AWARD NUMBER: W81XWH-16-1-0022

TITLE: Characterization of Clustered CTCs to Eliminate Breast Cancer Metastasis

PRINCIPAL INVESTIGATOR: Alvin Schmaier, M.D.

CONTRACTING ORGANIZATION: Case Western Reserve University
Cleveland, OH 44106

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This program aims to examine the role of platelets and IL-11 in breast cancer tumor cell clustering and metastasis to the organs such as lung. In year 1, we determined that 5 days of IL-11 treatment to C57BL/6, but not SCID or balb/c, mice resulted in a 2-3-fold increase in platelet counts. Thus, only IL-11induced thrombocytosis only occurs in C57BL/6 mice. In year 2 of the program, we then had to create a murine model for metastatic breast cancer in C57BL/6 mice. After establishing the institutional protocols to 1) label C57BL/6 tumor (EO771-LMB cells) with luciferase, 2) obtain Case Western Reserve University IACUC approval for these studies, 3) obtain Case Western Reserve University Institutional Biosafety Committee (IBC) approvals to inject stably transfected tumor cells into mice, and 4) Obtain re-approvals from DOD (ACURO) of our revised animal protocols, we began experiments using a syngeneic murine mammary cancer tumor in the C57BL/6. At this point, we have established a novel murine model to examine lung metastasis of breast cancer cells in mice with altered platelet counts and function. A detailed list of experiments is starting forthwith.

15. SUBJECT TERMS
IL-11, CD49b, CD44, miR30c, platelets, breast cancer stem cells, circulating tumor cells.

16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. INTRODUCTION:

Background: Circulating tumor cells (CTCs) with stem cell properties are considered the seeds of distant metastasis. The mechanisms how CTC clusters are generated are unclear. We aimed to determine if CTC clustering with lung metastasis is enhanced by platelets. Our aim is to examine the participation of IL-11/CD49b in the pathway for CTC/platelet clusters. We examined the association of platelets as well as IL-11 and CD49b with CTC clusters.

2. KEYWORDS:

Breast cancer stem cells; circulating tumor cells. IL11; CD49b; CD44; miR30c; platelets

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.

Task 1: Does IL11 promote CTC clustering and polyclonal metastasis in vivo. (1-4 months) – partial completion

Task 2: Are platelets activated by IL-11 to cluster with CD44+ CSCs/CTCs in vitro and in vivo? (4-8 months) – partial completion

Task 3: Is CD44 regulated by and required for IL-11 function in promoting CSC/CTC cluster formation? (8-12 months) – partial completion.

Task 4: Does IL11 induce CD49b expression in platelets and BCSCs? – not done yet

Task 5: Is CD49b important in IL-11 function in CSC/CTC clustering? – not done yet

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.

Our program is to examine the influence of IL-11 on platelets and there combined effect on breast cancer tumor clustering and metastasis to lung and other organs. In the first year of the award, we learned that only C57CL/6 mice, not SCID or balbc, are responsive to IL-11 with a 2-3 fold increase in platelet count. Thus we needed to establish a syngeneic, C57BL/6 murine model for breast cancer metastasis.

The literature provided a guide for such a model using EO771.LMB cells, a C57BL/6-mouse-derived model of spontaneously metastatic mammary cancer (Johnstone CN *et al*. Disease Models & Mechanisms (2015) 8:237. EO771.LMB tumors are derived from poorly metastatic parenteral EO771 mammary tumors. EO771.LMB cells were purchased from the ATCC. The cells are positive for EGFR and a mutant p53.

