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TITLE: Universal Influenza T Cell-Targeted Mucosal Vaccines

PRINCIPAL INVESTIGATOR: Daniel F. Hoft, MD PhD

CONTRACTING ORGANIZATION: Saint Louis University
St. Louis, MO 63103

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Fort Detrick, Maryland 21702-5012

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### Universal Influenza T cell Targeted Mucosal Vaccines

The goals of this work are to design and test novel T cell-targeted adenoviral (Ad)-based influenza vaccines with modified vector tropism [(designed to efficiently transduce dendritic cells (DC)]. Drs. Curiel and Dmitriev (WU) generated multiple replication deficient GFP-expressing Ad vectors incorporating camelid nanobodies specific for murine antigen presenting cells (CD40, Clec9a, and others). We tested the efficiencies of each using in vitro transduction assays in both total splenocytes and CD11c+ purified DC. We identified 2 different Ad vectors with enhanced DC transduction efficiencies. Control Ad and DC-targeted Ad vectors engineered to express conserved influenza CD4 and CD8 T cell epitopes are currently being generated and will soon be tested in HLA A2/DR1 transgenic mice. Cloning of the synthetic multi-epitope influenza vaccine genes was straightforward, however, rescue and propagation of recombinant DC-targeted Ad was problematic. Drs. Curiel and Dmitriev have designed a workaround to suppress expression of the multi-epitope influenza vaccine gene during virus rescue and propagation, and we expect to have all novel vaccines ready for study within the next two months. We will determine whether mucosal delivery of these DC-directed T cell-targeted influenza vaccines provide superior immunogenicity and protection against multiple influenza subtypes in the next reporting period.
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1. **INTRODUCTION:**

Influenza remains a significant public health threat, resulting in an estimated 250,000 – 500,000 deaths per year. Influenza vaccines that induce protection against seasonal and potential pandemic influenza strains are urgently needed. We have identified multiple T cell targets that are conserved in very diverse seasonal and potential pandemic strains that are immunogenic in humans and protect humanized mice against both H3 and H1 influenza strains. Dendritic cells (DC) are the most potent inducers of T cells, thus we propose development of novel vaccines targeting these antigen presenting cells. Furthermore, because mucosal lung immunity is highly relevant for influenza, we will study the mucosal delivery of novel DC-targeted, T cell-inducing influenza vaccines. Replication deficient adenovirus (Ad) vaccines designed to target DC and induce T cells will be developed at Washington University, and tested in humanized mice for immunogenicity and protection against diverse influenza strains at Saint Louis University.

2. **KEYWORDS:**

Influenza; T cell; pandemic; Dendritic cell (DC); vaccine; Adenovirus (Ad).

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

**Aim 1 Major Tasks:** In aim 1, we proposed the development of replication-deficient Adenovirus (Ad) vectors engineered to express camlaid nanobodies specific for murine DC. GFP-expressing Ad would be generated to study cell transduction efficiencies. DC-targeted Ad would also be engineered to express highly conserved influenza A T cell epitopes which we have previously shown to be relevant for human protection against diverse influenza A infection.

**Aim 1 Milestone:** Successful generation of DC-targeted T cell-based influenza vaccines.

**Timeline:** The major tasks of Aim 1 are 75% complete.

**Aim 2 Major Tasks:** The major tasks described in Aim 2 involved testing vaccines produced in Aim 1 in humanized HLA transgenic (Tg) mice. The goals are to determine whether 1) DC-targeted Ad vaccines expressing conserved influenza T cell epitopes are more effective than standard wild type attenuated Ad5 vaccines, and 2) whether mucosal (intranasal) delivery of these vaccines is more effective than systemic administration. The endpoints of such studies will be 1) T cell immunogenicity, and 2) protection against diverse influenza A strain challenges in HLA Tg mice.

**Aim 2 Milestone:** Demonstration that mucosal DC-targeted T cell-inducing influenza vaccines are immunogenic and protective against infection with diverse influenza strains.

**Timeline:** Awaiting Aim 1 completion (~6 month delayed onset)
What was accomplished under these goals?  
*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

Multiple GFP-expressing DC targeted Ad constructs were engineered to express camelid nanobodies with affinities for different molecules expressed on murine DC (unknown DC molecule, Clec9A, and CD40). To make DC-targeted Ad5-based vectors we employed a genetic fiber modification approach ablating the native tropism of Ad5 (PMCID: 114163). To this end, the knob domain of capsid fiber protein, which binds to a coxsackievirus and adenovirus receptor (CAR) (PMID: 9036860) was replaced with the C-terminal 95 amino acid long domain from the T4 phage fibrin protein required to maintain fiber trimerization (foldon domain) while allowing incorporation of targeting moieties (PMCID: 229360, PMCID: 296051, PMID: 16293334). To impart DC-binding capability to knob-deleted Ad5, we employed the camelid sdAb that were derived against murine CD40 (also known as TNFRSF5) or Clec9A (C-type lectin domain family 9 member A, also known as DNGR-1) essentially as we recently described (PMCID: 5972836). The engineered DC-targeted Ad5 genomes contained the eGFP reporter cassette in E3.

Next, replication deficient GFP-expressing Ad vectors were transduced into total murine splenocytes [50 – 5,000 virus particles (VP) per cell]. After 24 hours, cells were stained with anti-CD11c (DC marker), and analyzed by flow cytometry for determination of GFP expression in CD11c<sup>positive</sup> DC as well as CD11c<sup>negative</sup> non-DC. Shown below are frequencies of GFP expressing cells in DC (left) and non-DC (right). The highest GFP expression was found in DC transduced with Ad clones targeting an unspecified immature DC marker Unknown DC-targeted Ad1), murine CD40 (CD40-targeted Ad1), and Clec9a (Clec9a-targeted Ad1). In addition, co-stimulatory molecule expression (CD86, CD80) in DC were found to be greatly enhanced after transduction with these DC-targeted Ad vectors, but not by untargeted and WT Ad vectors (not shown).
These results collectively indicate that DC-targeted Ad vectors can efficiently transduce murine DC, and suggest that DC-targeted Ad vectors may provide greater T cell activation due to increased co-stimulatory molecule expression. The molecular surface receptor specificity of the FF1.8 camelid is unknown, thus relevance for future translation is diminished. We therefore focused on generating vaccines within DC targeted Ad vectors which bind to murine DC proteins with known human homologs (CD40, Clec9a).

