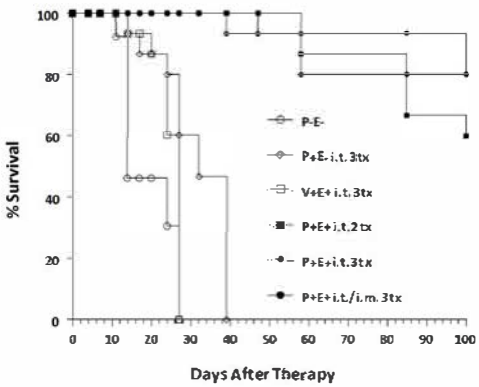



**Figure 1:** B16 F10 melanoma survival with repeated dosing. Mice were treated on days 0 and 7 or 0, 4 and 7. Results represent the combined data from three replicate experiments and error bars represent the standard error of the mean P=pUMVC3-mIL-12; V=control plasmid, pND2Lux; E=electroporation. i.t.= intra tumor; i.m.=intra muscular. The total number of samples for each treatment group was 15. Data is expressed for surviving mice on each day.

To evaluate the role of T lymphocytes in tumor regression, the experiment was repeated using athymic mice. B16.F10 tumor cells were subcutaneously injected and treatment performed on 3–5 mm diameter tumors. Mice received IT treatments with or without GET. No tumor regression was seen. No mice in any group survived longer than 30 days suggesting the need of a T-cell response for successful regression of B16.F10 melanoma tumors.

C.1.3 Translation to Clinical Study

In order to move the approach to clinical testing a preclinical toxicity study was performed[70]. Tumors were established in the left flank of C57Bl/6 mice and divided into treated and control groups. Similar to the efficacy study, three treatments were performed on days 1, 5 and 8. Extensive hematology and serum chemistry analysis was performed. Mice were sacrificed on days 9, 11, 16, 23 and 30 after treatment. Untreated mice showed decreased survival compared to partially treated (plasmid no elelctrotransfer) or treated mice (plasmid plus electrotransfer). All deaths were due to disease progression. No weight loss was seen in any of the groups. Histological examination of the heart, lungs, brain, bone, muscle and liver showed no evidence of abnormalities correlated with treatment. Mice in the group receiving plasmid IL-12 delivered by GET were generally in the best health and showed the least biochemical and hematology abnormalities.

C.1.4 First-in-Human Phase I trial of pIL-12 electrotransfer. A phase I trial was conducted at the Moffitt Cancer Center from December 2004 to February 2007[7]. Patients had metastatic melanoma with accessible cutaneous disease that was surgically unresectable. Twenty-four patients were treated in 7 cohorts (plasmid concentrations ranging from 0.1 mg/ml to 1.6 mg/ml). All patients had 3 treatments on days 1, 5 and 8 which included intra-tumor injection of plasmid pUMVC3-hIL-12-NGVL3 delivered using GET at 6 pulses 100 µs long and 1300 V/cm. No systemic treatment related adverse events were observed. Transient pain, erythema and bleeding at the GET sites following treatment were the only adverse events reported but all patients stated that the therapy was tolerable. Although the study was designed as a safety trial, over 70% of the treated lesions regressed. Nineteen of the 24 patients enrolled in this study had additional sites of disease outside the treated lesions. Of these nineteen patients, >10% showed complete regression of all metastases, both treated and untreated, and 42% showed disease stabilization or partial response. Post-treatment biopsies at various times after treatment (Days 11, 22 and 39) showed proportional increase in IL-12 protein with increased plasmid dose. Marked tumor necrosis and lymphocytic infiltrate were detected histologically. **The results from this study show pIL-12 delivery to melanoma patients is safe effective, reproducible and capable of inducing an effective anti-tumor immune response. Two patients showed long-term durable complete**

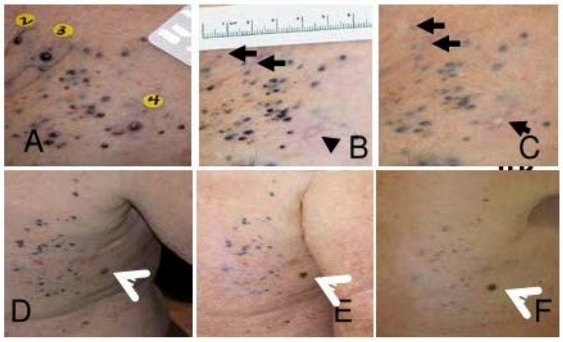



**Figure 2**: Cutaneous lesions in patients 09 from cohort 3 (panels A-F) Panels A,B ,C show the front chest and D,E ,F the back. A, D day 1 (pre RX) B , E day 256 and C ,F day 637. Electrotransfered lesions (2, 3, 4 in panel A) were resected (sites shown by black arrows). Untreated lesions flatten and fade away. On panels D, E and F the seborrheic keratosis (white open arrow) persists.

**response.** Two patients showed long-term durable complete response in both treated and untreated lesions. One patient had rapidly progressing cutaneous metastasis with over 50 nodules on his right chest and shoulder; following GET, all lesions flattened out and faded and no new lesions developed over a period of 18 months (Figure 2). A sample regressed lesion was biopsied and demonstrated residual pigment but no evidence of melanoma. He has no evidence of systemic disease six years following treatment. A third patient, had complete regression of all lesions (treated and untreated) and on a follow up CT scan had no evidence of disease five months post GET, after having received four cycles of dacarbazine.

C.1.5 Influence of GET delivery parameters on achieving positive therapeutic response

Our hypothesis is that specific GET parameters can be utilized to achieve a specific expression profile. To test this concept we utilized three GET protocols previously published GET protocols [5, 71-72] that resulted in different expression profiles. In addition, we utilized two plasmids pUMVC3-mIL12 (low expression) and pAG250-mIL12 (high expression) as well as two different electrode configurations. A circular configuration of 6 penetrating electrodes (N) or non-penetrating parallel plate caliper electrodes (C) were used to deliver pulses. The GET protocols that were chosen; EP1 (six rotating 1300 V/cm, 100 µs pulses), EP2 (ten unidirectional 600 V/cm, 5ms pulses) and EP3 (one 667 V/cm, 100 ms pulse). Three distinct patterns emerged. EP2(C) showed the highest expression which was significantly higher than the injection only control ($P < 0.01$) as well as the EP1(N) group ($P < 0.001$ at 24 hrs and $P < 0.01$ at 96 hrs) and was maintained at a high level for 96 hours. EP3 had high expression which began to drop by 96 hours. EP1 was higher than injection only but significantly lower than the other GET protocols and was close to background by 96 hours. No significant differences in expression were observed between EP2(C) and EP3(C) groups (Figure 3A). Delivery of pAG250-mIL12 resulted in higher IL-12 expression at certain time points compared to pUMVC3-mIL-12 (Figure 3b). Delivery using EP1(N) again resulted in the lowest gene expression of the three protocols tested and tapered off over the observation period. However, delivery of pAG250-mIL12 using this protocol resulted in higher expression than delivery of pUMVC3-mIL12 ($P < 0.001$). Delivery of pAG250-mIL12 using EP2(C) and EP3(C) resulted in similar levels of IL-12 expression that were only slightly higher, and not significantly different than delivery using EP1(N). The influence of the electrode design was tested by switching electrodes used for EP1 and EP2 (Figure 3c). Delivery of pUMVC3-mIL12 using EP1(C) and EP2(N) resulted in levels of IL-12 expression that were not statistically different from each other. Using EP1(C), expression levels at 24 hrs were significantly higher than when EP1(N) was used ($P < 0.001$). There was no significant difference in expression at 24 hrs when EP2(N) was used compared with EP2(C). However, at 48 and 96 hrs expression was significantly lower ($P < 0.01$) when the needle applicator was used instead of the caliper to deliver pulses.

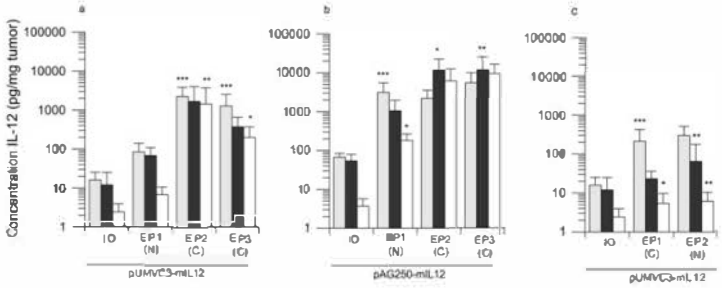



**Figure 3. Intratumoral expression of interleukin 12 over time after GET.** Gene expression is measured by ELISA from tumor homogenate 24 (□), 48 (■) and 96 (☐) hours after a single delivery of pIL-12 using various electroporation protocols: EP1, EP2, and EP3. Needle (N) or caliper (C) applicators used to deliver the pulses as indicated. Data are expressed as pg/mg of tumor ± standard deviation. $n = 5$ in each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for specified group compared to EP1 (N) (a), and comparable EP conditions used for delivery in a (b and c).

The key question was whether or not expression levels influenced therapeutic outcome. Three IT deliveries of pIL-12 on days 0, 4 and 7 using the EP1 and EP2 GET protocols were performed and tumor volumes were monitored for 9 weeks. Mice with tumors that expressed the highest concentration of the IL-12 transgene did not achieve 100% tumor regression and survival. Mice that received pUMVC3-mIL12 using EP2(C) showed 80% survival, mice that received pAG250-mIL12 using EP2(C) showed 30% survival, and mice that received pAG250-mIL12 using EP1(N) 89% survival (Table 1 (Tumor Free Day 50)). Mice with treated tumors that showed 100% survival were those that expressed relatively low levels of IT IL-12. These mice received pUMVC3-mIL12 delivered using EP1(N) and EP2(N). Mice from the control groups that received pIL-12

injection only or backbone plasmid delivered using GET protocols showed 40% survival or lower. Overall the choice of GET protocol (EP1 or EP2) used did not appear to impact survival of the groups tested following treatment of the primary tumor.