Several operational issues, however, presented itself upon the initiation of this work that has interfered with the research progress of the program in year 2. (1) My co-PI and collaborator on the DOD grant for the murine model studies, Dr. Huiping Liu, left Case Western Reserve University and transferred her project and support to Northwestern University in the late Spring, 2017. It has been originally planned that the murine metastasis models were to be performed in her laboratory. (2) I needed to submit to the IACUC the protocols for murine breast cancer metastasis that are novel for my laboratory to IACUC. The submission of these new protocols for the murine breast cancer metastasis to IACUC occurred at the same time that I needed to renew (done every 3 years) my entire animals protocol at Case Western Reserve University. (3) Since we were using transfected cells, the actual protocol also had to be reviewed and approved after the Animals' Protocol approval by the CWRU Institutional Biosafety Committee (IBC). (4) Once CWRU IACUC and IBC approvals were completed, DOD asked for an additional complete review of the newly approved Animals' Protocol from CWRU. During the time of when the institutional approvals were taking place (June 2017 through March 2018), NO ANIMAL RESEARCH ON THIS PROJECT COULD BE PERFORMED.

Time-Line for Completion of all Operational Mandatories.

7/14/17 – Submission of the Schmaier Animals' Experimental Protocol 2014-0089 renewal to IACUC. Expiration date of the old protocol was 8/8/18.
10/16/17 – IACUC approved the Animal Experimentation Protocol 2014-0089 titled: "Procedure to Examine Murine Thrombosis, Vascular Biology, and Cancer Metastasis in the Schmaier Laboratory".
10/17/17 – Submission of the Animals' protocol ("Lung Metastasis of Transfected Breast Cancer Cells") to the Institutional Biosafety Committee (IBC) to use stably transfected murine tumor cells to inject in mice.
11/8/17 – Submission of revised animal protocol to the USAMRMC Animal Care and Use Review Office (ACURO)
11/17/17 – Institutional Biosafety Committee (IBC) of Case Western Reserve University approval of "Lung Metastasis of Transfected Breast Cancer Cells".
11/24/17 – Email from ACURO that the entire new Animals's protocol needs to be reviewed by ACURO.
3/19/18 - In accordance with the above references, protocol BC150596P1 entitled, "Procedures to Examine Murine Thrombosis, Vascular Biology, and Cancer Metastasis in the Schmaier Laboratory," IACUC protocol number 2014-0089, Protocol Principal Investigator Alvin Schmaier, is approved by the USAMRMC Animal Care and Use Review Office (ACURO) as of 19-MAR-2018 for the use of mice and will remain so until its modification, expiration or cancellation.

Hence from June 1st 2017 until 3/19/18, we were unable to perform any animal experiments until all the institutional animal approvals were satisfied.

While the animal protocols were being reviewed and edited as results of the reviews, we labeled EO771.LMB cells by stably transfecting them with luciferase. These studies were performed in collaboration with Dr. Huiping Liu and her Research Assistant Wenjing Chen. Briefly, EO771.LMB tumor cells were stably transfected to express luciferase-2-tdTomato (L2T) using a lentivirus with the procedure from Addgene (https://www.addgene.org/protocols/lentivirus-production). These cells are hardy and viable and are easily transferred from the freezer to tissue culture. The cells are grown in DMEM high-glucose with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin.

Suffice-it-to-say, at this juncture, we are positioned to make progress in this program. Initial experiments have been performed with the new murine breast cancer cell metastasis model in C57BL/6 mice. Two murine models have been employed. (1) is a flank injection and (2) a tail vein injection.

Investigations with EO771.LMB cell Flank Injection.

L2T-labeled cells were grown to perform murine flank injections to monitor tumor growth. 5 X10^5 cells in 100 μl of HBSS were injected into the right flank using a 26 gauge needle on the supine position in C57BL/6 mice while under ~2% isoflurane/98% oxygen mixture. Growth was monitored by physical exam and by bioluminescence after the IP injection of luciferin (30 mg/ml stock) at a dose of 150 mg/kg. After 10 min, the mouse is scanned over the shaved body portion of interest. **Figures 1** and **2** below are representative bioluminescence scans and graphs,

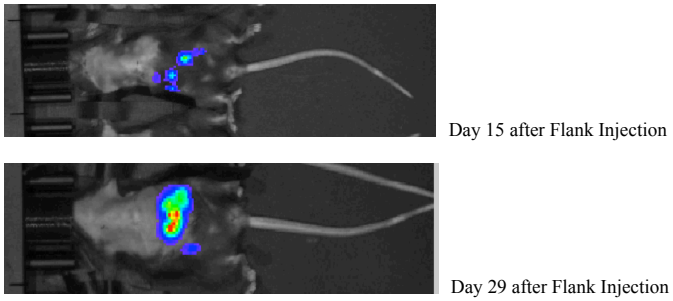

Figure 1. *Representative bioluminescent scans of flank tumors of injected EO771.LMB cells.* See Text for description.

