SUMMARY STATEMENT

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(Privileged Communication)

Release Date: **04/18/2017**
Revised Date:

Application Number: **1 F30 HL139017-01**

CULLEY,MIRANDA
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Review Group:	**ZRG1 F10A-A (20)** **Center for Scientific Review Special Emphasis Panel** **Fellowships: Physiology and Pathobiology of Cardiovascular and Respiratory Systems**
Meeting Date:	**03/23/2017**
Council:	**MAY 2017** ***PCC:*** **LLLJAN**
Requested Start:	**07/01/2017**

Project Title:	**Frataxin loss induces endothelial dysfunction to promote pulmonary hypertension**
	***Requested*: 4 Years**
Sponsor:	**Chan, Stephen Y**
Department:	**Medicine**
Organization:	**UNIVERSITY OF PITTSBURGH AT PITTSBURGH**
City, State:	**PITTSBURGH PENNSYLVANIA**
SRG Action:	**Impact Score:25**
Next Steps:	**Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects:	**10-No human subjects involved**
Animal Subjects:	**30-Vertebrate animals involved - no SRG concerns noted**

1F30HL139017-01 Culley, Miranda

RESUME AND SUMMARY OF DISCUSSION: This predoctoral (F30) application focuses on research and training in the area of pulmonary hypertension (PH) where the applicant proposes to test the hypothesis that hypoxic conditions at the pulmonary endothelium induces the presence of miR130b, which leads to mitochondrial and endothelial dysfunction by downregulating the mitochondrial protein frataxin (FXN), leading to PH. Though technical issues associated with the research plan were identified, the overall significance of this research was determined to be high. The reviewers widely agreed that the powerful cell and rodent models that are used in course of this study, contribute to great training. That said, there was some concern that the applicant, at times, did not demonstrate depth of thought in describing these studies. The applicant herself was widely considered outstanding, with stellar academic accomplishments and a strong record of research productivity. The sponsor, Dr. Chan, was equally impressive. However, there was some concern during discussion that the sponsor may be overcommitted, currently carrying 6 mentees. Overall, though there were minor weaknesses associated with the the research plan, it was strengths that included an outstanding applicant and mentor, a thoughtful research topic, and the availability of rich institutional resources that contributed to high training potential, leading the reviewers to consider this proposal as an excellent foundation for a future as a productive independent investigator.

DESCRIPTION (provided by applicant): Pulmonary hypertension (PH) is a deadly disease of the lung vasculature with a complex pathophysiology that remains largely undefined. My mentor's laboratory established the microRNA--130/301 family as a mediator of PH development and defined a separate mechanism by which iron--sulfur (Fe--S) cluster deficiency promotes PH. Fe--S clusters are bioinorganic cofactors essential to mitochondrial and cellular function. Frataxin (FXN) is a mitochondrial protein crucial to Fe--S biogenesis. Loss of FXN due to a trinucleotide repeat mutation causes Friedreich's ataxia (FRDA), a disease characterized by neurologic dysfunction and hypertrophic cardiomyopathy. Hypertrophic cardiomyopathy is often accompanied by PH, thought to be the result of left ventricular stiffening rather than direct dysfunction of the pulmonary vessels. However, I have found that hypoxia, a key trigger of PH, down--regulated FXN expression in pulmonary arterial endothelial cells. FXN was also decreased in the pulmonary vasculature of mice and humans with PH. Consequently, such FXN deficiency altered endothelial mitochondrial, vasomotor, apoptotic indices, thus leading to preliminary data regarding the alteration of PH in vivo. Taken together, there may be a direct role for FXN in PH. Hypothesis: FXN deficiency, induced by hypoxia or genetic mutation, disrupts endothelial metabolism and function to promote PH. Specific Aims: 1) Determine whether hypoxic down--regulation of FXN is controlled by miR--130b. I have found that the FXN transcript contains a possible binding site for the PH--relevant miR--130b. By gain-- and loss-- of--function methods in pulmonary arterial endothelial cells, I will determine whether hypoxia--induced miR--130b decreases FXN expression, thus defining a causative relationship among miR--130b, FXN, and Fe--S biogenesis. 2) Determine whether FXN loss attenuates mitochondrial respiration and endothelial function. In primary endothelial cells and inducible pluripotent stem cell--derived endothelial cells (iPSC--ECs) from FRDA patients, I will test the hypothesis that FXN deficiency induces Fe--S cluster--dependent mitochondrial dysfunction, resulting in endothelial phenotypic changes (e.g., apoptosis, proliferation). If successful, findings could establish a key link between hypoxia-- or genetically--driven FXN loss and endothelial dysfunction consistent with PH. 3) Establish whether FXN loss and resulting mitochondrial dysfunction predispose to PH in vivo. In a tamoxifen--dependent endothelial cell FXN knockout mouse model, I will test the hypothesis that FXN deficiency in the pulmonary endothelium promotes molecular, histologic, and hemodynamic changes consistent with PH. If successful, these results will validate an integral and direct role for FXN in the development of PH. Significance: This project is ideally structured to train me as a physician--scientist and bridge the gap between basic science and clinical medicine. I aim to contribute to the currently deficient understanding of Fe--S assembly proteins in endothelial function. I could also identify FXN as a key pathogenic factor in PH, offering the potential of diagnosing FRDA patients at risk for PH and defining FXN as a new drug target to benefit all PH patients.