Table 1

| Plasmid | Conditions | Electrode | Original n | Tumor Free Day 50 (n) | Tumor Free Post Challenge Day 50 (n) | % Overall Survival Post Challenge |
|---|---|---|---|---|---|---|
| pUMVC3-mIL12 | IO | - | 13 | 3 | 1 | 8 |
| pAG250-mIL12 | IO | - | 10 | 4 | 0 | 0 |
| pUMVC3 | EP1 | 6 needle | 10 | 0 | 0 | 0 |
| pUMVC3 | EP1 | Caliper | 9 | 2 | 2 | 22 |
| pUMVC3-mIL12 | EP1 | 6 needle | 10 | 10 | 7 | 70 |
| pUMVC3-mIL12 | EP1 | Caliper | 9 | 7 | 4 | 44 |
| pAG250-mIL12 | EP1 | 6 needle | 9 | 8 | 3 | 33 |
| pUMVC3 | EP2 | Caliper | 10 | 3 | 1 | 10 |
| pUMVC3 | EP2 | 6 needle | 10 | 1 | 1 | 10 |
| pUMVC3-mIL12 | EP2 | Caliper | 10 | 8 | 1 | 10 |
| pUMVC3-mIL12 | EP2 | 6 needle | 10 | 10 | 2 | 20 |
| pAG250-mIL12 | EP2 | Caliper | 10 | 3 | 0 | 0 |

The next critical question was to determine which protocol induced a protective immune response. To test this, mice that remained tumor-free for fifty days following treatment were inoculated with fresh B16.F10 melanoma cells on the opposite flank. If the mice developed tumors they were considered not to be resistant to challenge. Mice that did not develop tumors were monitored for 50 days, after which if no tumors developed they would be considered resistant to challenge. Treated mice with tumors that expressed high levels of IL-12 after treatment did not appear to be well protected from challenge (Table 1). In fact, the group that expressed the highest levels of local IL-12 had no animals that were protected from challenge. The highest overall survival among the relatively high expressing groups was 33% by the group that received pAG250-mIL12 using EP1(N). Mice given pUMVC3-mIL-12 using EP2(C) had only 10% that were resistant to challenge. The group that showed the greatest resistance to challenge received pUMVC3-mIL-12 using EP1(N) and expressed the lowest levels of local IL-12 among the GET groups. Of the control animals that received injection of plasmid DNA only, only one animal from the group that received pUMVC3-mIL12 DNA was resistant to challenge. Delivery of pUMVC3 vector control showed fewer than 22% of the animals resistant to challenge (Table 1). There are several important points to note, these results clearly show that gene transfer protocols are not just about getting the highest and longest expression. It is important to achieve the right expression of the transgene. The second is that when evaluating tumor responses it is important to look further than just the primary response. Many studies have reported an effective immune response but only examined the primary tumor. As can be seen from our results this can be misleading as it may not result in a protective response.

An additional interesting finding is that effective therapeutic outcome is also related to distribution. For GET this is directly related to electrode configuration and the target tissue. We tested this in the B16 model and utilized delivery parameters, including reduced concentration, to deliver AG250 (high expression) to match the expression profile of the successful delivery of pUMVC3. This did not result in as good of a therapeutic response but did result in a different distribution of expression. **While it may seem complicated to manipulate the many variables involved in developing a GET protocol, it is those variables that allow for better control over expression profile and distribution and can lead to better therapeutic outcomes**.

The preliminary studies, demonstrate that GET is an effective delivery tool. Expression profiles can be developed to achieve effective therapeutic responses. This project is designed to enhance this approach further. We will utilize the information obtained to improve clinical outcome. We will study the changes occurring in the tumor microenvironment following GET. In addition, it is apparent from the clinical studies that while effective, the therapeutic effect takes months to be achieved. Patients with visceral metastases or a short life expectancy are unlikely to obtain a complete response due to this delayed effect. This issue will be addressed by combining with another immune modulating agent that could enhance the systemic response leading to enhancing the effectiveness of this therapeutic approach.

### C.2 Specific Aim 1

*Determine the influence expression profile has in inducing an effective anti-tumor response and determine if a specific pattern of response can be identified.*

C.2.1.1 Rationale: We have recently documented the importance of controlling the expression profile in order to achieve a desired therapeutic response. Utilizing GET we have demonstrated that different delivery parameters results in distinct expression profiles and subsequently different therapeutic responses. This work culminated in the first in-vivo electrotransfer clinical trial in patients with metastatic melanoma utilizing a plasmid encoding IL-12. Based on the results GET appears to be safe, tolerable and efficient in gene transfer and clinically beneficial results.

Both the preclinical and clinical work has been very encouraging. It is clear from these results it is critical to achieve the right expression profile of the expressed transgene. With IL-12 it is **important to achieve a balance of stimulation while avoiding toxicity.** It is also clear that **overstimulation does not lead to a memory response.** Utilizing GET, it is possible to manipulate parameters including field strength, pulse width and electrode configuration to achieve a specific expression profile. It is also apparent that **manipulation of the tumor environment (electrode placement and pulses) also contributes to the response.** Therefore, to better understand this therapeutic approach and to improve clinical response rates it is important to fully characterize the events occurring during the delivery and subsequent events that contribute to the response. Characterizing the cellular components involved in the response would lead to more consistent and reproducible therapeutic outcomes. Work will include delineating the cellular profile and/or biomarkers following treatment in order to develop an indicator of proper delivery and treatment. We hypothesize that if appropriate delivery parameters are used to deliver plasmid IL-12 then a change in the tumor microenvironment will occur that will be associated with an appropriate therapeutic response. The induced immune response will be related to a change in the T-cell profile including a reduction of T-reg cells and enhanced activation of T effector and memory cells.

*C.2.1.2 Preliminary studies:* The phenotype of circulating lymphocytes following challenge of treated mice was determined. Samples were collected from resistant mice 50 days post challenge and from non-resistant mice after a palpable tumor was detected. CD4+ and CD8+ memory T cell subsets were evaluated using activation and memory markers CD45RB and CD62L. Low CD45RB expressing cells are characterized as memory cells and those expressing high CD45R are thought to be naive. Naïve cells also express high levels of CD62L which are lost on encounter with antigen. The sub-populations that were observed using this technique are delineated as follows: Effector memory cells (EM) are $CD45RB^{-}CD62L^{-}$, central memory cells (CM) are $CD45RB^{-}CD62L^{+}$, naïve cells (N) are $CD45RB^{+}CD62L^{+}$, and the activated effector cells (E) are $CD45RB^{+}CD62L^{-}$. The cell populations can be grouped based on clinical outcome rather than parameter and plasmid selection. If the mice were resistant to challenge and remained tumor free for the experiment duration, their phenotypic profile was similar regardless of treatment condition or plasmid and significantly different from their non-resistant counterparts (Figure 4a). Of the circulating $CD4^{+}CD3^{+}$ cell population, there were higher proportion of memory cells than naïve and activated effector cells. Resistant mice showed significantly higher proportion of circulating activated effector and effector memory cells ($p < 0.0001$) as well as a significantly lower proportion of naïve and central memory cells ($p < 0.05$ and $p < 0.0001$ respectively) than the non-resistant mice (Figure 4b). The circulating $CD8^{+}CD3^{+}$ population showed a higher proportion of naïve and activated effector cells than memory cells. The resistant mice showed a significantly higher proportion of activated effector and effector memory cells ($p < 0.0001$) and a significantly lower population of naïve cells ($p < 0.0001$) than the non-resistant mice. There was no significant difference noted between the CM populations of the two groups. It is noteworthy that the mice that remained tumor free post challenge had a higher proportion of circulating $CD8^{+}CD3^{+}$ activated effector cells compared to the mice that developed tumors and that these same mice had a depleted $CD8^{+}CD3^{+}$ naïve population compared to the non-resistant counterparts.

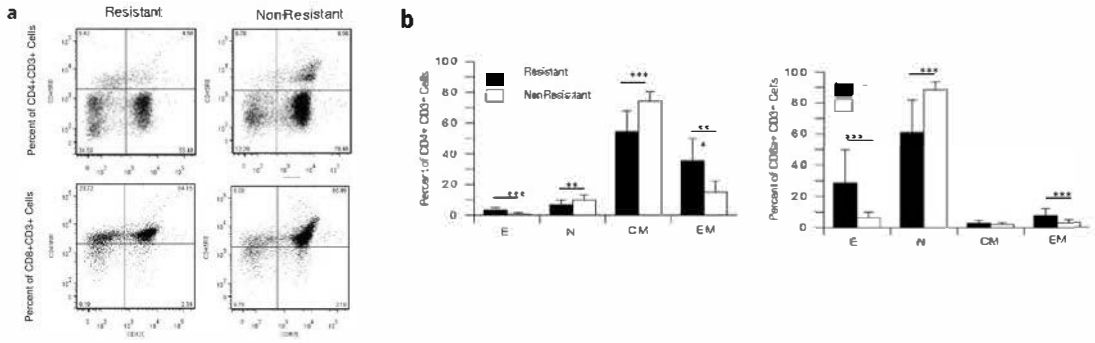



Figure 4. Phenotypic analysis of circulating lymphocytes in post challenge mice.
Dot plots (a) are representative of resistant and non-resistant mice. Each dot represents a cell gated on CD4+CD3+ or CD8+CD3+ parent populations. Cumulative data for the subpopulations are presented as mean percentage of the parent T cell population from resistant and non-resistant mice (b). Error bars represent standard deviation. E, effector; N, naïve; CM, central memory; EM, effector memory. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

*C.2.1.3 Research Design:* Our preliminary data showed that mice that were protected display a distinct T-cell profile from those that were not. We hypothesize that if IL-12 is present in the right concentrations within the tumor microenvironment then an increase in T effector cells will occur that will result in immune stimulation.