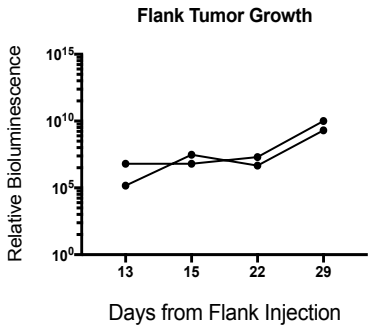

Figure 2. *Graphic representation of flank tumor growth.* See Text

respectively, of developing flank tumors. As can be seen there is relatively slow growth of the tumor from Day 13 to Day 29. In the end of the period of time, no metastasis is seen into the lung. The size of the local tumor is such that it presents a burden to the animal and, thus, the animal has to be euthanized. The local growth of the tumor is consistent with that proposed by Johnstone *et al.* We have not as yet have had the opportunity to perform lung autopsies on these animals. In the EO771.LMB murine model, metastasis is noted at two weeks if the primary tumor is resected at that time. If the primary tumor is not resected, the local tumor burden of the animal becomes excessive and it needs to be sacrificed at 25-30 days.

Investigations with EO771.LMB Tail Vein Injection.

L2T-labeled cells were grown to perform murine tail vein injections to monitor tumor growth. 5-10 X10^5 cells in 100 μl of HBSS were injected into the tail vein using a 26 gauge needle in C57BL/6 mice while under ~2% isoflurane/98% oxygen mixture. In these preliminary experiments we observed the time it takes to develop lung tumors. After the initial injection,

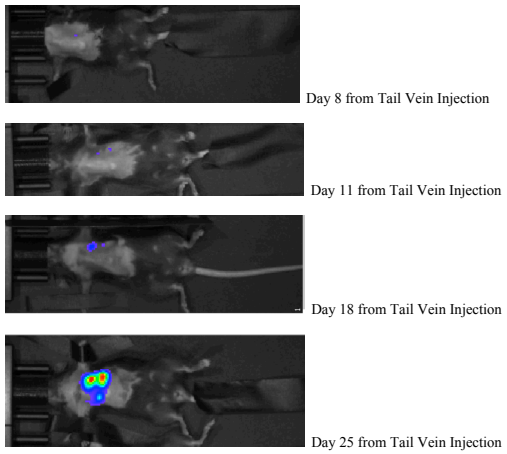

Figure 3. *Representative bioluminescence scans of mice after 0.5-1X10^5 EO771.LMB cells were injected into the tail vein of wild type C57BL/6 mice.* See Text.

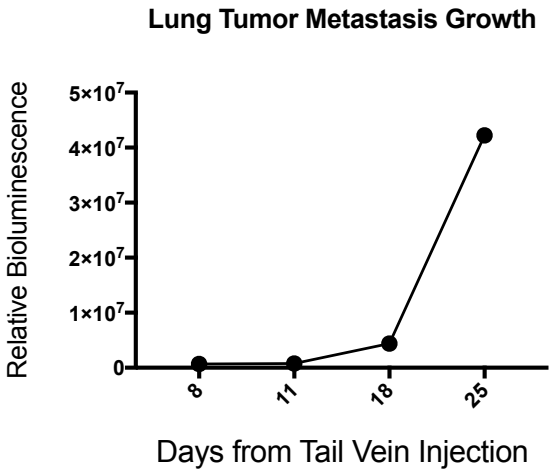

Figure 4. *Graphic representation of lung tumor growth after injection of EO771.LMB cells.* See text for description.