PUBLIC HEALTH RELEVANCE: Pulmonary hypertension is a deadly disease of the vessels that supply the lung that may in part be caused by a deficiency in iron--sulfur clusters – crucial metal complexes involved in a wide range of cellular functions. Frataxin, an iron--sulfur assembly protein, is mutated in a neurologic disease called Friedreich's ataxia where the presence of pulmonary hypertension has largely been neglected. This proposal aims to establish a direct molecular link between frataxin and the development of pulmonary hypertension to expand our understanding of diseases dependent upon iron--sulfur clusters, and, if successful, to improve management of Friedreich's ataxia patients and offer new drug targets for more effective treatment of pulmonary hypertension.

CRITIQUES: The written critiques of individual reviewers are provided in the essentially unedited form below. These critiques were prepared prior to the meeting and may not have been revised afterward. The "Resume and Summary of Discussion" above summarizes the final opinions of the committee.

CRITIQUE 1

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 5
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:
This is an F30 application from an MSTP student who is starting her first year in graduate school, having completed the first two years of medical school. The candidate has excellent academic performance as an undergraduate, an excellent USMLE Step 1 score, and published three co-authored manuscripts as an undergraduate. The sponsor has excellent productivity, current NIH funding, and has described a cohesive mentoring and career development plan for the applicant. There is some concern that the sponsor already has a total of six other mentees. However, his lab has the funding to easily support this number of trainees. This application proposes a novel mechanistic pathway for the development of pulmonary hypertension, which is a heterogeneous disease with multiple implicated signaling pathways. The candidate proposes that hypoxia induces miR130b, which in turn downregulates frataxin (FXN), leading to mitochondrial and endothelial dysfunction. She will examine this in primary cell culture, including inducible pluripotent stem cell-derived endothelial cells from human patients with Friedrich's Ataxia, which is a human disease with mutations in FXN. Additionally, the candidate will examine the effects of endothelial-specific knockout of FXN in the Sugen-hypoxia model of pulmonary hypertension. Enthusiasm for the research plan is tempered by several methodologic concerns. The primary concern is that all of the studies in cultured cells in Aims 1 and 2 are proposed to be done in 0.2% O_2, which represents near-anoxia. There is a robust literature describing that the mitochondrial response to hypoxia is different than that to anoxia. This has the potential to impact all of the data obtained in Aims 1 and 2, making it difficult to translate these findings to *in vivo* studies where the animals experience hypoxia, not anoxia. The candidate will learn many new techniques as well as skills in manuscript and grant preparation. There is a well-described training plan by the sponsor and co-sponsor that includes incorporating the candidate into the Angiopathy Training Program career development program. The institution and environment are sound.

1. Fellowship Applicant:
Strengths

- The candidate has an outstanding academic record with almost all A's as an undergraduate and graduating summa cum laude. Her medical school coursework is all graded Pass/Fail, but she recently scored a very impressive [redacted] on USMLE Step 1.
- The candidate has had an outstanding undergraduate research experience resulting in three co-author manuscripts in Cell Metabolism, Cell, and Nature Medicine.

Weaknesses

- None noted

2. Sponsors, Collaborators, and Consultants:
Strengths

- The primary sponsor has an excellent record of accomplishment of mentoring both predoctoral and postdoctoral trainees.
- The sponsor has a strong publication record of accomplishment with publications in high impact journals and currently has two R01 grants as well as an American Heart Association grant and several co-investigator projects.