We previously identified highly conserved HLA-A2 and HLA-panDR restricted influenza epitopes and have shown them to be immunogenic and relevant for protection against infection with diverse influenza A viruses. We generated codon-harmonized genes expressing the important influenza T cell epitopes and successfully sub-cloned these constructs into Ad shuttle plasmids. The genomes of adenovirus (Ad) vectors were constructed by homologous recombination in E. coli strain BJ5183 (PMCID: 190422) transformed with the plasmid carrying the Ad serotype 5 (Ad5) genome along with the shuttle plasmid containing the “universally relevant influenza” expression cassette construct. We used the pShuttle plasmid (PMCID: 19394) to clone the relevant expression cassette from Nature Technology Corporations (NTC’s) NTC8682 or NTC8684 vector. These vectors were designed to be responsive to Food and Drug Administration (FDA) regulatory guidance’s regarding DNA Vaccine vector composition (FDA 1996, FDA 2007; reviewed in Williams et al. PMCID: 2693335). These vectors also contain a novel chimeric promoter that directs superior mammalian cell expression (PMCID: 2767433; PMID: 21107439). NTC8682 vector targets encoded protein into the secretory pathway using an optimized tissue plasminogen activator (TPA) signal peptide. NTC8684 targets proteins to the proteosome by fusion of the C-terminal end of the protein to a destabilizing UbiquitinA76 tag. We generated the pShuttle-FluICS plasmid carrying the expression cassette for the synthetic secretory polypeptide with CD4-inducing, panDR-restricted influenza immunogenic consensus sequences (ICS). We also created the pShuttle-FluA2 plasmid carrying the expression cassette for HLA-A2/CD8-inducing polypeptide containing the N-terminal ubiquitin leader for protein degradation. First, Ad5-based viral genome containing the enhanced green fluorescent protein (eGFP) reporter gene expression cassette in place of the deleted E3 region was employed to introduce either the FluICS or FluA2 construct in place of E1 genes by homologous recombination with pShuttle-FluICS or pShuttle-FluA2 plasmid, respectively.

DC-targeted Ad vectors generated as described above were further modified to introduce each influenza multi-epitope construct in place of the E1 region by homologous recombination with pShuttle-FluICS or pShuttle-FluA2 plasmid as above. To rescue a replication incompetent non-targeted control Ad5 vectors containing wild-type fibers and expressing each multi-epitope influenza construct, we used 293 cells (PMID: 886304) to transfect the corresponding viral genomes. In order to rescue the DC-targeted Ad5 vector derivatives we employed 293F28 cells (PMCID: 229360) expressing both E1 and wild-type Ad5 fiber gene, which allows packaging of fiber-modified Ad vectors. The efficiency of virus rescue was assessed two weeks post-transfection by monitoring the relative number of GFP-expressing cells in transfected cell monolayers using epifluorescence microscopy. In this regard, seeing an increasing number of single cells and cell groups expressing the GFP reporter after transfection with viral genome indicates a successful Ad vector rescue. However, we observed a reduction of the number of GFP-positive cells transfected with viral genomes except for the control Ad5 vector expressing the cassette for the FluICS
synthetic secretory polypeptide. These data suggest that expression of the designed multi-epitope influenza transgenes using constructed Ad genomes can be deleterious to their own replication. This can result in the inhibition of virus rescue, reduced viral yields, or, in the worst-case scenario, make it impossible to construct a vector expressing the inhibiting transgene product (PMID: 10073694; PMCID: 102096). Thus, rescue of recombinant viruses expressing the conserved influenza T cell epitopes proved difficult.

A strategy has been developed to overcome this obstacle in which the CMV promotor driving expression of the vaccine gene is blocked during viral rescue and propagation. To accomplish this, the DNA sequences encoding the designed influenza T cell epitopes were subcloned into Ad shuttle plasmids under control of a CMV promotor containing tet operator sites (tetO) allowing tet repressor protein (tetR) binding. The constructed shuttle plasmids were used to insert the relevant influenza T cell epitope constructs in place of E1 genes of Ad5 genome while making it amenable to tetR-mediated repression of transgene expression while transfected into the T-REx™-293 cells (Life Technologies, USA). T-REx™-293 cells stably express tetR and exhibit extremely low basal expression levels in the repressed state, thus allowing rescue and production of vaccine and therapeutic Ad vectors expressing inhibitory transgenes that otherwise could not be generated. The constructed viral genomes will be used to rescue control and multi-epitope influenza vaccines essentially as described elsewhere (PMID: 17652790). We have used this system to produce vectors with high-level expression of protein that otherwise could not be rescued. These vectors were produced with a higher yield and without Ad contaminant proteins that had nonfunctional expression cassettes. Once these DC-targeted influenza vaccines are generated, we will proceed with immunogenicity and protection experiments in HLA-A2/DR1 transgenic mice as described above as well as in the original proposal and SOW.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.” Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased
knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

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

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The first major goal is to develop replication-deficient Adenovirus (Ad) vectors engineered to incorporate camelid nanobodies specific for murine DC, and express highly conserved influenza T cell epitopes. DC-targeted Ad vectors have been prepared, however, rescue of DC-targeted Ad viruses which also encode influenza vaccine inserts has proved difficult. This may be due to expression of the synthetic genes during Ad rescue. Our strategy to overcome this obstacle is to re-clone the multi-epitope vaccine genes under a direct CMV promotor with 2 tetracycline operator 2 (TetO2) sites, which can be blocked during viral rescue and propagation. The sequence of TetO2 serves as a strong binding site for the Tet repressor. T-Rex 293 cells stably express the tetracycline repressor protein, thus expression of the vaccine inserts will not occur in this cell line. We will further engineer T-Rex 293 cells to express the Ad fiber protein required for high recovery (rescue) of fiber-modified Ad such as those incorporating camelid DC-specific nanobodies. These efforts are underway, and vaccine virus rescue and propagation will continue into the next reporting period.

The second major goal is to determine whether mucosal delivery of DC-targeted Ad-based influenza vaccines are immunogenic and offer protection against influenza challenge in HLA Tg mice. First we will perform small scale vaccine dose optimization assays in which T cell interferon gamma ELISPOT assays will serve as the major endpoint. Next, larger groups of vaccinated HLA Tg mice will be vaccinated and challenged intranasally with H1N1. Lung viral burdens will evaluated within 1 week post-challenge by TCID50 assay. Additional experiments with optimal vaccines will be performed to evaluate disease after H1N1 challenge (weight loss and survival measurements). We will utilize H5N1 to assess heterotypic protection induced by the mucosal DC-targeted influenza A vaccines. A new stock of H5N1 will be prepared at SLU and used to challenge large groups of mice immunized with the DC-targeted flu multi-epitope vaccine (or matched control). One month later, mice will be challenged with H5N1 in the select agent animal suite, and lung viral burdens evaluated by TCID50 assay. Other H5N1 challenged mice will be utilized for study of weight loss and survival.
4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The recovery of recombinant adenoviral vaccines expressing complex synthetic genes can be problematic for even experienced molecular virologists. As described in sections above, expression of such genes can be silenced (suppressed) using novel molecular tools. This should result in higher virus recovery efficiency and enhanced vector propagation; however, the genes will be expressed in vivo post-vaccination.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- transfer of results to entities in government or industry;
- instances where the research has led to the initiation of a start-up company; or
- adoption of new practices.

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- improving public knowledge, attitudes, skills, and abilities;
• changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or
• improving social, economic, civic, or environmental conditions.