Table 2

| Group | Plasmid | Conc. mg/ml | Vol. (µl) | Elect. | GET Protocol |
|---|---|---|---|---|---|
| 1 | None (saline) | 0 | 50 | N | EP1 |
| 2 | pUMVC3-mIL-12 | 1.0 | 50 | None | None |
| 3 | pUMVC3-mIL-12 | 1.0 | 50 | N | EP1 |
| 4 | pUMVC3-mIL-12 | 1.0 | 50 | C | EP2 |
| 5 | pUMVC3-backbone | 1.0 | 50 | N | EP1 |
| 6 | pUMVC3-backbone | 1.0 | 50 | C | EP2 |

We have demonstrated that GET can be utilized to deliver pIL-12 to achieve the appropriate concentrations. It is important to determine the events within the tumor microenvironment occurring in response to this and leads to an effective anti-tumor response and stimulation of a memory response. We believe part of this activation is a result of increasing the Teff:Treg ratio to overcome T-reg-mediated immune tolerance by and/or by decreasing expression of CTLA-4, PD-1 and/or PD-L1, which are required for T-reg-mediated suppression. To test this, we will examine the cellular profiles of tumor, surrounding tissue and lymph nodes before and after treatment. We will also determine which cells are expressing the delivered IL-12 plasmid and if this has an impact on therapeutic outcome. Following growth of tumors, the mice will be divided into six groups (n=24 mice each) as described in Table 2. We will use delivery parameters that were found to give the best response (preliminary data). For comparison, we will also include delivery parameters that resulted in a high primary response but poor memory response. IL-12 plasmid will be injected IT at a concentration of 1 mg/ml and an injection volume of 50 µl. At specified time points after treatment, six mice from each group will be humanely euthanized and tumors and surrounding skin, lymph nodes and spleens removed and blood collected for both serum and peripheral mononuclear cells (PBMCs). Tissue samples will be processed for multi-color flow analysis to comprehensively evaluate leukocyte populations (i.e., helper, cytotoxic, memory, regulatory and activated T-cells as well as NK cells). In addition to delineating what cells are present, level of activation of these cells will be determined by marker analyses (i.e. CTLA-4, PD1, PDL1, etc.) as well as the presence of specific cytokines. Serum levels of IL-2, IL-4, IL-6, IL-10, MCP-1, IFNγ, TNF and IL-12 will also be measured. The analysis time points will be one day following treatment and then 10 and 50 days after the last treatment for those mice that respond and then 50 days post challenge from mice resistant to challenge and from non-resistant mice after a palpable tumor is detected. Intracellular cytokine and lymphoproliferative assays will be performed on the spleen cells and ELISPOT assays will be performed on PBMCs isolated from the blood sample. Based on these results, time points will be selected to perform gene arrays on the tumor samples to determine if changes occurred within the tumor following treatment. Results from all of these assays will provide information that can be used to determine the events occurring within and around the tumor as well as within the circulation following treatment.

*C.2.1.4 Anticipated results:* It is anticipated that there will be distinct differences in cell populations dependent on how mice are treated. The work performed as part of this aim will enable identification of which immune cells are present in the tumor and surrounding area at the time of GET treatment and after. Following effective treatment there will be a reduction in T-reg cells and increase in activated effector cells. We anticipate that these changes will occur in the peritumor area as well and not be restricted to within the tumor. Work in this part of the aim will also enable identification of cell populations expressing the delivered plasmid as well as other cytokines present. In addition, it is anticipated that there will be a distinct cytokine profile associated with a positive therapeutic outcome. Identifying effects of GET on the tumor microenvironment may lead to a better understanding of the mechanism for the positive effects of GET cytokine therapy.

*C.2.1.5 Potential problems and alternatives:* A major hurdle in observing distinct patterns will be timing. Samples will be taken prior to and at several points following treatment. However, it is possible that subtle changes could be missed. If this does occur, the comprehensive analysis should give enough information to delineate sequence of events and provide information related to what other time points should be evaluated. These results will also be useful in determining the mechanism related to response.

*C.2.2 Determine if the same patterns are detected in samples from ongoing melanoma clinical studies.*
C.2.2.1 Rationale: The first part of this Specific Aim will evaluate events occurring within the tumor microenvironment following treatment with pIL-12 GET. In addition to understanding the potential mechanism for the durable response, it is a goal to determine if there is a specific pattern or biomarker that can be identified that would indicate successful delivery and subsequent positive anti-tumor response. It is important to determine if the same tumor microenvironment changes and indicators are present in clinical samples following treatment with pIL-12 GET. This part of the aim will be focused on determining if the mouse data correlates with clinical samples. A Phase II study using GET to deliver pIL-12 has been initiated (trial itself is not part of this proposal). The investigators will have access to samples before and after treatment from this

Phase II study to determine correlation with murine studies. In preliminary studies, the C57Bl/6/B16.F10 model was shown to be accurate to determine translatability of this approach. Using this approach and model it was shown that regression of the primary tumor (treated lesion) would regress when treated and that if the plasmid was delivered appropriately about 70% of the mice were protected from new tumors forming. In a Phase I clinical study in a single dose cohort it was also shown that over 70% of the lesions that were treated completely regressed and in 66% of the patients untreated tumors went away and new tumors did not form.

C.2.2.2 Preliminary studies: Interim results from the Phase II clinical trial are confirming the results obtained in the Phase I study. Thus far 13 patients have reached at least 6 months follow-up and 2 have a complete response in that both treated and untreated lesions have completely regressed and both are now disease free. Two more are stable at 6 months. In addition 12 of the 13 patients had a partial or complete regression of treated lesions at 39 days. Samples from these 13 patients have been archived and will be used for the study below along with additional samples being collected currently and in the future. These results demonstrate the potential of this therapeutic response. As we have described in the preliminary studies, achieving appropriate expression is critical to achieving the correct response. Therefore, it is important to identify an indicator that correct delivery and subsequent expression was achieved.

C.2.2.3 Research Design: We are not proposing to perform a Phase II trial but to use samples from an existing, ongoing Phase II trial funded by Oncosec. Patients enrolled in the Phase II trial will have 2-4 lesions treated. There will be three treatments within one week as was done in mouse studies and the Phase I trial. On prestudy and Days 11 and 39 a punch biopsy will be taken of a treated lesion. On Day 39 and 180 a punch biopsy will be performed on an untreated lesion (if present). The tissue from the punch biopsies will be processed to evaluate leukocyte populations (T-regs, T-helper vs T-cytotoxic, memory and activated as well as NK cells). In addition to delineating what cells are present, marker analyses will be done to determine level of activation (i.e. CTLA-4, PD1, PDL1, etc.) as was done above. On Days 1, 39, 90, 120, 180, 270 and 360 blood will be collected to isolate serum and PBMCs. Serum samples will be evaluated with a Millipore Magpix multiplex. Isolated PBMCs will be assayed by ELISPOT to determine immune responses to melanoma antigens. All patients will be assessed for immune responses to peptide pools of gp100, Mart-1, and Mage-3.

C.2.2.4 Anticipated results: It is anticipated that the results obtained in this section will correlate with the results from the mouse studies. Cellular profiles will reveal a reduction in T-reg cells and an increase in memory cells over time. The data accumulated will be instrumental in determining the mechanism of the response. It will be interesting to evaluate the untreated lesions to determine if infiltrate is present and, if it is, will the profile be similar as in the treated lesions. Based on time to response seen in the Phase I, if there is a response in an untreated lesion it will probably be seen in the 180 day sample. It will be interesting to delineate the location of IL-12 expression and if a distinct distribution pattern will be associated with an effective response as was seen in the previous mouse studies. Work in this aim will also reveal possible mechanisms associated with a response in an untreated lesion. It is anticipated that a profile will emerge that could be used to identify which patients had the pIL-12 delivered correctly.

C.2.2.5 Potential problems and alternatives: As with the mouse studies, timing will be critical for delineating all of the profiles. Unlike the mouse studies, changing the timing of when samples are taken will not be as easily corrected. Responses will take longer to occur in humans compared to the mouse study and there will not be as much tissue to evaluate. Potential problem could be related to evaluation of untreated lesions. One issue could be if a patient has five or less cutaneous lesions we will not be able to get both biopsies of untreated lesions. If there is one untreated lesion we will take the biopsy at Day 180. The other issue is related to timing. It is possible that Day 180 may be too early to see signs of a response in an untreated lesion. If no response is seen, but there are indications of a systemic immune stimulation (results from other assays) then an additional lesion if available will be biopsied at Day 270.