Weaknesses

- The sponsor is currently the primary mentor for three postdoctoral fellows, 1 predoctoral student, and 2 undergraduate students. While his lab has funds to support all of these trainees, there is some concern whether he will have sufficient time for direct mentoring of this candidate.

3. Research Training Plan:
Strengths

- The candidate proposes a novel mechanistic pathway for the development of pulmonary hypertension, which is a heterogeneous disease with multiple implicated signaling pathways. The candidate proposes that hypoxia induces miR130b, which in turn downregulates frataxin (FXN), leading to mitochondrial and endothelial dysfunction.
- The candidate will make use of novel iPSC-derived endothelial cells from human patients with Friedrich's ataxia, a condition with a natural mutation in FXN. This will allow data which may be more rapidly translatable to humans.
- The candidate makes a good case for using tissue-specific FXN mice, specifically endothelial-specific FXN mice. She acknowledges that other tissue-specificity may be required and states that her mentor has funds available to support those studies.

Weaknesses

- In her preliminary data and in Aims 1 and 2, the candidate proposes to do her cellular studies in 0.2% O_2, which is not hypoxia but is instead nearly anoxia. Typically, hypoxia cellular studies are done with between 1.5-3% O_2. When you approach anoxia, the metabolism and ROS responses of the mitochondrial change dramatically. Thus, doing her studies at 0.2% O_2 will significantly impact her results and perhaps lead to results which cannot be extrapolated to organisms in hypoxia. This is a significant weakness of this proposal.
- In Aim 2, the candidate proposes to look at ROS production by DCFDA. There are multiple well-characterized problems with assessing ROS by the various fluorescent probes. The candidate would be better served by using a ratiometric probe for ROS measurements in live cells such as roGFP. The cells can then be imaged and ROS quantified both in hypoxia and normoxia within the same cells.

4. Training Potential:
Strengths

- The candidate puts forth clear career goals in her goals for fellowship training section. She has a clear understanding of what is needed for success and how an F30 award could help facilitate this training.
- The candidate will learn molecular biology skills in primary cell culture including iPSC and miRNA biology. She will additionally learn about modeling disease in mice and make physiologic measurements in mice, as well as tissue processing and analysis.
- The sponsor outlines a comprehensive training plan including graduate coursework, conference attendance, and manuscript preparation and goals.

Weaknesses

- There is a letter of support from the Biostatistics Core stating that they will "perform the statistical analysis." This is of concern as the candidate should be learning to do her own statistical analysis as none of the proposed studies require advanced biostatistics.

5. Institutional Environment & Commitment to Training:
Strengths

- The institutional environment at the University of Pittsburgh is excellent with an excellent record of accomplishment of training and research funding.
- The candidate has already been accepted to the Angiopathy Training Program and will benefit from the resources of the campus-wide program.

Weaknesses

- None noted

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

Vertebrate Animals:
YES, all four points addressed

- The animal species, mice, are the appropriate species for this work, and they are using one of the better rodent models for PH - the Sugen-hypoxia model.
- No animal welfare concerns.

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):

- The candidate has previously taken two courses in medical school: Ethics, Law, and Professionalism about informed consent, confidentiality, conflict of interest, and working with vulnerable populations and Professional Development focusing on biostatistics, proper documentation, and data sharing. Now in graduate school, she will take Ethics for Medical Students which consists of lectures and facilitate student discussions.

Comments on Subject Matter (Required):

- Her graduate school course includes general ethics concepts, analytical methods in ethical dilemmas, ethical decision making, animal experimentation, human subject research, conflict of interest.

Comments on Faculty Participation (Required):

- The graduate school course is led by the MSTP director, Dr. Richard Steinman.

Comments on Duration (Required):

- Four weekly two-hour sessions

Comments on Frequency (Required):

- States that she will have internet-based training modules require yearly centered on conflicts of interest, HIPAA, use of animals in research. No other specific comment on RCR retraining, but the grant is only for four years, so may not need to retrain prior to the end of fellowship training.

Resource Sharing Plans:
Acceptable

- The candidate adequately describes sharing of all data and any potential resources.