Nothing to Report

5. CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Nothing to report.

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Two delays have occurred in this project. First, multiple clones of DC-specific camelids were generated, and these camelids have different specificities for murine DC. Rather than just choosing one to move forward with, we tested each to determine which would have the greatest potential in our translational studies. We tested the in vitro transduction efficiencies of 7 different GFP-expressing, DC-targeted viruses. Based on those results, two DC-targeting Ad vectors which have different binding specificities (CD40 v Clec9A) were selected for further study. These assays were important to identify the most promising DC-targeted Ad constructs, but resulted in short delay prior to beginning generation of DC-targeted influenza vaccines. Second, engineering of the DC-targeted Ad vectors to further express conserved influenza A epitopes has proven more difficult than expected. Cloning of the synthetic gene was straightforward, however, the rescue of the DC-targeted influenza vectors has been problematic. We postulate, based on previous experiences, that expression of a complicated synthetic gene during virus generation and propagation negatively influences virus replication and production of virus particles. We therefore have re-cloned the vaccine inserts (containing multiple conserved influenza T cell epitopes) under the control of a CMV promoter which can be blocked during virus rescue and propagation. We are using Invitrogen’s T-Rex system where the vaccine genes are cloned under control of the CMV promoter with 2 tetracycline operator 2 (TetO2) sites that bind the Tet repressor. T-Rex 293 cells, which express the tetracycline repressor protein, will be engineered to express the Ad fiber, which is required for increased recovery (rescue) of fiber-modified Ad such as those incorporating camelid DC-specific nanobodies. Though delayed, we expect to successfully generate control and multi-epitope influenza vaccines in each of the following Ads early in the next reporting period.
Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Because of the delays noted above, some funds originally scheduled for use in year 1 will be utilized in the next period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals
Because of the delays noted above, some experimental animals originally scheduled for use in year 1 will be utilized in the next period.

Significant changes in use of biohazards and/or select agents
Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- Publications, conference papers, and presentations
  Report only the major publication(s) resulting from the work under this award.
**Journal publications.** List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

**Books or other non-periodical, one-time publications.** Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

**Other publications, conference papers and presentations.** Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

- Nothing to Report

  publications already specified above in this section.

Nothing to Report

**Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report
Inventions, patent applications, and/or licenses
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

Other Products
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
- data or databases;
- physical collections;
- audio or video products;
- software;
- models;
- educational aids or curricula;
- instruments or equipment;
- research material (e.g., Germplasm; cell lines, DNA probes, animal models);
- clinical interventions;
- new business creation; and
- other.

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
**Funding Support:**

The Ford Foundation (Complete only if the funding support is provided from other than this award.)

<table>
<thead>
<tr>
<th>Name: Daniel Hoft, M.D., Ph.D.</th>
<th>Project Role: Principal Investigator</th>
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<tbody>
<tr>
<td>Researcher Identifier: NA</td>
<td>Nearest person month worked: 0.4</td>
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<tr>
<td>Contribution to Project: Dr. Hoft served as PI for this project. He provided oversight for all aspects.</td>
<td></td>
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<tr>
<td>Funding Support: NA (effort funded by this award)</td>
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<tr>
<th>Name: Christopher Eickhoff, MS</th>
<th>Project Role: Co-Investigator</th>
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<tr>
<td>Researcher Identifier: NA</td>
<td>Nearest person month worked: 1</td>
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<tr>
<td>Contribution to Project: Chris performed experiments to evaluate the adenovirus vectors designed by Drs. Curiel/Dmitriev.</td>
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<tr>
<th>Name: Igor P. Dmitriev</th>
<th>Project Role: Co-Investigator</th>
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<tr>
<td>Researcher Identifier: NA</td>
<td>Nearest person month worked: 2</td>
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<td>Contribution to Project: Dr. Dmitriev worked closely with Dr. Curiel to design the genomes of the adenoviral vectors.</td>
<td></td>
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<td>Funding Support: NA (effort funded by this award)</td>
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<tr>
<th>Name: Elena Kashentseva</th>
<th>Project Role: Senior Research Technician</th>
</tr>
</thead>
<tbody>
<tr>
<td>Researcher Identifier: N/A</td>
<td>Nearest person month worked: 3</td>
</tr>
<tr>
<td>Contribution to Project: Ms. Kashentseva worked with Dr. Dmitriev to construct and validate the adenoviral vectors.</td>
<td></td>
</tr>
<tr>
<td>Funding Support: NA (effort funded by this award)</td>
<td></td>
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</tbody>
</table>
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Daniel Hoft, M.D., Ph.D.
Effort has ended on R01HL111523.
Effort has ended on Aeras A-051.
Revised other support page attached.

Chris Eickhoff, M.S.
2015661 “Efficacy of Sublingual BCG vaccination against Mycobacterial challenge” ended as planned on 5/13/18.
Effort has ended on R01HL111523.
Effort has ended on Aeras A-051.
Revised other support page attached.

David Curiel, M.D.
UG3 HL141800, which had been pending was awarded 9/1/17.
550003 CMMN which had been pending was awarded 9/1/17.
OC170200 was awarded 7/1/18.
R41TR001869 was awarded 9/18/18
20-FY19-01 was awarded 1/1/19
18-06 was awarded 1/1/19
PR182272 was awarded 4/1/19

Igor Dimitriev, M.D.
UG3 HL141800, which had been pending was awarded 9/1/17.
OC170200 was awarded 7/1/18.
20-FY19-01 was awarded 1/1/19
18-06 was awarded 1/1/19
PR182272 was awarded 4/1/19

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”
Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

**Organization Name:**

**Location of Organization:** (if foreign location list country)

**Partner’s contribution to the project (identify one or more)**

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner’s facilities for project activities);
- Collaboration (e.g., partner’s staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and
- Other.

Organization: Washington University
Location: St. Louis, MO, USA
Contribution: Dr. Curiel’s group at Washington University serves as a subcontract site on this project. They are responsible for creation of the replication deficient DC-targeted Adenovirus vaccine encoding conserved influenza A T cell epitopes.

8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS:** N/A
Study Goals: To design and test novel T cell-targeted adenoviral-based influenza vaccines with modified vector tropism [designed to efficiently transduce dendritic cells].

Specific Aims:

- **Aim 1:** Engineering of dendritic cell (DC)-targeted adenovirus (Ad) incorporating HLA-restricted conserved flu epitopes.
  - Generate DC-targeted Ad vectors
  - Further engineer DC-Ad to express conserved influenza T cell epitopes

- **Aim 2:** In vivo assessment of mucosal DC-targeted adenovirus vaccines for influenza.
  - Compare T cell responses induced by:
    - Ad v DC-Ad flu vaccines
    - vaccines delivered systemically vs. mucosally
  - Determine whether new vaccines provide protection to humanized mice against diverse influenza strain challenges.

Key Accomplishments and Outcomes:

1) Generated multiple replication deficient GFP-expressing Ad vectors incorporating camelid nanobodies specific for murine DC (CD40, Clec9a, and others).
2) Tested efficiencies of each using in vitro transduction assays in both total splenocytes and CD11c+ purified DC.
   - Identified two Ad vectors with enhanced DC transduction efficiencies.
3) Control Ad and DC-targeted Ad vectors designed to express conserved influenza T cell epitopes are currently being generated for testing in HLA A2/DR1 transgenic mice.
   - Cloning of the synthetic multi-epitope influenza vaccine genes was straightforward, however, rescue and propagation of recombinant DC-targeted Ad was problematic.
   - We designed a workaround to suppress expression of the multi-epitope influenza vaccine gene during virus rescue.
   - We expect to have all novel vaccines ready for study within the next two months.
   - Resulted in year 1 underspending (remaining year 1 funds to be spent in year 2 for original proposed research).

