NCATS Example Summary Statement

SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **03/18/2015**

Personal Info

Application Number: **1 R41 TR001338-01**

Principal Investigator

FUJII, GARY PHD

Applicant Organization: MOLECULAR EXPRESS, INC.

Review Group: **ZRG1 IMST-S (12)**
Center for Scientific Review Special Emphasis Panel
Small Business: Basic and Integrative Bioengineering

Meeting Date: **02/26/2015** ***RFA/PA:*** **PA14-308**
Council: **MAY 2015** ***PCC:*** **OSA13**
Requested Start: **07/01/2015**
Dual IC(s): **EB**

Project Title: **Development of a Gene and Oligonucleotide Delivery System**

SRG Action: **Impact Score:** Impact Score
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	201,814	324,133
TOTAL	**201,814**	**324,133**

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R41TR001338-01 Fujii, Gary

RESUME AND SUMMARY OF DISCUSSION: This application seeks to develop functional enveloped virus-like-particles (EVLPs) in which anti-sense oligonucleotides or other types of genetic medicines are conjugated with viral capsid proteins and encapsulated using liposome-like delivery devices. A proof-of-concept study was proposed in an investigation of newly constructed EVLPs and their HIF-1α knockdown activities using cultured cell models. If successful, the proposed EVLPs would be useful for delivering genetic medicines to targeted cells or tissues, and protecting them from pre-mature loss that other non-encapsulated delivery systems suffered. The panel noted that the focus of this proposal was highly significant since it aimed to address an unmet challenge, i.e. to deliver genetic materials to targeted cells *in vivo*; and the idea of capsid-conjugated encapsulation appeared novel. The team of investigators is made by field experts, some with impressive SBIR/STTR track records; the resources and environment are excellent. Enthusiasm from reviewers was high despite some identified weaknesses. There were some concerns in the shortage of comparative analysis for the proposed approach over competitive methods; the *in vitro* feasibility study might provide limited insights on its *in vivo* performance; and non-specific hybridization of single strand DNA in organelles was not discussed. Overall, the panel was excited about the significance of the application; the identified weaknesses did not reduce enthusiasm; a final score was received reflecting a R&D program expected to have a high impact in the areas of genetic medicine delivery and targeted therapies of disease such as cancer.

DESCRIPTION (provided by applicant): The knockdown of targeted genes by anti-sense oligonucleotides (ODNS) and genetic medicines (collectively, G-MEDS) holds promise for a variety of therapies. The delivery of effective quantities of ODNS to specific cells, however, has proved to be challenging. We propose here a novel approach to ODN delivery that involves enveloped virus-like-particles (EVLPs). These delivery agents are prepared by the *in vitro* self- assembly of pure G-MEDS and pure viral capsid protein (CP) into virus-like nanoparticles particles (VLPs). The capsid protects the contents yet, as we have demonstrated, is capable of giving up its contents in the cytoplasm of mammalian cells. The particles, which are highly mono-disperse, are then enveloped by lipid bilayers that can suppress the immunogenicity of the VLPs and are capable of being functionalized for targeting and uptake by mammalian cells of interest. In preliminary experiments, we have demonstrated our ability to prepare EVLPs using the CP of the cowpea chlorotic mottle virus and a model antisense ODN for vascular endothelial growth factor (VEGF), a protein over-expressed in many cancer cells to stimulate angiogenesis a facilitate tumor survival under low- oxygen conditions. We propose to optimize the assembly and to functionalize the lipid bilayers with epidermal growth factor (EGF), which binds the EGF receptor that is over-expressed on cancer cells, especially those of breast cancer. The effectiveness of the EVLP will be demonstrated by assaying the reduction in the secretion of VEGF in cultured breast cancer cells.

PUBLIC HEALTH RELEVANCE: The development of the proposed system for delivering genetic medicines such as genes and oligonucleotides would be an important medical advance. Its development would facilitate the targeted delivery of genetic medicines to specific cells, tissues and organs, thus enabling the development of healthcare products which will exert a significant impact on the practice of medicine.