**C.3 Specific Aim 2**

*Evaluate expression patterns following delivery of plasmids encoding anti-PD1, anti-PD-L1 or anti-CTLA4.*

C.3.1 Rationale: Results from the Phase I clinical study and interim results from the Phase II trial indicate that local delivery of pIL-12 directly to tumors can induce an effective immune response. Both treated and untreated lesions completely regressed and 3 patients from the Phase I have been tumor free for over 3 years. Similar results have been seen in the Phase II although follow up is still limited to under a year. While these

results are encouraging, the time to respond is greater than six months. For patients with visceral metastases or with life expectancy of less than 6 months this approach will not be effective. Therefore, in order to improve this therapeutic approach it is important to add a component to the therapy that can enhance the systemic response or at least stabilize the patients long enough to allow the pIL-12 therapy to have an effect. Antibodies that target T-cell blocking signals such as anti-CTLA-4, anti-PD-1 and anti-PD-L1 have shown effectiveness in melanoma patients with advanced disease resulting in stable disease or even inducing a durable response [8-11, 45-48]. As with many melanoma therapies these results are very encouraging but it is important to increase the number of patients with positive outcomes. It should also be noted that with some of these approaches there are associated toxicity. While anti-PD-1 has shown the best responses[11] it is important to evaluate several candidates to find the right combination. In addition, some will also require multiple injections over extended period of time. To address this issue we propose to deliver these agents in the form of plasmid DNA achieving an appropriate expression profile as we developed for pIL-12. In addition, we hypothesize that combining these agents with local delivery of pIL-12 will further enhance both therapeutic modalities. We will utilize our knowledge of GET to achieve the appropriate transgene expression. In addition, to being able to control the transgene expression by manipulating the GET parameters it is also possible to influence the profile by careful selection of the target tissue. The greatest success has occurred delivering the antibodies systemically. For PD-1, continuing administration for a long period has been the most successful. Therefore, to achieve systemic levels of these molecules we will test delivery in muscle and skin. We have significant experience using these tissues and can select parameters to achieve the expression profiles needed[73-75]. Both skin and muscle will be tested as they each give different kinetic profiles. Muscle will typically result in longer expression than skin.

C.3.2 Research Design: The initial study will evaluate expression profiles of the three plasmids. The therapy will require systemic expression of the transgene. Therefore, the target tissues will be muscle and skin. Muscle will result in longer expression. Between the two we can obtain high and low expression as well as long and short term expression. An additional advantage of skin could be the ability to deliver peritumorally. Plasmids will encode anti-CTLA-4, anti-PD-1 and anti-PD-L1. For each plasmid there will be 8 experimental groups which will include 3 skin GET protocols, one skin injection only group and 3 muscle GET and one muscle injection only group as described in Table 3. Plasmid will be injected at a concentration of 1 mg/ml in a volume of 50 ul for muscle and at a concentration of 2 mg/ml in a volume of 50 ul for skin. There will be 8 mice/group and the mice will be followed for 21 days. Blood samples will be taken at Days 2, 5, 7, 10, 14, 17 and 21 to determine serum levels of the expressed protein.

Table 3

| Group | Tissue | Elect. | GET Protocol |
|---|---|---|---|
| 1 | Muscle | None | None |
| 2 | Muscle | Needle | mEP1 20 ms 100 V/cm |
| 3 | Muscle | Needle | mEP2 100 us 1300 V/cm |
| 4 | Muscle | Needle | mEP3 10 ms 300 V/cm |
| 5 | Skin | None | None |
| 6 | Skin | Plates | sEP1 150 ms 175 V/cm |
| 7 | Skin | Plates | sEP2 20 ms 200 V/cm |
| 8 | Skin | Plates | sEP3 150 ms 125 V/cm |

The next set of experiments will determine the potential for multiple treatments. Same plasmids and groups will be used for this set of experiments. Treatment timing will be based on the results from the previous experiment. Delivery will be repeated when the serum levels drop and the mice followed for another 21 days.

Once the expression profile has been established effect on tumor growth will be evaluated. Four expression profiles will be tested for each plasmid which will cover long and short expression and high and low expression. Treatment will be initiated once B16.F10 melanomas reach a size of at least 5 mm diameter on the flank. Parameters and multiple treatments will be administered as determined in the above experiments. We will utilize 6 groups for each plasmid to include treatment with 4 GET protocols, one injection only and a no treatment group. There will be 12 mice per group. Five days after treatment, 4 mice will be humanely euthanized, tumor removed and PBMCs isolated. The tissue and PBMCs will be evaluated for the presence of activated T cells. The other eight mice will be followed for at least 50 days or until the tumors grow to a volume greater than 1,000 $mm^3$. Mice that are tumor free at 50 days will be challenged by injecting new B16.F10 cells in the opposite flank and followed for another 50 days.

C.3.4 Anticipated results: It is anticipated that distinct expression profiles will be obtained with each of the GET protocols for each of the tissues. It will be interesting to determine if the profiles will differ between plasmids. Serum levels should be maintained with multiple treatments. With respect to the tumor therapies, it is anticipated that we should see at least a reduction or slowing of tumor growth with some of the GET protocols. It is possible that we will see a complete response with one or more of the GET protocols. The measure of success will be to have a demonstrated increase in T-cell activation in the periphery and within the tumor. In

addition, we would also anticipate having a reduction in the presence of T-reg cells. In addition to these criteria, a successful outcome will also include at least a slowing of tumor growth. We do not believe we need to achieve complete regression with these agents, although it would be a bonus, as we intend to combine it with pIL-12 therapy in Aim 3. Slowing or stopping tumor growth will be an important addition to the pIL-12 therapy and could enhance the success seen clinically.

C.3.5 Potential problems and alternatives: We do not expect any issues with the methods as we are well experience in this work. It is possible that we will not achieve the 4 distinct expression profiles for each of the plasmids. We may not see the type of immune response in order to determine successful protocols. It is also possible that we may see toxicity with some of the delivery protocols which would be indicated by mice having significant weight loss, being lethargic or even sudden unexplained death. If any of these three events occurs we will modify the GET parameters to achieve different expression profiles. It may also be necessary to modify the plasmid construct to also adjust the expression profile.

**C.4 Specific Aim 3**

*Therapeutic efficacy of the approach in a metastatic mouse model.*

C.4.1 Rationale: It is critical to test the potential of this therapeutic approach in a regional and/or distant metastatic disease model. The first two aims as well as previous studies have demonstrated the potential of pIL-12 delivered with GET. In both the preclinical studies and the Phase I clinical trial it was clear that an effective distant response stimulated by this therapeutic approach took time to develop. For patients with aggressive disease or established visceral metastases, six to twelve months to achieve a response may be too long. Therefore, to enhance the effectiveness of IL-12 GET, it would be advantageous to add one or more agents that would stabilize or further enhance the immune response for patients with advanced melanoma. To fully develop an effective therapy, we will test a combination approach that can be effective against multiple lesions and the combination of both subcutaneous and visceral disease. Specific aim 2 will have tested the use of plasmids encoding anti-CTLA4, anti-PD1 or anti-PD-L1 antibodies and have identified appropriate delivery protocols to achieve immune stimulation. In this specific aim, we will combine IL-12 GET with the administration of plasmids encoding anti-CTLA4, anti-PD1 or anti-PD-L1 antibodies. Based on the previous studies with these agents and our preliminary results it would appear that together with pIL-12 therapy an additive or even synergistic effect could be achieved leading to a more effective therapy for metastatic melanoma. Therefore, in this aim, we will examine the limits of this therapeutic approach with respect to distant and regional disease.

C.4.2 Evaluate combination therapy to effectively treat distant cutaneous metastases and regional disease

*C.4.2.1 Preliminary studies:* Experiments were performed to evaluate if GET of IL-12 could be used to block the formation of new tumors prior to the regression of the primary tumor. Three days after mice received an injection B16.F10 cells in the left flank a second injection of B16.F10 cells was administered to the right flank. Mice were evaluated for regression of the first tumor as well as prevention of establishment of the second tumor. Secondary tumors developed in 50% of mice receiving two or three IT GET treatments. 100% of mice receiving IT injection of IL-12 plasmid without electroporation, 87.5% of the no treatment group and 75% of the mice receiving an IT injection of control plasmid followed by electrotransfer developed secondary tumors. Only mice receiving three IT GET treatments compared to the 3 control groups showed a significant increase ($p < 0.05$) in survival (Figure 5). Mice receiving only two IT GET treatments showed no differences from any of the other groups[68].

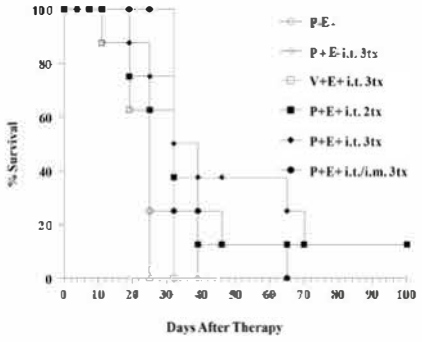



Figure 5: Prevention of Second Tumor Induced Prior to Initiation of Therapy. Two tumors established one on each flank and only tumor on left flank treated. Percent survival of mice following treatment. P=pIRES IL-12; V=control plasmid, pND2Lux; E=electroporation.

*C.4.2.2 Research Design:* We will utilize the best protocol from Specific Aim 2 for each of the three plasmids tested individually or in combination with pIL-12 delivery. *This is based on the assumption that one of the dosing combinations for each plasmid will show a significant effect. In the event that one or more of the plasmids does not show a significant anti-tumor effect then it will not be included in the study conducted in this aim. In addition, antibodies for each of the three will be used as controls.* The anti-CTLA-4, anti-PD-1 and anti-PD-L1 will be injected IP at doses of 250 µg for anti-PD-1 and anti-PD-L1 and 100 µg for anti-CTLA-4. B16.F10 melanoma cells will be injected in the left flank of mice and three days later the same number of cells injected into the right flank. Once tumors have formed on both sides, mice will be divided into 15 groups of 8

mice each (Table 4). GET IL-12 will be performed only on the left flank tumor. By combining with the plasmids encoding anti-CTLA-4, anti-PD-1 or anti-PD-L1 *we predict that that percentage of animals that are tumor free or have significantly reduced growth can be increased compared to treating with pIL-12 GET alone (preliminary data).* Mice will be monitored twice weekly for health status and size of tumor(s). Mice will be followed for 100 days or until tumors reach 1,000 $mm^3$ at which point they will be humanely euthanized.