Publications: none to date
Patents: none to date
Funding Obtained: none to date
9. APPENDICES:
   NONE
HOFT, DANIEL F.

Ongoing Research
1) HHSN272201300021I (Hoft) 9/16/13 – 9/15/23 3.9 calendar
   NIH/NIAID
   No Overlap
   “Vaccine and Treatment Evaluation Units (VTEU)”
   The major goal of this IDIQ contract is to evaluate control measures against infectious
diseases other than AIDS. This contract has multiple ongoing clinical trial and laboratory
projects.
   Stacey Esters

2) OPP1118659 (Hoft) 04/24/15-11/01/19 1.8 calendar
   Bill & Melinda Gates Foundation
   No Overlap
   “Novel Antigens That Induce Mtib Inhibiting gamma/delta T cells: Further Ag Identification
   and Test of Protection Against TB Infection/Disease in NHPs”
   The major goal of this grant is to develop novel vaccines with the potential to significantly
   reduce TB infection and disease.
   Lynda Stuart, Lynda.stuart

3) R01AI048391 (Hoft) 07/01/00-02/29/20 2.4 calendar
   NIH/NIAID
   No Overlap
   “Role of gamma/delta T cells in Vaccine Induced Immunity”
   The major goal of this grant is to develop novel vaccines with the potential to significantly
   reduce TB infection and disease.
   Carine Normil

4) U24 AI 118665 (Murthy) 06/20/15-05/31/19 0.4 calendar
   NIH/NIAID
   No Overlap
   “Automated Patient-Specific Dendritic Cell Generation for Transcriptomics-Driven
   Vaccinology”
   This milestone-driven technology development project will design an instrument to convert
   blood-derived monocytes into dendritic cells for mechanistic studies involving the BCG
   vaccine and T cell response.
   Cheryl Y. Wall

5) R21AI128270 (Hoft) 12/01/16-11/30/19 1.0 calendar
   NIH/NIAID
   No Overlap
   “Protective and pathologic effects of Th17 cells specific for an intracellular pathogen”
The major goal of this grant is to produce results that could lead to the development of protective and safe T. cruzi vaccines, immunotherapies capable of preventing or treating chagasic disease, and a better understanding of the general importance of these T cell subsets for other chronic diseases associated with immunopathology.

Devon R. Bumbray-Quarles

6) 1U54TR001961-01 (B. Evanoff, K. Moley, PIs) 06/01/17- 05/31/22 0.3 calendar
NIH/NCATS
No Overlap
“Washington University Institute of Clinical and Translational Sciences”
The purpose of this subcontract is to mentor and train junior scientists in ‘Omics research (transcriptomics, metabolomics, lipodomics, and proteomics).
Gloria F. Velez

7) D01 W81XWH1810140 (Hoft) 5/1/2018 – 10/31/2019 0.5 calendar
DOD
No Overlap
“Universal Influenza T Cell Targeted Mucosal Vaccines”
The purpose of this grant is to develop an efficient vaccination platform to deliver mucosally a T cell based vaccine.

Pending
R21 Application Pending (Liu) 0 calendar
NIH/NIAID
No Overlap
“Regulating Mycobacterium tuberculosis-mediated immune responses by RNA-binding protein MCPIP1”

Completed
1) R21AI105605 (Hoft) 04/15/13 – 03/31/16
NIH/NIAID
“Universal T Cell Targeted Influenza Vaccine”
The major goals of this project are to identify highly conserved T cell epitopes restricted by common HLA present in widely diverse influenza A strains, prepare vaccines encoding these conserved epitopes for testing, and determine if these new vaccines can protect against H1N1, H3N2 and H5N1 strains of influenza in HLA humanized mice.
Juan A. Rodriguez

2) R21AI099514 (Hoft) 03/15/12 – 02/28/15
NIH/NIAID
“Development of Novel Chagas Vaccines”
Goal is to design novel vaccines encoding CD4+ and CD8+ T cell epitopes and test their prophylactic and therapeutic efficacies in “humanized” HLA A2/DR1 dual transgenic mice. The outcome will provide key data for proceeding with Phase 1 Chagas vaccine trials.

3) AGR-404680 (Hoft) 01/01/11 – 12/18/14
MedImmune
"Clinical Research Grant Agreement”
Goal is to conduct clinical trial and related assays for: A Randomized, Open-Label, Study to Evaluate the Immunogenicity of One Dose of Live Attenuated Influenza Vaccine (LAIV)
Compared to One Dose of Trivalent Inactivated Influenza Vaccine (TIV) in Adults 18-49 Years of Age.

4) HHSN266200700022C/NO1-AI-70022 (Boom) 05/01/07 – 04/30/14
   "Tuberculosis Research Unit"
   Goal to study correlates of protective TB immunity and support development of new TB drugs/vaccines.
   Role: Co-Investigator

5) Aeras Foundation (Hoft) 12/01/10 – 06/26/14
   "Phase I Trial of a new recombinant BCG, AERAS-422"
   Gates funded TB vaccine development.

6) R01AI048391 (Hoft) 07/01/00 – 02/28/13
   NIH/NIAID
   "Role of gamma delta T cells in Vaccine Induced Immunity"
   Goals to study inhibitory effects, helper effects, and Ag specificity of TB- & viral-specific human γδ T cells.
EICKHOFF, CHRISTOPHER

Ongoing Research

1) OPP1118659 (Hoft) 04/24/15-11/30/19 2.4 calendar
   Bill & Melinda Gates Foundation
   No Overlap
   “Novel Antigens That Induce Mtb Inhibiting gamma/delta T cells: Further Ag Identification and Test of Protection Against TB Infection/Disease in NHPs”
   The major goal of this grant is to develop novel vaccines with the potential to significantly reduce TB infection and disease.
   Lynda Stuart

2) R01AI048391 (Hoft) 07/01/00-02/29/20 3.00 calendar
   NIH/NIAID
   No Overlap
   “Role of gamma/delta T cells in Vaccine Induced Immunity”
   The major goal of this grant is to develop novel vaccines with the potential to significantly reduce TB infection and disease.
   Carine Normil

3) U24 AI 118665 (Murthy) 06/20/15-05/31/19 3.6 calendar
   NIH/NIAID
   No Overlap
   “Automated Patient-Specific Dendritic Cell Generation for Transcriptomics-Driven Vaccinology”
   This milestone-driven technology development project will design an instrument to convert blood-derived monocytes into dendritic cells for mechanistic studies involving the BCG vaccine and T cell response.
   Cheryl Y. Wall

4) R21AI128270 (Hoft) 12/01/16-11/30/19 1.2 calendar
   NIH/NIAID
   No Overlap
   “Protective and pathologic effects of Th17 cells specific for an intracellular pathogen” The major goal of this grant is to produce results that could lead to the development of protective and safe T. cruzi vaccines, immunotherapies capable of preventing or treating chagasic disease, and a better understanding of the general importance of these T cell subsets for other chronic diseases associated with immunopathology.
   Devon R. Bumbray-Quarles

5) HHSN272201300021I (Hoft) 9/16/13 – 9/15/23 0.60 calendar
   NIH/NIAID
   No Overlap
   “Vaccine and Treatment Evaluation Units (VTEU)”
The major goal of this IDIQ contract is to evaluate control measures against infectious diseases other than AIDS. This contract has multiple ongoing clinical trial and laboratory projects.