Tumor cell growth was slow for the first 18 days. From 18 to 25 days, there was exponential tumor cell growth. These data are consistent to what has been observed in Johnstone *et al*. These combined data indicate that we have established a syngeneic, C57BL/6 murine mammary cell carcinoma models that can be used for metastasis studies that can be influenced by modification of *in vivo* platelet counts.

Going forward, we propose that each experiment will consist of 10 treated mice and 10 control mice. A double-sided power of 90% (p value between 0.01 to 0.001) will require 5 to 8 animals in each group. At most, 10 animals total (10 in one condition and 10 mice with a comparison condition) will be needed for each experimental condition.

Since each experiment will take ~1 month, we propose the following schedule over the next 12 months. Initial experiments will be with 10 control and 10 IL-11-treated mice. We will determine if elevation of the murine platelet count by IL-11 injections (100 micrograms/kg subcutaneous) in less than 100 microliters per mouse daily for 7 days influences metastasis (Blood 81:27, 1993). Normal C57BL/6 mice will be treated with IL-11 for 7 days followed by tail vein injection of 10 X 10^5 EO771.LMB cells. Platelet counts before and after IL-11 injections will be determined by venipuncture of the inner canthus of the eye using microhematocrit tubes. Control mice will not be treated with IL-11. Luciferin treated mice will be scanned weekly for three weeks followed by scans twice weekly. The rate and extent of lung metastasis will be determined. At sacrifice, the lungs will be harvested for histologic studies.

Subsequent experiments will examine the influence of thrombocytopenia on tail vein-induced pulmonary metastasis by injection of Moab MwReg30 to platelet integrins alpha 2b beta 3. The

Moab MwReg30 antibody is given by IV or IP injection or osomotic minipump. 1) A single intravenous infusion at 0.04 to 0.4 mg/kg results in a 12 to 24 h 80% thrombocytopenia (Abuqayyas L et al., International J of Pharmacology 444:185, 2013); 2) Intraperitoneal (IP) administration at 100 microliters serum (~0.1 microgram/ml) lowered the platelet count 25% within 1 h, but it returned to baseline within 5 h (Nieswandt B et al., Blood. 94:684, 1999).

Subsequent plans will follow proposal as originally written.

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.

The project allowed us to develop the murine bioluminescence imaging technique.

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

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

We intend to utilize the murine C57BL/6 circulating EO771.LMB tumor cells model for breast cancer metastasis into one syngeneic with C57BL/6 cells. Once demonstrating increased lung metastasis by modifying platelets counts with IL-11 and other platelet elevating agents, such as romiplostim an eltrombopag , we will examine the effect of IL11 on platelet and megakaryocytes CD44 and CD49b. Additional studies will be performed as written in the original proposal.

4. IMPACT:

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.

Nothing to Report.

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

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:

No changes in planned protocol to date

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.

Actual problems in Year 2 that led to delay in research as been outlined above in the progress report.

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.

The costs of the program have increased because expected collaborator has left. There has been no increase in funding for more activities and greater expenses for this project.

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

Not relevant for this project.

Significant changes in use or care of vertebrate animals

No changes in protocol since the last review. All changes in the animal protocols have already been reviewed by ACURO, 3/19/2018.

Significant changes in use of biohazards and/or select agents

No changes in use of biohazards and/or select agents since the last review.

6. PRODUCTS:

- **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).*

None at this time

  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).*

None at this time

  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.*

None at this time

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Not relevant

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

Not relevant

- **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.

None at this time

- **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.*

Not relevant

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.)*

See Next Page

Dr. Alvin H. Schmaier MD
Project Role: PI
ORCID ID: 0000-0002-3884-6234
Nearest person month worked: 1 month
Contribution to the project: Designed the studies, trained the Research Assistant, analyzed the data; wrote the grant and progress report.