Table 4.

| GROUP | AGENT | GET |
|---|---|---|
| 1 | None | N |
| 2 | pIL-12 | N |
| 3 | pAnti-CTLA4 | Y |
| 4 | pAnti-PD-1 | Y |
| 5 | pAnti-PD-L1 | Y |
| 6 | pIL-12 | Y |
| | pAnti-CTLA4 | Y |
| 7 | pIL-12 | Y |
| | pAnti-PD-1 | Y |
| 8 | pIL-12 | Y |
| | pAnti-PD-L1 | Y |
| 9 | pIL-12 | Y |
| 10 | pIL-12 | Y |
| | Anti-CTLA-4 Antibody | N |
| 11 | pIL-12 | Y |
| | Anti-PD-1 Antibody | N |
| 12 | pIL-12 | Y |
| | Anti-PD-L1 Antibody | N |
| 13 | Anti-CTLA-4 Antibody | N |
| 14 | Anti-PD-1 Antibody | N |
| 15 | Anti-PD-L1 Antibody | N |

We next will evaluate multiple lesions to better simulate the clinical situation. Three tumors will be established on the left flank and one on the right. Two lesions on the left flank will be treated with IL-12 GET. For the combination therapy we will utilize the combination(s) that worked the best in the two tumor model and the treatment timing that worked the best. We anticipate that there will be 6 groups (8 mice/group). The other aspects of follow up will be the same as described above.

Once the appropriate therapy is established, both experiments will be repeated (using controls and only treatment that worked) to evaluate the immune response. Assume there will be 8 groups and 18 mice/group. The timing for evaluation will be based on results from Specific Aim 1 and will include three time points. At specified time points after treatment, six mice from each group will be humanely euthanized, tumors (and surrounding skin) and spleens removed and blood collected for both serum and peripheral mononuclear cells (PBMCs). The evaluation will be the same as described in Specific Aim 1 Section C.2.1.3.

*C.4.2.3 Anticipated results:* It is anticipated that there will be a reduction in the growth of untreated tumors both distant and regional. A successful protocol will be one that results in a disease free survival for 100 days in at least 50% of treated mice. Following therapy, it is anticipated that there will be a large lymphocytic infiltrate at treated and untreated tumors. The infiltrate will appear later at the untreated site versus the treated site. Analysis of lymphocyte populations should reveal an increase in activated and decrease in T-reg cells (69). We would also expect at the later time points to see an increase in T cells with a memory phenotype.

*C.4.2.4 Potential problems and alternatives:* One possibility is that combination therapy may not lead to increased response of untreated tumors. One reason could be related to the timing between IL-12 delivery and pAnti-CTLA4, pAnti-PD-1 or pAnti-PDL1 treatment. If this is the case, different timings will be attempted. To enhance the therapeutic effect, we may also administer the antibody expressing plasmids at other doses established in Specific Aim 2 as well as test additional combinations (more than 2 plasmids) i.e. pIL-12, pAnti-CTLA4 and pAnti-PD-1. We may also need to repeat the therapy an additional time to achieve the appropriate response. Another possibility is that the combinations could result in detectable toxicity. If this is the case, we will also modify the dose and/or the timing of administering the two therapies.

C.4.3 Determine best protocol to effectively treat distant visceral metastases in a metastatic model

*C.4.3.1 Preliminary studies:* C57Bl/6 mice were injected with $10^5$ B16-F10 cells via the tail vein. Immediately after injection, mice were administered an intramuscular (IM) treatment with 50 µg of plasmid encoding IL-12 and GET. Four days later a second treatment was performed. Mice were humanely euthanized 21 days later and their lungs examined for tumor nodules. Eighty-seven percent (7 out of 8) untreated mice and 75% of those receiving an IL12 plasmid without GET developed lung nodules. By contrast, only 37.5% (3 out of 8) of mice receiving IL-12 by GET developed lung nodules.

*C.4.3.2 Research Design: Mice will have standard B16.F10 cells or B16.F10 that stably express luciferase injected subcutaneously behind the ear. This will establish a cutaneous lesion and metastatic spread from the injection site[76]. Subcutaneous tumors will receive pIL-12 GET as part of the combination therapy. The groups will be those that had a positive response in 4.2.2 and were used in the multiple tumors and immune response assessment. We assume with controls that there will be 8 groups. There will be a total of 22 mice/group. The purpose is to determine which treatment results in largest reduction of metastatic spread. Protocol will be done first with B16.F10 cells with luciferase (6 mice/group) to document metastatic spread and response. Then repeated with standard B16 cells (16 mice/group) to determine if there was an adjuvant effect of luciferase. Using the luciferase expressing cells will enable monitoring mice multiple times to determine the course of metastatic spread and efficacy of treatment and establish timeline of spread and response that will facilitate timing when using standard B16.F10 cells. Mice will be followed for up to 42 days and evaluated twice per*

*week for health status and through the use of whole body IVIS imaging to determine metastatic spread. Mice observed to have significant level of metastases will be euthanized prior to 42 days.* At 7 and 14 days 5 mice (non-luciferase) will be euthanized and blood and tissue taken to evaluate immune response as in previous aims.

*To fully evaluate the effectiveness of this therapeutic approach, a model that will more closely match the immune competence that would potentially be seen in an expected patient will be tested. To accomplish this assessment, the metastatic experiment will be repeated using one year old mice instead of the 6-7 week old mice used in the previous specific aims. For this experiment we will utilize the combination(s) that worked the best in the first metastatic experiment above (assume 5 groups including controls with 20 mice/group).* Timing and evaluations will be the same as described above except at 7 and 14 days 5 mice will be euthanized and blood and tissue taken to evaluate for an immune response as in previous aims.

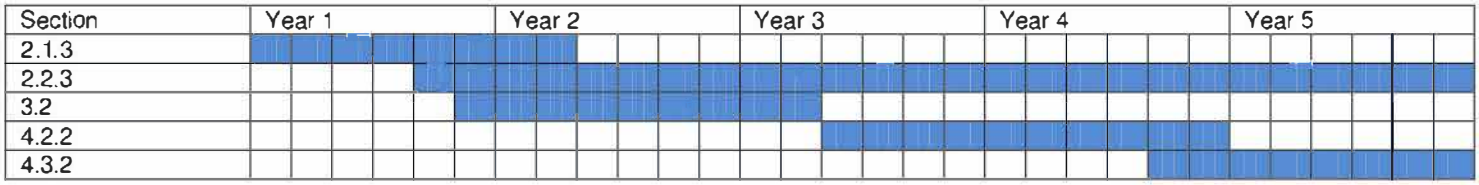
*C.4.3.3 Anticipated results:* It is anticipated that the combination of pIL-12 GET and antibody therapy will result in fewer metastases and greater survival. Previously, treating via an IM route we found no lung colonies in 62.5% of mice, however, we did not test the potential for treating when both cutaneous lesion and metastases were present. We believe that the IT route will have a lower potential for toxicity. We anticipate that combining pIL-12 GET IT with plasmids encoding the antibodies will be as effective as or better than the IM route and will be more reflective of the clinical situation. This will be more apparent in the more difficult to treat metatstatic model. There will be a correlation between local responses at the subcutaneous lesion and absence or reduction of metastatic spread. The analysis will compare the effectiveness of this approach on treated and untreated distant lesions. These results will define how this therapy may be used in the future.

*C.4.3.4 Potential problems and alternatives:* It is possible that the combination does not result in reduction in *metastatic spread*. As with the cutaneous approach this could be related to timing or dose. If either of these is the case we will modify dose or timing and repeat the experiment. It is also possible that the IM route is found to be more effective than IT route alone. If this occurs, we will add IM route to other combinations and repeat. *It is possible that we do not get sufficient metastatic spread in the "ear" model. If this occurs, we will utilize either Braf transgenic mouse model that was developed at UCSF that will facilitate multiple lesions and eventual metastatic spread[77,78] or inject B16.F10 cell intraperitoneally together with subcutaneous injection[79].*

## C.5. Summary

*While there have been major advances in gene therapy, improving clinical responses is still a significant hurdle.* We strongly contend that finer control over transgene expression can directly lead to improved therapeutic outcome. To achieve this control by manipulation of delivery parameters would be an important advance in the field. We believe this is possible with the use of GET. In this proposal, we will utilize this approach to enhance immunotherapy of malignant melanoma, a life threatening disease without an effective therapy. We have demonstrated that utilizing GET to deliver a plasmid encoding IL-12 to achieve the correct expression pattern can result in positive therapeutic outcome. IL-12 is a potent immune stimulator, but in previous trials utilizing recombinant protein it was found to cause serious adverse events. We have demonstrated a way to harness the immune stimulating properties of IL-12 while reducing or eliminating the associated toxicity. *While we have demonstrated success, there is still a need for improvements to benefit more patients and to enhance the effectiveness in patients with distant metastatic disease. By increasing our understanding of the events occurring within the tumor microenvironment following treatment and combining pIL-12 therapy with other immune stimulating approaches will result in improvements of this therapeutic approach. An exciting aspect of this study is the availability of clinical samples from melanoma patients treated with pIL-12 GET. By evaluating clinical samples we can determine if there is any correlation between mouse model results and what is being observed clinically. The investigative team includes a Medical Oncologist (Dr. Daud) who is an expert in melanoma; a Medical Oncologist (Dr. Fong) who is an expert in immunotherapy and an expert in gene transfer (Dr. Heller) who has developed the use of GET.*

## C.7 Timeline



**Human Subjects Research**

Samples from a clinical trial will be collected for evaluation in this study (Specific Aim 1). This will include blood and tumor biopsies. Samples will be coding and blinded. Samples analyzed at ODU will not have patient identification. The clinical trial itself is not part of this grant application.