Stacey Esters

Pending
None.

Completed

1) R21AI105605 (Hoft) 04/15/13 – 03/31/16
   NIH/NIAID
   No Overlap
   “Universal T Cell Targeted Influenza Vaccine”
   The major goals of this project are to identify highly conserved T cell epitopes restricted by common HLA present in widely diverse influenza A strains, prepare vaccines encoding these conserved epitopes for testing, and determine if these new vaccines can protect against H1N1, H3N2 and H5N1 strains of influenza in HLA humanized mice.
   Juan A. Rodriguez

2) R21AI099514 (Hoft) 03/15/12 – 02/28/15
   NIH/NIAID
   No Overlap
   “Development of Novel Chagas Vaccines”
   Goal is to design novel vaccines encoding CD4+ and CD8+ T cell epitopes and test their prophylactic and therapeutic efficacies in “humanized” HLA A2/DR1 dual transgenic mice. The outcome will provide key data for proceeding with Phase 1 Chagas vaccine trials.

3) 2015661 (Hoft) 01/14/16-05/31/18 1.0 calendar
   La Crosse Community Foundation
   No Overlap
   “Efficacy of Sublingual BCG vaccination against Mycobacterial challenge”
   The goal of this project is to evaluate the efficacy of sublingual BCG vaccination against aerosolized Mtb challenge in mice.
Curiel, David T.  
Active

**OTM11023 (Dmitriev)**  
DNATrix  
6/27/2016-6/26/2019  
0.60  
A Universal Cell Line for Targeted Adenovirus Virotherapy Agents  
Immune checkpoint blockade is one of the most promising approaches achieving spectacular results in cancer therapy. Recent studies also suggested that its therapeutic efficacy in cancer patients could be enhanced by tumor inflammation.  
Role: Co-Investigator  
Specific Aims:  
1. DNX-2401 (AdΔ24-RGD) incorporating a humanized single chain antibody  
2. A Universal Cell Line for Propagation of Targeted Adenoviral Virotherapy Agents  
Program Official:  
Admin Official:  
Brett Ewald 6

**R21 AI131254 (Curiel)**  
National Institutes of Health  
3/20/2017-2/28/2020  
1.20  
Gorilla Adenovirus Zika Vaccine for Humans  
A key question that will be explored in this proposal is whether protective humoral responses will be greater when soluble E or the prM/M-E subviral particle (SVP) is the immunogen in the context of Ad-based vaccines.  
Role: Principal Investigator  
Specific Aims:  
Aim 1. Generate GAd-vectored vaccine to express ZIKV E or SVPs (prM-E) and compare immunogenicity and protective efficacy against ZIKV challenge in adult mice.  
Aim 2. Engineer DC-targeted GAd expressing ZIKV proteins and evaluate its protective efficacy against ZIKV infection in mouse models of maternal-fetal transmission.  
Program Official:  
Admin Official:  
Patricia Repil; Kevin Roy Heath,

**R01 CA211096 (Curiel)**  
National Institutes of Health  
6/19/2017-5/31/2022  
1.50  
Novel targeted adenovirus  
The goal of this project is to develop targeted adenoviral vectors and thereby address key proof-of-principle issues of field wide relevance.  
Role: Principal Investigator  
Specific Aims:  
1. To construct adenoviral vectors, derived from Ad and GAd, with engineered specificities based upon the combination of liver un-targeting and transcriptional targeting, plus sdAb-based transductional targeting, and to evaluate their vector properties in vitro.  
2. To evaluate the in vivo gene delivery biology of the targeted Ads and validate synergistic gains in cell specificity.  
3. To validate the therapeutic index gains which accrue from synergistic targeting in a murine model of metastatic prostate cancer gene therapy to establish key proof-of-principle.  
Program Official:  
Admin Official:  
Rachelle Salomon, Alania Foster

**(Aboody)**  
The Ivy Foundation  
7/1/2017-6/30/2020  
0.12  
Neural stem cell- oncolytic virotherapy for brain tumors  
The overall objective of this application is to significantly advance NSC-mediated virotherapy as a novel treatment for newly diagnosed and recurrent glioma patients, with potential application to pediatric and metastatic brain tumors.  
Role: Subaward Principal Investigator
Specific Aims:
1-Construct conditionally replicative adenovirus (CRAd) with chimerism for fiber knob (type 5 substitute with type 3) and SPARC promoter configured in place of native adenoviral promoter to provide transcriptional control of adenoviral E1A/B genes
2-Rescue and upscale 3/5 SPARC CRAd and validate genomic and protein compositional elements
3-Validate tumor specific replication of CRAd in tumor cells (SPARC-expressing) and normal control cells
4-Validate targeted cytotoxicity of CRAd in tumor cells (SPARC-expressing) and normal control cells

Program Official: Catherine Ivy
Admin Official:

**UG3 HL141800 (George/Rentschler/Curiel)-awarded**

1.0
9/1/2017-8/31/2022 National Institutes of Health calendar

A 3D in vitro disease model of atrial conduction

The central objective of this proposal is to create and validate a robust 3D in vitro microphysiological model of human atrial conduction utilizing patient-derived induced pluripotent stem cells. The model can be used to test the safety and efficacy of drugs to treat atrial arrhythmias such as atrial fibrillation (AF) in a precision medicine format.

Role: MPI/ Subcontract

Specific Aims: SA1. Create Benchmarks: characterize the transcriptome, epigenome, and electrophysiology of adult human atrial cardiomyocytes (normal and AF).
SA2. Model development: create a 3D in vitro disease model of human atrial conduction leveraging human iPS cell-derived atrial cardiomyocytes (iPS-aCM) and atrial regulatory gene expression.
SA3. Atrial-specific “Drug” (adenovirus) delivery: design and test an adenoviral gene delivery strategy to specifically target atrial (not ventricular or nodal) cardiomyocytes.

Program Official: Christine A Livingstone
Admin Official: Ann Marie Brasile

**UG3 HL141800 S1 (George/Rentschler/Curiel)-awarded**

0.60
9/20/2018-7/31/2019 National Institutes of Health Calendar

A 3D in vitro disease model of atrial conduction- Impact of Opioids on Atrial and Ventricular Conduction

The primary goal of this project is to investigate the impact of opioids on the electrical conduction patterns in the heart (both atra and ventricles) using our novel in vitro and ex vivo model and viral vector drug delivery systems.

Specific Aims:
1. Examine impact of opioids with varying signaling biases (e.g., methadone vs hydrocodone) on conduction velocity, action potential duration, and the transcriptome using an in vitro iPS-derived model of atrial and ventricular conduction.
2. Examine impact of opioids with varying signaling biases on conduction velocity, action potential, and the transcriptome of the adult human atra using an ex vivo human model of atrial and ventricular conduction.
3. Create a new adenovirus with capacity to selectively impact the expression of opioid receptors in the atra.