Budget and Period of Support:
Recommend as Requested

CRITIQUE 2

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 3
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:
This is an F30 application from an MSTP student at University of Pittsburgh Medical Center. She is pursuing her Ph.D. studies in the lab of Dr. Stephen Chan. She is currently in the 3rd year of her MD/Ph.D. training (1st year of Ph.D. phase). The goal of her research is to identify whether frataxin deficiency, induced by hypoxia or genetic mutation, disrupts endothelial metabolism and function to promote pulmonary hypertension (PH) development. This applicant is outstanding. She has worked in excellent labs, and her studies during her undergraduate training resulted in co-authorships in Cell, Cell Metabolism, and Nature Medicine. Her academic performance during undergraduate training has been very strong. Her letters are highly supportive. The sponsor is a highly regarded and highly funded expert in the study of microRNAs and use of network approaches in PH. The research plan is very well written and is supported by robust preliminary data. Several innovative techniques are being proposed that will provide an excellent training experience. The training plan is outstanding and is individualized to the applicant's needs. The proposed studies will allow the applicant to learn a large number of new methods and concepts, such as induced pluripotent stem cell differentiation and culture, in situ hybridization and immunofluorescence microscopy, molecular biology techniques and systems are driven bioinformatics. The scientific environment at UPMC is extremely rich and stimulating. There, however, are a few weaknesses; these include the fact that the sponsor has not yet been able to demonstrate that his mentees can launch successful careers as independent scientists. However, since the sponsor and his mentees are still "early" in their careers, this is not considered a major weakness. There is also a minor concern that the sponsor may be overcommitted with regards to his mentoring load. The research plan has some minor weaknesses that pertain to the insufficient description of the origin of the human PH lungs, lack of specifics on dosing and time courses in SA1a, and concerns about the relevance of the mouse SuHx model. However, these weaknesses are considered minor, and the enthusiasm for this proposal is very high.

1. Fellowship Applicant:
Strengths

- The applicant is an MD/Ph.D. student in the Cellular and Molecular Pathology (CMP) graduate and Program and Angiopathy Training Program at the University of Pittsburgh. She is pursuing her Ph.D. studies in the lab of Dr. Stephen Chan. She is currently in the 3rd year of her MD/Ph.D. training (1st year of Ph.D. phase).
- The applicant has worked in excellent labs, including the Hazen lab at the Cleveland Clinic
- The applicant has received several scholarships and awards during her undergraduate studies.
- Strong publication record in undergraduate research. The applicant is a co-author on manuscripts published in Cell, Cell Metabolism, and Nature Medicine. She also has published a book chapter with the sponsor.
- Very strong academic performance.
- Involved in several leadership activities.
- Extremely strong letters. Sponsor puts her in the top 1-2% of pre-doctoral trainees that he has met. Dr. Stanley Hazen labels her the “very best in any entering [MSTP] class.”

Weaknesses

- The applicant has not yet presented any of her data from this project at a local or national meeting.

2. Sponsors, Collaborators, and Consultants:
Strengths

- The sponsor is an expert in the study of microRNAs and use of network approaches in PH. He is one of the emerging leaders in the field and is well known for his innovative mechanistic studies in animal models and cell culture systems. He also serves as the Director of the Center for Pulmonary Vascular Biology and Medicine at Pitt.
- The sponsor has mentored 7 postdoctoral fellows, 4 pre-doctoral students, and 6 undergraduate students.
- The sponsor has sufficient funding to support the proposed studies.

Weaknesses

- The sponsor has not yet been able to demonstrate that his mentees can launch successful careers as independent scientists. However, since the sponsor and his mentees are still “early” in their careers, this is not considered a major weakness. In fact, the sponsor started mentoring early in his career, which is considered a sign of his commitment to the mentoring and training process.

3. Research Training Plan:
Strengths

- The research plan is very well written.
- Strong preliminary data.
- Good discussion of Potential Pitfalls and Alternative Approaches.
- Use of state-of-the-art methods and endpoints.

Weaknesses

- In fig. 1, it would be important to state the WHO group of the patients from which lung tissue was isolated. Were these PAH patients or patients with non-PAH-PH?
- SA1a does not provide specifics on dosing and time courses. A less profound degree of in vitro hypoxia would probably be more physiologically relevant.
- It would be important to know whether the decrease in FXN is hypoxia-specific or if this can be replicated with other inducers of PH development (e.g. pro-inflammatory cytokines, growth factors, etc.).