Dr. Daud a Co-Investigator on this application is the principal investigator and sponsor of the ongoing clinical trial that will be the source of samples. Dr. Daud holds the IND (IND-BB 11322) for this clinical trial. pUMVC3-hIL-12 is an investigational plasmid that was has been investigated in a phase I setting with an acceptable toxicity profile. pUMVC3 is the backbone plasmid. Both are manufactured under GMP grade facilities at the City of Hope Biotechnology Facility, Duarte, CA.

*Although the clinical trial is not part of this proposal, since we will be evaluating samples obtained from the clinical study we have included all of the information pertaining to the trial.*

## Protection of Human Subjects

RISKS TO THE SUBJECTS

**a. Human Subjects Involvement and Characteristics**

**i. Proposed involvement of human subjects**

Human subjects are being asked to participate in this study if they have metastatic melanoma. Their decision whether or not to participate has no effect on the quality of medical care that is provided. The purpose of this research study is to see if treatment with pUMVC3-hIL-12 can induce the immune system to target melanoma. We will evaluate the response to treatment in 50 patients with metastatic melanoma. Other standard therapies for patients with metastatic melanoma include chemotherapy.

**ii. Patient Population**

We plan to enroll up to 50 patients with metastatic melanoma and a good performance status. Subjects enrolled in this study will be seen in the UCSF Cutaneous Oncology Clinic / Melanoma Center by Dr. Daud or one of his staff.

ii.1 Inclusion Criteria:

- Pathologically documented malignant melanoma with AJCC Stage IIIB-C or IVA disease with cutaneous tumor nodules accessible to electroporation.
- >18 years old
- ECOG Performance Status ≤2
- Any prior therapy must be stopped 4 weeks prior to electroporation
- ≥2 and ≤4 tumors may be treated, any number of tumors may be present. Patient must have at least 2 metastatic tumor nodules.

ii.2 Exclusion Criteria:

- Any significant active infection at the time of study (e.g. pneumonia or cellulitis)
- Creatinine > 2 mg/dl, serum bilirubin > 1.4 mg/dl
- Absolute neutrophil count <1000/mm3, platelets <100,000/mm3
- Pregnant or breast feeding women are excluded because effects of this treatment on the fetus or passage through milk are unknown
- Women of child bearing potential must use highly effective contraception and have a negative serum or urine pregnancy test. Men who have a partner of child bearing potential must use highly effective contraception.
- Patients with electronic pacemakers or defibrillators are excluded. Caution should be used in patients with a known history of seizure disorder or serious cardiac tachyarrhythmia or bradyarrythmia. Any patients with Seizure within the past 5 years are excluded. Patients with a history of ventricular arrhythmia, cardiac asystole or life threatening atrial arrhythmia are excluded.

ii.3 Inclusion of special classes of subjects. Patients that are incarcerated or institutionalized may be considered for this study so long as they meet the inclusion and exclusion criteria.

ii.4 Collaborating sites: The patient recruitment, treatment, and clinical management will be performed at the UCSF Cutaneous Oncology Clinic / Melanoma Center. UCSF will perform immunologic monitoring assays on samples derived from the patients.

**iii. Sources of Materials**
Blood will be obtained from subjects by standard phlebotomy. Data will include radiologic studies, blood tests and physical exam findings. Archived tissues derived from prior biopsies/resections will be requested where available. Subjects demographics, clinical data, medical history, clinical response to treatment, toxicity, and immune response will be recorded in a secure database. Clinical outcomes will be collected from the medical record by trained physicians, nurses and clinical research assistants The clinical and correlative biological outcome data will be linked to subjects as they are primary and secondary outcomes of the study. Coded subject identifiers will be generated by the investigators. Only those investigators and medical personal that need to know the identity of subjects for clinical care will have access to direct source data.

**iv. Potential Risks**
**Risks Associated with pUMVC3-hIL-12 Treatment**
In a phase I clinical trial, in vivo electroporation with pUMVC3-hIL-12 was associated with minimal systemic toxicity. No dose limiting toxicity has been noted to date in patients treated with pUMVC3-hIL-12. No hematologic abnormalities have been observed.

**Likely(occurring in > 20% of patients):**
- Pain during the electroporation procedure
- Mild to moderate localized bleeding at the treatment site

**v. Alternative Therapies**
Melanoma is resistant to many commonly used cytotoxic drugs. The single most active agent in melanoma is the alkylating agent, dacarbazine (DTIC). Unfortunately, even dacarbazine is only modestly effective. Other chemotherapy agents such as organoplatinum complexes, vinca alkaloids, taxanes and nitrosoureas have shown limited efficacy. Combination chemotherapy regimens such as the cisplatin, dacarbazine, BCNU and tamoxifen combination (referred to as the Dartmouth Regimen) was not more effective than DTIC alone In randomized trials. Similarly, biochemotherapy, a combination of multiagent chemotherapy with IL-2 and Interferon-α 2B has also not shown a survival advantage over chemotherapy alone in prospective randomized trials. High dose IL-2 is highly toxic, but yields objective tumor responses in 17% of patients with approximately 5% of patients enjoying durable long term remissions. Other alternatives include: supportive care or participation in another investigational treatment.

ADEQUACY OF PROTECTION AGAINST RISKS
**a. Recruitment and Informed Consent**
**i. Subject Recruitment.** Subjects enrolled in this study will be those seen in the UCSF Cutaneous Oncology Clinic / Melanoma Center by Dr. Daud or one of his staff. Subjects will be reviewed for eligibility for this study during their new patient appointments or follow-up appointments. In order to identify which subjects to approach for this study, it is necessary for research nurses from the study team to access medical records prior to screening them. Once it has been ascertained that subjects are eligible for the study, we will obtain their authorization to use PHI for this study and we will obtain their informed consent.

**ii. Consent Process.** All subjects who choose to participate in this study will sign a Subject's Authorization for Research and an IRB-approved consent prior to beginning study treatment. Subjects may take the informed consent home to review prior to signing and may take as long a time as necessary to make an informed decision. The treating physician will review the consent form item by item with prospective subjects. Subjects are required to verbalize their understanding of the risks/benefits. Subjects may sign the consent form at the clinic during their new patient appointment or follow-up appointment. The subject will be given a copy of the signed consent and the original copy will be a part of the permanent medical record. Each subject will be consented by his research physician.

**b. Protection Against Risk**

**i. Oversight and Monitoring Plan.** The UCSF-CCC Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-CCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data in each cohort
- Quarterly review for progress and safety
- Review of all serious adverse events
- Minimum of a yearly audit

**ii. Monitoring and Reporting Guidelines.** Investigators will conduct continuous review of data and patient safety at monthly study group or site committee meetings where the results of each patient's treatment are discussed and the discussion is documented in the minutes. The discussion will include the number of patients, significant toxicities as described in the protocol, doses adjustments, and observed responses. Quarterly summaries will be submitted to the DSMC for review. All grade 3-5 AEs and SAEs will be entered in the UCSF-CCC Velos eResearch database.

**iii. Review and Oversight Requirements**

**1. Adverse Event Monitoring**

Adverse Events (AEs) will be recorded on the Velos eResearch database, all grade 3-5 expected and unexpected AEs will be recorded and updated at each visit.

**2. Serious Adverse Event Reporting**

Serious Adverse Event reporting will be in accordance with the UCSF- Committee on Human Research Regulations and Code of Federal Regulation Title 21 Volume 5 Part 312.32.
UCSF CHR website for guidance in reporting serious adverse events http://www.research.ucsf.edu/chr/Guide/chrA_AE.asp

FDA website for guidance in reporting serious adverse events
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32

MedWatch forms and information: http://www.fda.gov/medwatch/getforms.htm
Serious Adverse events will be reported on the MedWatch form. A copy of the MedWatch report and CHR forms must be sent to CCC- DSMC at Box 1297. The date the SAE was sent to all required reporting agencies will documented on Velos eResearch, hard copies of the report will be maintained in the regulatory files.

If the SAE is death, and is determined to be possibly, probably or definitely related to the investigational drug or any research related procedure, the event must be reported to the DSMC Chair or his designee with in 24 business hours. The reporting procedure is by personal communication via phone or in person with written documentation of the one-on-one communication via e-mail with a copy of the e-mail to DSMC Administrator and DSMC Coordinator.

ii. Review of Adverse Event Rates. If the study has an increase of unexpected or expected Adverse Event grade 3 or 4 above the rate reported in the Investigational Brochure or package insert. The increase rate of AEs will be reported to the DSMC at the time of Identification. The Chair and PI will discuss the finding and proceed with a written course of action. Each quarterly report will indicate if the AE incidence is within the scope of the investigational brochure or package insert. If at any time the Investigator stops enrollment or stops the study due to safety issues the DSMC Chair and Administrator must be notified within 24 business hours via e-mail. The DSMC must receive a formal letter within 10 business days and the CHR must be notified. If any of the above action occurs in a multiple-institutional clinical trial coordinated by the UCSF-CCC, the Study Coordinator will insure that all participating sites are notified.

**iv. Serious Adverse Events**

A serious adverse event is an undesirable sign, symptom or medical condition which:

1. is fatal or life-threatening
2. required or prolonged hospitalization
3. results in persistent or significant disability/incapacity
4. constitutes a congenital anomaly or a birth defect
5. is medically significant, may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events not considered to be serious adverse events are hospitalizations for the:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen
- treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission.

Pregnancy, although not itself a serious adverse event, should also be reported on a serious adverse event form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication). Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to study drug (or therapy) is suspected.