Program Official: Christine A Livingstone
Admin Official: Ann Marie Brasile

**550003-Curiel-awarded**

5.10
9/17/2018-8/31/2019 CMMN Pilot Award-Cancer Center calendar

Targeted magnetic nanoparticles for disseminated myeloma

We propose an adeno-nano delivery vehicle to accomplish effective gene delivery for the genetic treatment of treatment of multiple myeloma.

Role: PI

Specific Aims:
Specific Aim #1: To develop and incorporate a range of magnetic nanomaterials into adenoviral capsids utilizing various coupling strategies.

Specific Aim #2: To further advance adenoviral capsid utilities by integrating targeting motifs into their design.

Specific Aim #3: To analyze the functionality of the developed multifunctional systems in stringent animal models of MM.

Program Official: Admin Official: Paige Isom

OC170200 (Curiel)-awarded 7/1/18-12/31/19 1.8
Department of Defense Novel ovarian cancer therapy
These studies will test a hypothesis regarding the biologic basis of virotherapy action that is of field-wide relevance. In addition, we will realize the database rationalizing translational development of a novel virotherapy agent for carcinoma of the ovary.
Role: PI
Specific Aims
Specific Aim 1: To construct an ovarian cancer CRAd based upon gorilla adenovirus. Specific Aim 2 To characterize the tumor selectivity of the gorilla CRAd in vitro and in vivo. Specific Aim 3 To evaluate the ability of CRAd-based virotherapy to induce anti-tumor immunity in a syngeneic immunocompetent murine model of carcinoma of the ovary.
Program Official Admin
Christopher Baker Official: Kevin R. Moore

R41TR001869 (Curiel)-awarded 9/18/2018-9/17/2019 1.20
National Institutes of Health NOVEL PLATFORM TECHNOLOGY FOR HEMOPHILIA GENE THERAPY
We propose to develop a novel vector approach that addresses the key limitations to current methods and utilizes the unique capacity to target pulmonary endothelium for reconstituting deficient serum factors. We will accomplish this by combining technologies from Washington University and Precision Virologics, Inc. In Phase I we will demonstrate the feasibility of the new platform technology to efficiently deliver to pulmonary endothelium and achieve stable long-term correction of factor VIII deficient mice.
Specific Aims:
Specific Aim #1 – To construct a capsid modified adenoviral vector targeted to the pulmonary endothelium and evaluate its capacity to accomplish effective augmentation of FVIII levels in FVIII hemophilia deficient mice.
Specific Aim #2 – To functionally configure the CRISPR/Cas9 into pulmonary targeted capsid modified Ads and demonstrate in vivo gene editing of target cells of the pulmonary endothelium.
Specific Aim #3 – To employ the composite vector system, which embodies pulmonary vascular targeting and CRISPR/Cas9, to achieve stable long term correction of factor VIII deficient mice.

20-FY19-01(Gillanders/Curiel)-new award 1/1/2019-12/31/2019 1.20
Siteman Cancer Center Evaluation of a Novel Personalized Vaccine Strategy for Breast Cancer
The goal is to activate immune cells capable of recognizing and killing breast cancer using the "prime/boost" neoantigen vaccines, and then take the "brakes" off these immune cells using checkpoint blockade therapy. This combination has the potential to be a synergistic and highly effective strategy in TNBC, and in other cancers, particularly cancers resistant to checkpoint blockade therapy alone.
Role: Principal Investigator
Specific Aims:
Specific Aim 1: Test the safety, feasibility, and immunogenicity of a neoantigen vaccine strategy consisting of
a simian adenovirus vaccine "prime," followed by a plasmid DNA vaccine "boost" in a phase 1 clinical trial. Specific Aim 2: Test the hypothesis that specifically targeting tumor-associated macrophages (TAM) will enhance the efficacy of breast cancer neoantigen vaccines in the setting of established disease.

Program Official: 
Admin Official: 
Aleatha Harris, aharris@wustl.edu

18-06, Curiel-new award 

University of Missouri 
TARGETED GENE THERAPY FOR SPINAL TUMORS 
The goal of this project is to advance targeting to tumor endothelial cells to realize vector technology that will make effective gene therapy for intramedullary glioma feasible. 
Role: Principal Investigator 
Specific Aims: 
Specific Aim 1: To derive an adenoviral vector with engineered specificity based upon the combination of liver un-targeting and ROBO4 transcriptional targeting with anti-CD276 sdAb transductional targeting, and to evaluate its' vector targeting properties in vitro.  
Specific Aim 2: To evaluate the in vivo gene delivery biology of the targeted Ad and validate synergistic gains in cell specificity in a rodent model of glioma IMSCT.  
Specific Aim 3: To validate the therapeutic index gains which accrue from synergistic targeting in a rodent model of IMSCT glioma gene therapy to establish key proof-of-principle and rationale for 
Program Official: 
Admin Official: 
Ashley Berg

PR182272 (Abate)-new award pending subaward 

4/1/2019-9/30/2020 Department of Defense 
A novel approach to enhance TB lung immunity 
A prime-pull approach using chemokines to recruit immune cells into the lungs will help control TB infection and disease.  
Role: Co- Investigator 
Specific Aims: 
Aim #1- Evaluate the effects on T cell recruitment by lung chemokine delivery during systemic TB vaccination.  
Aim #2- Evaluate the effects of lung chemokine delivery during BCG vaccination on Mtb infection & 
Program Official: 
Admin Official: 
Cecilia Dupecher

Recently Completed

DARPA-16-33-Office-Wide-BAA-FP-042 (Curiel)-completed 

7/1/2017-6/30/2018 Defense Advanced Research Projects Agency (DARPA) 
An inducible Genetic Bioshield for Flexible and Rapid Protection of Body Portal Against Bioweapons Attack of Diverse Types 
The proposed project offers a novel, broadly applicable countermeasure system against bioweapons threats of diverse categories. Our studies herein will expedite the realization of a strategy that offers significant advantages for biodefense in battlefield settings: (1) inducible; (2) fast-acting; (3) high potency; (4) broad-based defense activities; (5) readily adaptable to new threat contexts.  
Role: Principal Investigator 
Specific Aims: 
1. Tropism modification of GAd to accomplish selective gene expression in pulmonary epithelium  
2. Construction of pulmonary endothelial targeted GAd encoding biodefense genes  
3. Employment of control element to achieve inducible expression of genes at pulmonary epithelium  
4. Utilization of CRISPR/Cas9 for in vivo gene editing to allow regulated long term gene expression
R01 CA154697 (Curiel/Mathis)- completed 9/26/2011-7/31/2017
National Institutes of Health
Targeted-and Image-Based Adenovirus Cancer Therapeutic Vectors
The goal of this application is to develop a multi-functional adenovirus vector that combines both imaging and targeted therapy for treating cancer.
Role: Principal Investigator
Specific Aims: Aim 1: To develop Ad vectors capable of cell-specific targeting, by incorporating targeting single chain antibodies (scFv) into the viral capsid in combination with liver detargeting; Aim 2: To develop an imaging modality for dynamic pharmacokinetic assessment of Ad vectors in vivo based on a novel capsid incorporation method that exploits metallothionein as a functional imaging motif; and Aim 3: To determine in vivo the quantitative value of our imaging modality and the therapeutic index gains from our targeted tumor transduction, using a murine model of metastatic cancer.
Program Official:
Admin Official:
Keyvan Farahani, Silvia Torres,

