AWARD NUMBER: *W81XWH-17-2-0003*

TITLE: Photosensitization of Bacterial Pathogens through Small Molecule Activators of Heme Photosynthesis

PRINCIPAL INVESTIGATOR: Eric Skaar, Ph.D, M.P.H.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37203-6856

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Fort Detrick, Maryland 21702-5012

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Photosensitization of Bacterial Pathogens through Small Molecule Activators of Heme Biosynthesis

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**6. AUTHOR(S)**

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**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Vanderbilt University Medical Center
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Nashville, TN
37203-6856

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U.S. Army Medical Research and Materiel Command
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**14. ABSTRACT**

Gram-positive bacteria cause the majority of skin and soft tissue infections (SSTIs), resulting in the most common reason for clinic visits in the United States. Recently, it was discovered that Gram-positive pathogens utilize a unique heme biosynthesis pathway, which implicates this pathway as a novel target for development of antibacterial therapies. We report here the identification of a small molecule activator of coproporphyrinogen oxidase (HemY) from Gram-positive bacteria, an enzyme essential for heme biosynthesis. Activation of HemY induces accumulation of coproporphyrin III and leads to photosensitization of Gram-positive pathogens. In combination with light, small molecule HemY activators reduce bacterial burden and tissue ulceration in murine models of SSTI. Thus, small molecule activation of HemY represents an effective strategy for the development of light-based antimicrobial therapies. In addition, we have conjugated photactivators to mAbs that target the surface of *S. aureus* increasing the antibacterial activity of light against *S. aureus*. Future work will focus on optimizing the combined activity of these small molecule and mAb photosensitizers.

**15. SUBJECT TERMS**

Gram-positive bacteria, antibiotic, photosensitization, heme biosynthesis, photodynamic therapy
Antibiotic

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

*Staphylococcus aureus* is a Gram-positive commensal bacterium that colonizes the anterior nares of approximately 30% of the human population. *S. aureus* is the most prevalent pathogen isolated from skin and soft tissue infections, is a leading cause of food borne illness, and is the leading cause of infectious endocarditis and hospital-acquired infections in the United States. In keeping with this, *S. aureus* is a tremendous threat to our Armed Forces. Post-traumatic infections are a source of considerable morbidity to Service Members and U.S. Veterans. *S. aureus* and other Gram-positive bacteria are some of the most common organisms causing post-traumatic infections in Service members. Unfortunately, rapid acquisition of antibiotic resistance compromises effective antimicrobial therapy. It is therefore imperative to develop new therapies for post-traumatic infection that are effective against antibiotic-resistant *S. aureus*. The objective of this proposal is to develop new photoactivatable antibiotics for the treatment of *S. aureus* infections. One promising area of research focuses on *S. aureus* heme synthesis, which is vital to *S. aureus* survival within the host. Studies proposed in this application will lead to the design of molecules that disrupt heme homeostasis and inhibit staphylococcal disease as well as diseases caused by a variety of other important infectious threats.

**KEYWORDS:**

- Gram-positive bacteria
- Antibiotic
- Photosensitization
- Coproporphyrinogen oxidase (CgoX, formally known as HemY)
- Heme biosynthesis
- Pathogenesis
- Antimicrobial photodynamic therapy
- Skin infection model
- Iron regulated surface determinant (Isd) system
- Antibodies

**ACCOMPLISHMENTS:**

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

| **Specific Aim 1: Synthesize and test analogs of '882 for CgoX activating potential.** | **Timeline** | **Site 1:** | **Site 2:** |
|---|---|---|---|
| **Major Task 1: Synthesize '882 analogs** | Months | **Vanderbilt University Medical Center (VUMC); Vanderbilt University (VU)** | **WRAIR** |
| Subtask 1: Work with VU Chemical Synthesis Core to create molecular analogs based on the '882 scaffold. Collaborate with Dr. | 1-24 | Dr. Skaar (VUMC)<br>Dr. Sulikowski(VU) | Dr. Sciotti |