**v. Study Progress – Quarterly Review**

Principal Investigators are required to submit quarterly study progress reports regarding the trial to the DSMC. These reports must include an update on accrual, information about any new amendments or updated consents and a summary of grade 3 and 4 toxicities (expected and unexpected) and all internal SAE reports. At the time of the quarterly report, all external DSMB reports and/or external formal audit reports that were received during the reporting quarter are to be sent to the committee. These quarterly reports are reviewed at Data Safety Monitoring Committee meetings. These reports are required: February 1, May 1, August 1, and October 1. Failure to submit such reports may result in trial suspension.

**vi. Special Safety-Related Procedures**

**Instructions for Rapid Notification of Serious Adverse Events**

The principal investigator of the clinical trial has the obligation to report all serious adverse events to the FDA, IRB, and the Epidemiology department.

In IND studies, all serious adverse events must be reported to the FDA by the investigator as required by 21 CFR 312.32. These reports are to be filed utilizing the Form FDA 3500A (MedWatch Form), available at http://www.accessdata.fda.gov/scripts/medwatch/. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

In addition, any serious adverse event occurring in a patient after providing informed consent, while receiving study drug, and until four weeks after stopping study drug must be reported by fax or email to the manufacturer of pUMVC3-hIL-12 within 24 hours of learning of its occurrence, even if it is not felt to be drug related.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

**Instructions for Completing Adverse Event Case Report Forms**

Each adverse event is to be reported on an Adverse Event Case Report Form. Refer to the Case Report Form or to the Case Report Form Completion Guideline for details.

**vii. Protocol Amendments, Other Changes in Study Conduct**

Any changes to the protocol will be made in the form of an amendment. Changes in study conduct are not permitted. Any unforeseen changes in study conduct will be recorded in the clinical study report. All changes must be reviewed and approved by the manufacturer of pUMVC3-hIL-12.

**viii. Protocol Modifications/Deviations**

In the event of any modification(s) the protocol and/or informed consent form, the Institutional Review Boards (IRB) at the University of California, San Francisco.

All modifications must detail the necessity for the change and be signed by the Principal Investigator prior to local IRB and FDA amendment submission. A revised protocol and informed consent form will also be submitted, as applicable. Local IRB must be obtained before patients can be accrued onto the amended protocol.

Inadvertent protocol deviations may impact patient safety and eligibility for continuation in the study. Upon discovery, all protocol deviations must be reported to and reviewed by the Principal Investigator and by the Medical Monitor. The deviation and outcome must be reported to the local IRB.

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

The potential benefit to research subjects participating in this study is shrinkage or disappearance of their cancer (response) and perhaps improved survival. Because electroporation using pUMVC3-hIL-12 has yielded objective tumor responses in a phase I clinical trial, there is a possibility that the patients will benefit from participating in this research. Safeguards have been built into the trial such that if no reasonable possibility of observing a benefit occurs, then the trials will be stopped early.

IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

If clinical efficacy of the procedure that is the subject of this proposal can be demonstrated by the research proposed, this would be an important advance as the currently available treatments for patients with metastatic melanoma are not efficacious. Moreover, by identifying antigens that are relevant for immune responses to IL-12, we may develop surrogate markers that may either predict responders to treatment or be used to assess the immunologic efficacy of treatment. Moreover, identified antigens could one day represent novel vaccine candidates.

The risks to subjects are reasonable in relation to the importance of the knowledge that reasonably may be expected to result because metastatic melanoma is an incurable disease with limited treatment options. The potential treatment-associated toxicities observed thus far after electroporation with pUMVC3-hIL-12 are mild, transient, and manageable.

BIOHAZARD

Given that plasmid IL-12 and UMVC3 will be used, the IBC (Institutional Biosafety Committee) and NIH OBA guidelines will be followed. Plasmid disposal will be done in biohazard containers and any deviation will be reported. Plasmid will be stored in secure freezers. Any breakdown in the freezer will be reported. Sterile technique including sterile gloves, gowns and masks will be used for all injections. Any spills will be reported to the IBC and OBA.

## Inclusion of Women and Minorities

There will be no exclusion on the basis of gender or minority. The composition of the trial population will reflect the demographics of the San Francisco bay area. This includes: 30% Hispanic Americans, 20% Asian Americans, 8% African Americans, and 42% Caucasian Americans. Melanoma, however, is known to occur less frequently in African Americans so this group will be underrepresented in the study cohort.

OMB Number: 0925-0002

# Planned Enrollment Report

**Study Title:** A Multicenter Phase II Trial of Intratumoral pIL-12 + Electroporation in Advanced Stage Cutaneous and In Transit Malignant Melanoma

**Domestic/Foreign:** Domestic

**Comments:**

| Racial Categories | Ethnic Categories | | | | Total |
|---|---|---|---|---|---|
| | Not Hispanic or Latino | | Hispanic or Latino | | |
| | Female | Male | Female | Male | |
| American Indian/Alaska Native | 0 | 0 | 0 | 0 | **0** |
| Asian | 2 | 4 | 0 | 0 | **6** |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | **0** |
| Black or African American | 0 | 0 | 0 | 0 | **0** |
| White | 16 | 18 | 4 | 6 | **44** |
| More than One Race | 0 | 0 | 0 | 0 | **0** |
| **Total** | **18** | **22** | **4** | **6** | **50** |

Study 1 of 1





Funding Opportunity Number: PA-13-302. Received Date: 2014-03-04T18:30:26.000-05:00

## Inclusion of Children

No subjects under the age of 18 will be enrolled. Children over 18 will be allowed entry into this trial. Metastatic Melanoma is very unusual in children under 18 years of age.

## VERTEBRATE ANIMALS

### 1. Description of proposed animal work

The mice involved in this study will be used to test a new waveform that could be used to deliver plasmid DNA to solid tumors. C57Bl/6 and C57 albino mice will have B16.F10 murine melanoma tumor cells injected into their flanks to elicit tumor formation. The procedure will be performed as follows: to produce solid tumors, $10^6$ B16.F10 cells in sterile saline in a total volume of 0.05 ml will be injected subcutaneously into flanks of mice. The mice will be monitored until tumors are formed approximately 7-10 days after injection. For lung colonization experiments, $10^5$ B16-F10 cells that have been stably transfected with a luciferase gene will be diluted in sterile saline in a total volume of 0.05 ml and injected into the tail vein of C57 albino mice.

Following tumor formation these mice will be placed into several groups as described in the experimental design. These groups include both treated and untreated controls. Plasmid DNA, when applicable, will be injected directly at the tumor site using a 25 gauge, 3/8 inch length needle. All $E^+$ animals will be anesthetized with a mixture of 3% isofluorane and 97% oxygen prior to receiving electric pulses. The animals will be monitored carefully until the anesthesia wears off and then routinely through the course of the experiment.

For the experiments described in this study C57Bl/6 or C57 albino mice will be used. The mice will be 6-7 week old female or male. The number of animals needed for this study is 1161 C57Bl/6 and 193 C57 albino mice and 110 aged C57Bl/6 mice. The breakdown is described below.

Specific Aim 1 is divided into two parts.
The first part described in Section C.2.1.3 will evaluate the immune response following delivery of plasmid IL-12 with GET. The concept is to determine events occurring in the tumor microenvironment. The other aspect is to determine if a marker can be delineated that would indicate when proper delivery was achieved that would lead to an appropriate response. This will require 6 groups (Table 2) with 24 mice/group. Six mice will be evaluated at each of the four time points. This experimental set will require 144 mice. **Total of 158 (144 +10% to account for possible mice with no tumor) C57Bl/6 mice will be needed for C.2.1.3.**

The second part of Specific Aim 1 is described in Section C.2.2.3 and is designed to evaluate samples from an ongoing clinical trial. There will be no mice needed for this part of the study.
**Total of 158 C57Bl/6 mice will be needed for Specific Aim 1.**

Specific Aim 2 is designed to evaluate delivery of plasmids encoding anti-CTLA-4, anti-PD-1 and anti-PD-L1. There will be three experimental sets performed and both are described in Section C.3.2. The first experimental set is to determine expression patterns of each for the three plasmids following GET delivery to either skin or muscle. Mice will be divided into 8 groups as described in Table 4 for each plasmid and there will be 8 mice in each group. This experimental set will require 192 mice. The second aspect to be evaluated is to determine the potential of multiple treatments. This will include the same groups and number of mice/group. This experimental set will require 192 mice. The final experimental set will evaluate the anti-tumor response following treatment with these plasmids. Mice will be divided into 6 groups with 12 mice in each group for each of the three plasmids. At two time points, 4 mice will be evaluated for immune response. This experimental set will require 216 mice. **Total of 660 (600 +10% to account for possible mice with no tumor) C57Bl/6 mice will be needed for C.3.2.**

Specific Aim 3 is divided into two parts.
The first part described in Section C.4.2.2 will evaluate the combination of GET IL-12 together with the plasmids tested in Specific Aim 2. The first aspect is to evaluate the combination in a two tumor model with a tumor on each flank and only one tumor treated. Mice will be divided into 15 groups as described in Table 4. There will be 8 mice in each group. This experimental set will require 120 mice. The second aspect to be evaluated is to determine the effectiveness of the therapy on a multiple tumor model with three lesions on one flank and one on the opposite flank and two lesions treated. Mice will be divided into 6 groups with 8 mice in each group. This experimental set will require 48 mice. The final experimental set will evaluate the immune response following the combination treatment. Mice will be divided into 8 groups with 18 mice in each group. At three time points, 6 mice will be evaluated for immune response. This experimental set will require 144

mice. **Total of 343 (312 +10% to account for possible mice with no tumor) C57Bl/6 mice will be needed for C.4.2.2.**