R01 DK104698 (Warner)- completed 3/15/2015-2/28/2019
National Institutes of Health
Angiogenesis in Intestinal Adaptation
After massive intestinal resection, the remnant bowel tries to compensate via a process termed adaptation. The overall goals of this project are to fully understand the process of adaptation and intestinal re-growth. This information is critical for the development of new therapeutic strategies to enhance this process, thereby saving lives of patients with short gut syndrome.
Role: Co-Investigator
Specific Aims:
1. Determine the mechanism for angiogenesis in response to SBR-induced intestinal hypoxia
2. Determine the mechanism for increased CXCL5 expression following SBR
3. Determine the contribution of CXCL5 expression and angiogenesis to functional adaptation by elucidating the metabolic consequences of perturbed angiogenesis. We will also define the effect of adenoviral-directed endothelial overexpression of HIF1a, EGFR, and CXCL5 on resection-associated angiogenesis and adaptation responses.
Program Official: Patricia Greenwel; Karin Mastrangelo
Admin Official:

R33 HL120760 (Cole)- completed 3/1/2016-2/28/2019
National Institutes of Health
Lipidomic Screening For Functional Surfactant Gene Mutations
The goals of this grant are to develop and implement a human model system that uses static and dynamic lipidomic signatures for functional screening of mutations that disrupt surfactant metabolism and that could be used for testing small molecules to correct mutation-encoded, functional defects in any gene expressed in the human alveolar type 2 cell.
Role: Co-Investigator
Specific Aims: R33 Phase Aim: Characterize differences in lipidomic signatures and kinetics, lamellar body phenotype, and secreted surfactant function between ABCA3 wild type- and mutation-rescued human alveolar type 2 cells.
Program Official: Gan, Weiniu, Connolly, Amy
Admin Official:

R21 AI117459 (Moreno)- completed 4/1/2016-3/31/2018
National Institutes of Health
Novel DC Targeted Adenovirus Vector for Malaria Vaccine Development
Subaward from Emory U. (Curiel)
The goal of this project is to develop a novel immunization regimen for a Plasmodium vivax malaria vaccine candidate.
Role: Subcontract PI/Co-Investigator
Specific Aims: 1) Construct an sdAb myeloid DC-targeted Ad encoding a multi-stage P. vivax chimeric antigen and validate cell specific gene delivery using murine DCs; 2) Characterize and compare the impact of DC targeting on the immunogenicity and efficacy in a stringent murine malaria model of protection

Program Official: Annie X, Y, Mo

OTM12067 (Curiel)-contract ended
12/29/2016-12/28/2018
GenVec
Endothelium Gene Editing Delivery for Novel Hemophilia Therapeutics
Specific work scope retargeting GenVec’s multi-deleted GC45 gorilla vector for combined gene editing and payload delivery
Role: Principal Investigator
Specific Aims:
Aim 1: Construct tropism modified GC45 gorilla adenovector incorporating the best MBP pulmonary endothelium targeting designs and the GenVec multi-deleted adenoviral product candidate vector designs.
Aim 2: Test the new GC45.MBP adenoviral vector designs for myeloid moieties binding specificity.
Aim 3: Biodistribution analysis of the new GC45.MBP adenoviral vector designs in murine models
Aim 4: Proof of principle construction of blood factor IX (FIX) gene editing components within a single GC45.MBP multi-deleted genome and in vitro testing
Aim 5: Proof of principle construction of blood factor VIII (FVIII) gene editing components – in vitro testing
Program Official: Douglas Brough
Admin Official: Rena Cohm
Dmitriev, Igor
Active

OTM11023 (Dmitriev) 6/27/2016-6/26/2018 0.24
DNAtrix
A Universal Cell Line for Targeted Adenovirus Virotherapy Agents
Immune checkpoint blockade is one of the most promising approaches achieving spectacular results in cancer therapy. Recent studies also suggested that its therapeutic efficacy in cancer patients could be enhanced by tumor inflammation.
Role: Principal Investigator
Specific Aims:
1. DNX-2401 (AdΔ24-RGD) incorporating a humanized single chain antibody
2. A Universal Cell Line for Propagation of Targeted Adenoviral Virotherapy Agents
Program Official: Admin Official:
Brett Ewald

R21 AI131254 (Curiel) 3/20/2017-2/28/2020 0.6
National Institutes of Health
Gorilla Adenovirus Zika Vaccine for Humans
A key question that will be explored in this proposal is whether protective humoral responses will be greater when soluble E or the prM/M-E subviral particle (SVP) is the immunogen in the context of Ad-based vaccines.
Role: Co-Investigator
Specific Aims:
Aim 1. Generate GAd-vectored vaccine to express ZIKV E or SVPs (prM-E) and compare immunogenicity and protective efficacy against ZIKV challenge in adult mice.
Aim 2. Engineer DC-targeted GAd expressing ZIKV proteins and evaluate its protective efficacy against ZIKV infection in mouse models of maternal-fetal transmission.
Program Official: Admin Official:
Patricia Repil, Kevin Roy Heath

R01 CA211096 (Curiel) 6/19/2017-5/31/2022
National Institutes of Health
Novel targeted adenovirus
The goal of this project is to develop targeted adenoviral vectors and thereby address key proof-of-principle issues of field wide relevance.
Role: Co-Investigator
Specific Aims:
1. To construct adenoviral vectors, derived from Ad and GAd, with engineered specificities based upon the combination of liver un-targeting and transcriptional targeting, plus sdAb-based transductional targeting, and to evaluate their vector properties in vitro.
2. To evaluate the in vivo gene delivery biology of the targeted Ads and validate synergistic gains in cell specificity.
3. To validate the therapeutic index gains which accrue from synergistic targeting in a murine model of metastatic prostate cancer gene therapy to establish key proof-of-principle.
Program Official: Admin Official:
Rachelle Salomon; Alania Foster

UG3 HL141800 (George/Rentschler/Curiel)-awarded 9/1/2017-8/31/2022 2.0
National Institutes of Health
A 3D in vitro disease model of atrial conduction
The central objective of this proposal is to create and validate a robust 3D in vitro microphysiological model of human atrial conduction utilizing patient-derived induced pluripotent stem cells. The model can be used to test the safety and efficacy of drugs to treat atrial arrhythmias such as atrial fibrillation (AF) in a precision medicine format.
Role: Co-Investigator

Specific Aims:

SA1. Create Benchmarks: characterize the transcriptome, epigenome, and electrophysiology of adult human atrial cardiomyocytes (normal and AF).

SA2. Model development: create a 3D in vitro disease model of human atrial conduction leveraging human iPS cell-derived atrial cardiomyocytes (iPS-aCM) and atrial regulatory gene expression.