- It is unclear to this reviewer whether the applicant is planning to knock down FXN in iPSCs from Friedreich ataxia patients. If so, the rationale would be unclear, as cells from these patients would be expected to have less FXN to start with.
- The SuHx mouse model of PH is not very robust and many labs cannot reproduce this model. The applicant should provide data that this is established in the sponsor's lab.

4. Training Potential:
Strengths

- The proposed studies will allow the applicant to learn a large number of new methods and concepts, such as induced pluripotent stem cell differentiation and culture, in situ hybridization and immunofluorescence microscopy, molecular biology techniques and systems are driven bioinformatics.
- The training plan is well-rounded and harnesses the rich scientific environment at UPMC. Enrollment in the T32 Angiopathy Training Program is considered one particular strength and indicates dedicated support by the institution.
- The selected courses will provide valuable complementary knowledge skills.
- Strong and individualized training plan with a strong letter from the sponsor.
- Strong thesis committee with a broad area of scientific and professional expertise.

Weaknesses

- Not all required coursework has been completed.
- In addition to the applicant, the sponsor serves as the primary research mentor for 3 postdoctoral fellows, 1 pre-doctoral student, and 2 undergraduate students. This is considered a fairly high mentoring load.

5. Institutional Environment & Commitment to Training:
Strengths

- The applicant is currently funded through a T32 grant, indicating strong institutional support.
- The environment at the University of Pittsburgh, UPMC's Heart and Vascular Institute, and the Vascular Medicine Institute is outstanding. The applicant has multiple intellectual and infrastructural resources available through the sponsor, the MSTP program and through the various institutes.

Weaknesses

- None noted

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

Vertebrate Animals:
YES, all four points addressed

- The vertebrate animal section is very short and could provide more detail

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):

- Medical Scientist Training Program (MSTP) curriculum has several structured courses and workshops on this topic interspersed throughout the medical and graduate school.
- completed a semester-ˇlong course titled "Ethics, Law, and Professionalism" (Fall 2014; two hours each week for 15 weeks).
- complemented by other courses and online modules

Comments on Subject Matter (Required):
- all important topics are covered

Comments on Faculty Participation (Required):
- Faculty from the bioethics department, medical school, and law school
- MSTP director

Comments on Duration (Required):
- will take throughout graduate school

Comments on Frequency (Required):
- yearly renewal

Resource Sharing Plans:
Not Applicable (No Relevant Resources)

Budget and Period of Support:
Recommend as Requested

Additional Comments to Applicant (Optional):
- Note the redundant "improve my ability to independently ask a pertinent question and" on page 31.

CRITIQUE 3

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 1
Research Training Plan: 2
Training Potential: 1
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:
This is a new application from an MD/Ph.D. student at the University of Pittsburgh working in the lab of Dr. Stephen Chan. The applicant performed research during her undergraduate years at Case Western with Dr. Hazen and was listed as a middle author on 3 very high impact publications. She also lists several awards and has good grades. The sponsor and environment are outstanding and will ensure a successful training experience. The sponsor is well-funded and well-published and has trained numerous physician-scientists in the past. The applicant details nicely what skills she has performed already and what she will be learning for the proposed project. The research plan is well written and the topic is interesting and timely. One major weakness is the deletion of FXN in all endothelial cells will affect other organs as well and how that will be reconciled was not discussed. Nonetheless, successful completion of the project will generate valuable information about the processes involved in the development of pulmonary hypertension.

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

Vertebrate Animals:
YES, all four points addressed

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):
- None noted

Comments on Subject Matter (Required):
- None noted

Comments on Faculty Participation (Required):
- None noted

Comments on Duration (Required):
- None noted

Comments on Frequency (Required):
- None noted

Resource Sharing Plans:
Not Applicable (No Relevant Resources)

Budget and Period of Support:
Recommend as Requested

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

VERTEBRATE ANIMALS: ACCEPTABLE
COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 F30 HL139017-01; PI Name: Culley, Miranda Kay

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.

MEETING ROSTER
Center for Scientific Review Special Emphasis Panel

CENTER FOR SCIENTIFIC REVIEW
Fellowships: Physiology and Pathobiology of Cardiovascular and Respiratory Systems
ZRG1 F10A-A (20)
03/23/2017 - 03/24/2017

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html and NOT-OD-15-106 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html, including removal of the application from immediate review.

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