The second part of Specific Aim 3 is described in Section C.4.3.2 and is designed to evaluate the ability to treat visceral disease utilizing the combination of GET IL-12 together with plasmids encoding either anti-CTLA4, anti-PD-1 or anti-PD-L1. The first experimental set will evaluate blocking lung colonization (B16.F10 cells injected intravenously) when a cutaneous tumor is treated. Mice will be divided into 8 groups as described in the experimental plan. There will be 22 mice in each group. This experimental set will require 176 mice. The other experiment in this part of Specific Aim 3 will evaluate utilizing this therapeutic approach to treat older mice with existing disease. Mice will be divided into 5 groups with 20 mice in each group. This experimental set will require 100 mice. **Total of 193 (176 +10% to account for possible mice with no tumor) C57 albino mice will be needed for C.4.3.2 and a total of 110 aged C57Bl/6 mice.**
**Total of 343 C57Bl/6 and 193 C57 albino and 110 aged C57Bl/6 mice will be needed for Specific Aim 3.**

Therefore, the requirements for this project for are: 158 C57Bl/6 mice for aim 1, 660 C57Bl/6 mice for aim 2 and 343 C57Bl/6 mice 193 C57 albino mice and 110 aged C57Bl/6 mice for Aim 3 for a total of **1161 6-7 week old C57Bl/6 mice; 193 6-7 week old C57 albino mice; 110 aged C57Bl/6 mice.**

This number of animals is the minimum number that can be done to obtain adequate data to perform statistical analysis. The results will be expressed as the mean+/- standard error of the mean. The difference between two groups will be tested by the Student's t-test. Differences among more than two groups will be tested by ANOVA. Multiple comparisons between groups within any one group will be performed with a Bonferroni modification of the t-test. A value of $p < 0.05$ will be considered significant. To determine the number of animals required to obtain statistical significant we utilized PS version 2.1.31 (computer program) and Power Sample Size Analysis was done to obtain a p-value $\leq 0.05$ with a power of 0.8.

**2. Justification of animal use**

The research is designed to determine if a new immunotherapeutic approach would be effective for treating metastatic melanoma. Previous work utilizing this approach has been performed using the C57Bl/6 murine melanoma model (B16-F10 cells with C57Bl/6 mice). In preliminary studies, this model has been shown to be accurate to determine translatability of a therapeutic approach. In previous preclinical studies, using this approach and model it was shown that regression of the primary tumor (treated lesion) would regress when treated and that if the plasmid was delivered appropriately about 70% of the mice were protected from new tumors forming. In a Phase I clinical study in a single dose cohort it was also shown that over 70% of the lesions that were treated completely regressed and in 66% of the patients untreated tumors went away and new tumors did not form. With respect to the therapeutic response, there was some correlation between findings in this mouse model and in a small sample of patients in a Phase I study. In this project, we will further examine this therapeutic approach and look for immune correlations between the mouse model and treatment of melanoma patients. Therefore this model has been chosen. The number of animals used has been minimized by working with small experimental groups. Additionally, a medline search was performed to find alternatives. The work performed during this project will yield important information that can be used for the future development of new therapies for skin malignancies and other cancers.

**3. Veterinary care**

Animals will be housed, fed and watered at the Old Dominion University Vivarium. This is a new state-of-the-art 10,000 sq ft animal facility located in the same building as the Center for Bioelectrics. The OLAW and AAALAC certified facility includes several procedure rooms as well as an imaging suite and surgical facilities. The facility is staffed by SoBran, Inc. (Fairfax, VA) per NIH guidelines. SoBran has 13 years of experience furnishing the onsite management and professional personnel necessary to perform the duties associated with animal research support services. They are the largest provider of onsite laboratory animal research support at the National Institutes of Health. The animals will be cared for under NIH guidelines. The vivarium is well staffed and the animals receive daily care. The vivarium employs a full time veterinarian.

**4. Minimizing discomfort**

The procedures involved in this project will not cause the animals any pain or discomfort. The animals will always be treated humanely and will be handled in a gentle manner. Anesthesia will be used prior to exposing

the tumors to the electrical fields to allow manipulation of the mice without trauma. General anesthesia will be administered using isoflurane. Mice will first be placed in an induction chamber that is charged with a mixture of 3-4% isoflurane in oxygen for several minutes. These mice will then be fitted with a standard rodent mask and kept under general anesthesia using a mixture of 2-3% isoflurane and oxygen.

**5. Euthanasia**

At the completion of this study, all the mice will be humanely euthanized. This will be accomplished by exposure to $CO_2$. Following cessation of respiratory and cardiac movements a thoractomy will be performed. This form of euthanasia adheres to guidelines of the AVMA panel.

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**Consortium**

This project involves a consortium of two institutions: Old Dominion University and University California San Francisco. Two of the investigators (Heller and Daud) on this project have a history of working together when they were both at the University of South Florida and the H. Lee Moffitt Cancer Center and Research Institute. Drs Daud and Fong have been collaborating for several years. Letters of Support are included in the application to show the commitment of each investigator.

The project will be led by the PI, Dr. Richard Heller. He will have overall responsibility for overseeing the project. Dr. Heller will be directly responsible for directing the work done in Specific Aims 1-3. These are all of the murine studies that will be performed at Old Dominion University. He will also oversee the analysis of the clinical samples collected by Dr. Daud at UCSF. All three investigators will discuss the results and the significance with respect to clinical potential. Dr. Fong's immunology expertise will be critical for determining the significance of the immunological response data.

Given the established connections among the consortium members and the clearly defined roles that each member has in the project, we expect this research consortium to have a productive structure and collegial relationship.

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February 18, 2014 P0062199

Old Dominion University Research Foundation
PO Box 6369
Norfolk, VA 23508

Dear Mr. Facenda:

We are presenting for your review a request for support of the following project:

Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression

PRINCIPAL INVESTIGATOR: Adil Daud, MD

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the pertinent Federal regulations and policies and are prepared to establish written inter-organizational agreements that will ensure compliance with all such policies. The investigators shall comply with all applicable regulatory requirements, conform to current recommendations of the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and the Occupational Safety and Health Administration (OSHA) with respect to minimum standards, and adopt additional biological safety policies as appropriate. All studies conducted on animal subjects or material will be subject to approval by the UCSF Committee on Animal Research. All studies on human subjects will be subject to approval by the UCSF Committee on Human Research, UCSF's Institutional Review Board. The University of California, San Francisco submits this proposal with the understanding that it is unclassified, fundamental research as defined by the Export Control regulations. As such there should be no limitation on the freedom to publish research results or restrictions on the citizenship or national origin of those performing the research.

We reserve the right to negotiate the final contract. Your favorable consideration will be appreciated.

Any Award documentation or correspondence should be sent directly to:
Krista Roznovsky
Contracts and Grant Officer
UCSF – Office of Sponsored Research | Research Management Services
3333 California Street, Suite 435 | San Francisco, CA 94118
Telephone: (415) 502-0690 | e-Mail: CGAwardTeam@ucsf.edu

Any financial payments should be sent directly to:
The Regents of the University of California
Mail Remittance Cashier; Accounting Office UCSF :
1855 Folsom Street, Suite 425 | San Francisco, CA 94143-0815
Tax ID Number: 94-6036493W
Jean DeMartini – Accounts Receivable Manager | Telephone: (415) 476-9641 | e-Mail: jean.demartini@ucsf.edu

Please direct questions to Martha White at martha.white@ucsf.edu.

Sincerely,

Digitally signed by Krista Roznovsky
DN: cn=Krista Roznovsky, ou=UCSF, ou=Research Management Services, email=Krista.Roznovsky@ucsf.edu, c=US
Date: 2014.02.21 13:24:28 -08'00'

Krista Roznovsky
Contracts and Grants Officer

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Please direct questions to Martha White at martha.white@ucsf.edu.

Sincerely,

Krista Roznovsky
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February 18th, 2014

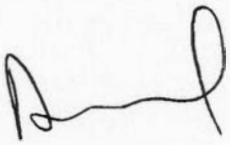
Richard Heller Ph.D.
Director Frank Reidy Research Center for Bioelectrics
Professor, School of Medicine Diagnostics and Translational Science
Old Dominion University
4211 Monarch Way, Suite 300
Norfolk, VA 32508

Dear Rich,

I enthusiastically support your revised NIH R01 application titled " Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression" and am pleased to act as co-investigator for the proposed work. As a collaborator with you over the last decade in clinical and translational research into delivery of DNA using electroporation in vivo, I have been impressed at the efficacy of this method in inducing an immune response leading to tumor regression and efficient expression of cytokines in the tumor microenvironment in human subjects. I look forward to collaborating with you in better understanding and exploiting this technique to someday provide melanoma patients with a desperately needed option for safe and effective treatment. Your R01 application, I believe, holds promise in better understanding the conditions needed for successful treatment and hence could translate to clinical trials with better outcomes.

In summary, I enthusiastically support this project and believe that it has great promise in helping understand the biology behind electroporation induced immune activation.

Sincerely,



Adil I Daud MD

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February 18, 2014

Richard Heller, Ph.D.
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Norfolk, VA 32508

Dear Richard:

I enthusiastically support your revised NIH R01 proposal entitled "Efficient Delivery of Plasmid DNA to Achieve Appropriate Transgene Expression" and am pleased to act as Co-Investigator for the proposed work. As you are well aware, my group has a long-standing interest tumor immunotherapy. We are already collaborating with you and Dr. Adil Daud on clinical trials with IL12 plasmid electroporation in melanoma. I look forward to continuing our collaboration through this application including providing advice and assistance in interpretation of the results from both the murine and human studies. Your new therapeutic approach shows great promise in inducing potent anti-tumor immune response.

This is an important project and I am excited at the prospect of working together with you. There is an urgent need to have a better understanding of the events occurring within the tumor microenvironement following treatment. You have done an excellent job in bringing together the appropriate expertise to complete the stated aims of the project. I look forward to collaborating with you on this exciting project.

Sincerely,