SA3. Atrial-specific "Drug" (adenovirus) delivery: design and test an adeno-viral gene delivery strategy to specifically target atrial (not ventricular or nodal) cardiomyocytes.

UG3 HL141800 S1 (George/Rentschler/Curiel)-awarded  
9/20/2018-7/31/2019 National Institutes of Health calendar

A 3D in vitro disease model of atrial conduction - Impact of Opioids on Atrial and Ventricular Conduction

The primary goal of this project is to investigate the impact of opioids on the electrical conduction patterns in the heart (both atria and ventricles) using our novel in vitro and ex vivo model and viral vector drug delivery systems.

Role: Co-I

Specific Aims:
1. Examine impact of opioids with varying signaling biases (e.g., methadone vs hydrocodone) on conduction velocity, action potential duration, and the transcriptome using an in vitro iPS-derived model of atrial and ventricular conduction.
2. Examine impact of opioids with varying signaling biases on conduction velocity, action potential, and the transcriptome of the adult human atria using an ex vivo human model of atrial and ventricular conduction.
3. Create a new adenovirus with capacity to selectively impact the expression of opioid receptors in the atria.

Program Official: Christine A Livingstone  
Admin Official: Ann Marie Brasile

OC170200 (Curiel)-awarded  
7/1/18-12/31/19 Department of Defense calendar

Novel ovarian cancer therapy

These studies will test a hypothesis regarding the biologic basis of virotherapy action that is of field-wide relevance. In addition, we will realize the database rationalizing translational development of a novel virotherapy agent for carcinoma of the ovary.

Role: Co-I

Specific Aims
Specific Aim 1: To construct an ovarian cancer CRAd based upon gorilla adenovirus. Specific Aim 2 To characterize the tumor selectivity of the gorilla CRAd in vitro and in vivo. Specific Aim 3 To evaluate the ability of CRAd-based virotherapy to induce anti-tumor immunity in a syngeneic immunocompetent murine model of carcinoma of the ovary.

Program Official: Christopher Baker  
Admin Official: Kevin R. Moore

20-FY19-01(Gillanders/Curiel)-new award  
1/1/2019-12/31/2019 Calendar

Siteman Cancer Center

Evaluation of a Novel Personalized Vaccine

Strategy for Breast Cancer

The goal is to activate immune cells capable of recognizing and killing breast cancer using the "prime/boost" neoantigen vaccines, and then take the "brakes" off these immune cells using checkpoint blockade therapy. This combination has the potential to be a synergistic and highly effective strategy in TNBC, and in other cancers, particularly cancers resistant to checkpoint blockade therapy alone. Role: Co-Investigator

Specific Aims:
Specific Aim 1: Test the safety, feasibility, and immunogenicity of a neoantigen vaccine strategy consisting of a simian adenovirus vaccine "prime," followed by a plasmid DNA vaccine "boost" in a phase 1 clinical trial. Specific Aim 2: Test the hypothesis that specifically targeting tumor-associated macrophages (TAM) will enhance the efficacy of breast cancer neoantigen vaccines in the setting of established disease.

Program Official: 
Admin Official:
Aleatha Harrisu

18-06, Curiel-new award  
University of Missouri 
1/1/2019-12/31/2020 1.2 
TARGETED GENE THERAPY FOR SPINAL TUMORS 
The goal of this project is to advance targeting to tumor endothelial cells to realize vector technology that will make effective gene therapy for intramedullary glioma feasible. 
Role: Co- Investigator 
Specific Aims: 
Specific Aim 1: To derive an adenoviral vector with engineered specificity based upon the combination of liver un-targeting and ROBO4 transcriptional targeting with anti-CD276 sdAb transductional targeting, and to evaluate its' vector targeting properties in vitro. 
Specific Aim 2: To evaluate the in vivo gene delivery biology of the targeted Ad and validate synergistic gains in cell specificity in a rodent model of glioma IMSCT. 
Specific Aim 3: To validate the therapeutic index gains which accrue from synergistic targeting in a rodent model of IMSCT glioma gene therapy to establish key proof-of-principle and rationale for 
Program Official: 
Admin Official: 
Ashley Berg

P18-04949 (Dmitriev)-new award  
11/4/2018-11/3/2019 Unleash Immuno Oncolytics 0.60 Oncolytic Virotherapy Viruses (for Cancer) 
The goal of this study is to construct a class of conditionally replicative human adenovirus for active anti-tumor immunization and validate in vitro and in vivo. 
Role: Principal Investigator 
Specific Aims: 
1.Construct ovarian cancer CRAds with SPARC controlled replication 
2.Validate tumor selective replication in vitro 
3.Validate tumor selective replication and study therapeutic index in murine models 
Program Official: 
Admin Official: 
Daniel Katzman

PR182272 (Abate)-new award pending subaward  
4/1/2019-9/30/2020 Department of Defense 1.8 
A novel approach to enhance TB lung immunity 
A prime-pull approach using chemokines to recruit immune cells into the lungs will help control TB infection and disease. 
Role: Co- Investigator 
Specific Aims: 
Aim #1- Evaluate the effects on T cell recruitment by lung chemokine delivery during systemic TB vaccination. 
Aim #2- Evaluate the effects of lung chemokine delivery during BCG vaccination on Mtb infection & 
Program Official: 
Admin Official: 
Cecilia Dupecher.

Recently Completed 
R21 AI117459 (Moreno)-completed  
National Institutes of Health 4/1/2016-3/31/2018 1.80
Novel DC Targeted Adenovirus Vector for Malaria Vaccine Development
Subaward from Emory U. (Curiel)
The goal of this project is to develop a novel immunization regimen for a Plasmodium vivax malaria vaccine candidate.
Role: Co-Investigator
Specific Aims: 1) Construct an sdAb myeloid DC-targeted Ad encoding a multi-stage P. vivax chimeric antigen and validate cell specific gene delivery using murine DCs; 2) Characterize and compare the impact of DC targeting on the immunogenicity and efficacy in a stringent murine malaria model of protection
Program Official: Annie X, Y

DARPA-16-33-Office-Wide-BAA-FP-042 (Curiel)-completed 3.00 7/1/2017-6/30/2018 Defense Advanced Research Projects Agency (DARPA) calendar
An inducible Genetic Bioshield for Flexible and Rapid Protection of Body Portal Against Bioweapons Attack of Diverse Types
The proposed project offers a novel, broadly applicable countermeasure system against bioweapons threats of diverse categories. Our studies herein will expedite the realization of a strategy that offers significant advantages for biodefense in battlefield settings: (1) inducible; (2) fast-acting; (3) high potency; (4) broad-based defense activities; (5) readily adaptable to new threat contexts.
Role: Co-Investigator
Specific Aims:
1. Tropism modification of GAd to accomplish selective gene expression in pulmonary epithelium
2. Construction of pulmonary endothelial targeted GAd encoding biodefense genes
3. Employment of control element to achieve inducible expression of genes at pulmonary epithelium
4. Utilization of CRISPR/CFas9 for in vivo gene editing to allow regulated long term gene expression
Program Official: James Gimlett
Admin Official: Fumiko Hall