CRITIQUE 1:

Significance: 3
Investigator(s): 1
Innovation: 3
Approach: 2
Environment: 1

Overall Impact: This is a strong proposal that addresses a prominent problem for genetically derived medications. The approach is innovative, combining targeted liposome encapsulation with capsid/g-med particle formation. The group has demonstrated the expertise to synthesize the particles and provides a sound plan for demonstrating the efficacy of the particles *in vitro*. A better sense of how this work will position the company in the marketplace and move towards translational trials would be beneficial.

1. Significance:
Strengths
- The proposal outlines an approach for antisense RNA delivery that addresses existing challenges within the field. Through efficient sequence independent encapsulation of RNA within a cowpea chlorotic mottle virus capsid (ccmc CP) and subsequent creation of a targeting envelope, the team presents a convincing achieving efficient delivery and suppress. immunogenicity.
- The uniformity of CP-G-med particles is impressive.

Weaknesses
- A clear sense is not provided on how this effort will practically move beyond the progress that has been shown by the host of other antisense experiments and technologies that have surfaced over the last decade. The PI should work to paint a clear picture of how this work will position this technology compared to the other contenders within the field.
- What are the gold standards in the field, and how might a direct comparison to field leaders be carried out in Aim 3.

2. Investigator(s):
Strengths
- The PI exhibits a history of managing SBIR/STTR grants and aggressively pursuing IP in the field (in lieu of publishing). An appropriate infrastructure and support team appear to be in place.

Weaknesses
- None noted.

3. Innovation:
Strengths
- The combination of liposome encapsulation for targeted delivery combined with the capsid encapsulation of the chosen G-meds appears to be innovative.

Weaknesses
- None noted.

4. Approach:
Strengths
- The approach seems appropriate moving from a refinement of formulation demonstrated in preliminary data (Aims 1 & 2) towards a proof of principle demonstration *in vitro* (Aim 3)

Weaknesses
- The approach to minimizing cationic lipids within the formulation and subsequently substituting other cationic lipids at the same concentration seems overoptimistic. I would imagine the different cationic lipids will be stable at different compositions and one would expect that the ‘minimization process’ described for DOTAP would need to be repeated for any substitutes.

5. Environment:
Strengths
- Molecular Express will leverage their own resources against those available at UCLA. Facilities and equipment seem adequate for the proposed work.

Weaknesses
- None noted.

Phase II (Type 2 R42 and Type 2 R44 applications):
Not Applicable

Fast Track (Type 1 R42 and Type 1 R44 applications):
Not Applicable

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Vertebrate Animals:
Not Applicable (No Vertebrate Animals)

Biohazards:
Not Applicable (No Biohazards)

CRITIQUE 2:

Significance: 3
Investigator(s): 2
Innovation: 3
Approach: 3
Environment: 2

Overall Impact: This STTR Grant, a collaboration between Molecular Express, Inc. and the University of California at Los Angeles, will focus on the delivery of antisense oligonucleotides (ODNS) and genetic medicine (G-MEDS) that involves enveloped virus-like-particles (EVLPs). These EVLPs are composed of the assembly of pure G-MEDS and pure viral capsid protein into virus like molecules which are then enveloped by lipid bilayers that could be functionalized for targeting to specific cells. The investigators are well prepared to conduct this work, but the proposal is confusing to read is it mixes results with methods. It is also a very complex project given the various elements and not sure how *in vitro* results will link to *in vivo* results.

1. Significance:
Strengths
- The delivery of ODN and G-Meds can serve as an important therapeutic modality that continues to advance given new delivery technologies and approaches.

Weaknesses
- The proposed delivery method for these G-MEDS is complex and there are many challenges toward future clinical uses of these delivery systems. There will always be the concern on how these will be scaled up and tested for quality control and quality assurance as well as the difference between what will happen *in vitro* and what will happen *in vivo*.

2. Investigator(s):
Strengths
- It is a long-lasting collaboration of Dr. Fujii with Drs. Gelbart and Knobler on the development of the EVLP technology.