Alona Merkulova
Project Role: Research Assistant IV
ORCID ID: none
Nearest person month worked: 7 months
Contribution to Project: Performed all the experiments, animal care, obtained the final data for analysis; responsible for all activities related to this program.

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."

Active Support.

BC150596P1, Schmaier (PI) 06/01/16-5/31/19 0.32 Calendar mo
CDMRP, Department of the Army
Characterization of Clustered CTCs to Eliminate Breast Cancer Metastasis
This program seeks to characterize the role of platelets in participating in breast cancer metastasis. It is a novel project that will examine the roles of IL11, CD49b and CD44 in platelets and circulating breast cancer cells to determine if these cells conjoin to promote metastasis. There is no overlap with the current proposal.
Total Support: $317,359; Direct Support: $200,247.

R01 HL126645-01 , Simon (PI) 12/01/15-11/31/20 1.2 Calendar mo
NIH/NHLBI
"MRP-14, CD36 and Thrombosis"
The overall objective of this proposal is to define the role of MRP-14 in vascular inflammation and thrombosis.
Role: Co-PI
Total Support: Salary support only

R01 AI130131-01 (Kazura, PI) 04/01/17-3/31/22 1.2 Calendar Mo
NIH/NAI
"*Kruppel-Like Factor 2 Counters Vascular and Immunologic Dysfunction in Child Cerebral Malaria*"
The overall goal of this program is to examine how the head domain of the malaria parasite influences the constitutive anticoagulant nature of vascular endothelium.
Role: Co-PI
Total Support $71,484 for year 1.

VeloSano Award (Schmaier, PI) 06/1/17-05/31/19 0 Calendar Mo
Case Comprehensive Cancer Center
"*Tyrosine Kinase Inhibitors and Cardiovascular Events in CML*"
The overall objective of this award is to examine how ponatinib induces cardiovascular events in 30% of patients who receive the medication. The goal of the award is to define mechanism(s) of action and characterize an antidote to ponatinib's negative cardiovascular effects.
Direct Support: $50,000 for 1 year.

- *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.*

R13 HL140902, Schmaier (PI) 04/01/18-03/31/19 0 Calendar mo
NIH/NHLBI
"KININ2018CLE"
The overall objective of this award is to provide travel awards for junior investigators to KININ2018CLE that will be held in Cleveland, OH on the campus of Case Western Reserve University. This meeting will present and discuss the major issues in the kallikrein/kinin, contact activation, and renin angiotensin field.
Direct Support:

Shire Investigator-Initiated Support (Schmaier, PI) 7/1/2018-6/30/2019 0.32 Calendar Mo.
"*Prolylcarboxypeptidase Activates Prekallikrein.*"
This project critically examines if endothelial cell prolycarboxypeptidase has the ability to activate PK to plasma kallikrein to generate bradykinin and factor XIIa. We examine how ambient C1 inhibitor levels influence PRCP activation of PK and indirectly FXII.
~~Total Support:~~

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A

Abstract. This program aims to examine the role of platelets and IL-11 in breast cancer tumor cell clustering and metastasis to the organs such as lung. In year 1, we determined that 5 days of IL-11 treatment to C57BL/6, but not SCID or balb/c, mice resulted in a 2-3-fold increase in platelet counts. Thus, only IL-11induced thrombocytosis only occurs in C57BL/6 mice. In year 2 of the program, we then had to create a murine model for metastatic breast cancer in C57BL/6 mice. After establishing the institutional protocols to 1) label C57BL/6 tumor (EO771-LMB cells) with luciferase, 2) obtain Case Western Reserve University IACUC approval for these studies, 3) obtain Case Western Reserve University Institutional Biosafety Committee (IBC) approvals to inject stably transfected tumor cells into mice, and 4) Obtain re-approvals from DOD of our revised animal protocols, we began experiments using a syngeneic murine mammary cancer tumor in the C57BL/6. At this point, we have established a novel murine model to examine lung metastasis of breast cancer cells in mice with altered platelet counts and function. A detailed list of experiments is starting forthwith.