Weaknesses

- The specific role of Dr. Gelbart and Dr. Knobler in the Senior and Key Personal Profile has not been identified in the proposal on these forms.
- There is a concern that Dr. Fujii has the time to work on the current project considering the current funded work (including those with no cost extension) and the series of current proposals.
- Minor point - Dr. Gelbart NIH Biosketch is not accurate as it talks about the contribution to a SBIR Fast Track collaborative proposal.
- The proposal needs to have an NIH Biosketch for Su Ming Chiang and Thai Q Do as they have a significant involvement in this work given the current budget proposal and the scope of their work involving the lipid wrapping studies and the analysis of the lipid components of EVLP's.

3. Innovation:

Strengths

- The methods are one commonly used in these types of studies, but the twist is the use of the capsid protein cowpea chlorotic mottle virus (CCMV), specifically they will use an antisense that targets the expression of Hypoxia-Inducible Factor 1α with his capsid protein, followed by coating with a lipid bilayer which is functionalized by the use of epidermal growth factor (EGF) as the targeting agent to the receptor overexpressed in breast cancer cells.
- There previous work has shown success with using the VEGF antisense in this process.
- They are also using a relevant molecule in these studies, specifically the Hypoxia-Inducible Factor 1α, which is in use in clinical trials.

Weaknesses

- It is a standard process by which one forms a delivery system protected by lipids and then functionalized for targeted drug delivery.

4. Approach:

Strengths

- The methods are one commonly used in these types of studies ranging from encapsulating the ODN, followed by wrapping with the lipid bilayer and then adding the targeting agent to the lipid bilayer to target specific receptors and test for the extent of protein expression and toxicity. It seems very ambitious that they will be able to achieve over 95% encapsulation efficiencies that will result in a successful therapeutic outcome.
- They have demonstrated some initial success with these approaches in using VEGF antisense and now will include Hypoxia-Inducible Factor 1-alpha in specific aim 1.
- They do have extensive experience in the various experimental methods.

Weaknesses

- The proposal is confusing to read as they mix the results from previous studies with the currently proposed studies.
- It would strengthen the proposal if they would have identified specifically the conjugatable lipid anchors that will be formulated with the liposomes so it could be used to conjugate with the model targeting agent. This is the one of the weakest part of the proposal. While they do provide a basis of the conjugation chemistries available, it does not necessarily ensure that there will be success in this process.

5. Environment:

Strengths

- The resources and equipment available at Molecular Express and the University of California at Los Angeles laboratories of Drs. Gelbart and Knobler, as well as the Nanosystems Institute, is well suited to complete the proposed scope of work.

Weaknesses

- None noted.

Phase II (Type 2 R42 and Type 2 R44 applications):
Not Applicable

Fast Track (Type 1 R42 and Type 1 R44 applications):
Not Applicable

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Vertebrate Animals:
Not Applicable (No Vertebrate Animals)

Biohazards:
Acceptable

Budget and Period of Support:
Recommend as Requested

CRITIQUE 3:

Significance: 3
Investigator(s): 2
Innovation: 2
Approach: 3
Environment: 2

Overall Impact: This proposal is based on the development of lipid encapsulated (what appears to be a liposome-like delivery device) single strand DNA (ssDNA) conjugated with the capsid protein of cowpea chlorotic mottle virus that will target Hypoxia Inducible Factor 1α. The PI paid attention to use minimal cationic lipid in enveloping the ssDNA conjugate. Cytotoxicity and VEGF expression protocols are appropriate but targeting cannot be established adequately using a mixture of cancerous and non-cancerous cells. Although there is no targeting approach in this proposal, this technology will help a lot in experimental approach in delivery of ssDNA and siRNA. Given that hypoxia inducible factor is a regulator for angiogenesis, it is appropriate to assay the expression of VEGF, however, a direct assay of hypoxia inducible factor using Western and/or RT-PCR would be desirable. In addition, a few disadvantages of ssDNA are known for non-specific hybridization and inefficient endocytosis in the organelles. Are there any suggestions to minimize these disadvantages? Enthusiasm is high for this proposal.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWER'S WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

- Additional budget justification for the graduate student was requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.