| | | | |
|---|---|---|---|
| Sciotti (WRAIR) to ensure VU synthetic chemistry efforts are complimentary with the Army research team. | | | |
| Milestone(s) Achieved: Synthesize approximately 150 ‘882 analogs. Approximately 100 compounds will be synthesized by Vanderbilt team and approximately 50 compounds will be synthesized by the WRAIR team. | | 113 ‘882 analogs have been synthesized | 33 ‘882 analogs have been synthesized |
| **Major Task 2: Determine the activity of ‘882 analogs against live *Staphylococcus aureus*** | Months | **VUMC** | **WRAIR** |
| Subtask 1: Test all ‘882 analogs for *hrtAB* activating potential | 12-24 | Dr. Skaar (VUMC): 146 of the 146 ‘882 analogs have been tested | |
| Subtask 2: Test the ability of ‘882 analogs to increase coproporphyrin production in *S. aureus* | 12-24 | Dr. Skaar (VUMC): 61 of the 146 ‘882 analogs have been tested. | |
| Subtask 3: Test the ability of ‘882 analogs to activate CgoX enzymes from diverse CARB priority pathogens *in vitro.* Test ‘882 analogs against human protoporphyrin oxidase to ensure specificity against bacterial enzymes. | 12-24 | Dr. Skaar (VUMC): 12 of the compounds have been tested for activity against other microorganisms. | |
| Milestone(s) Achieved: Identify ‘882 analogs with potent activity against CgoX from multiple bacterial pathogens with minimal to no activity against human enzyme. | | This milestone has not been achieved. | |
| **Major Task 3: Test the ability of optimized ‘882 analogs to photosensitize *Staphylococcus aureus* to light-based killing.** | Months | **VU and VUMC** | **WRAIR** |
| Subtask 1: Design and build devices to deliver light for photodynamic therapy. This will include antibacterial experiments, tissue delivery devices, and deep tissue penetration devices | 1-48 | Dr. Jansen (VU): We continue to develop multiple additional lights, including lights that simultaneously deliver blue, green and red light to increase tissue penetration while ensuring light-dependent bacterial killing. Current work is focused on | |

| | | | |
|---|---|---|---|
| | | building lights for in vivo infection studies. | |
| Subtask 2: Test ‘882 analogs for light-based anti-bacterial activity. This will involve testing for the best antibacterial activity against numerous pathogens and then sending those ‘882 analogs to WRAIR for further testing. | 1-36 | Dr. Skaar (VUMC): These experiments are on-going in an iterative process as new molecules are synthesized and new lights are developed. | |
| Milestone(s) Achieved: Identification of highly active ‘882 analogs that exhibit potent antibacterial activity in the presence of newly created light delivery devices and are metabolically stable to mouse and human microsomes. | | This milestone has not yet been achieved. | |
| **Specific Aim 2: Define the mechanism by which ‘882 activates CgoX and use this information to design improved analogs.** | **Timeline** | **Site 1:** | **Site 2:** |
| **Major Task 1: Purify recombinant CgoX from various bacterial pathogens** | Months | **VUMC** | **WRAIR** |
| Subtask 1: Purify CgoX wild type and point mutations from *S. aureus* and CARB priority pathogens | 1-6 | CgoX wild type and point mutants from *S. aureus* have been purified. (see Surdel *et. al.* Fig 2A,G) | |
| Milestone(s) Achieved: Purify CgoX from four distinct bacterial pathogens | | CgoX was successfully purified from *P. acnes* and *B. subtilis*. (see Surdel *et. al.* Fig 2B,G) | |
| **Major Task 2: Solve the co-crystal structure of CgoX bound to ‘882 analogs** | | **VUMC** | **WRAIR** |
| Subtask 1: Solve the crystal structure of ‘882 analogs bound to CgoX from *S. aureus*. Dr. Skaar’s group will purify *S. aureus* CgoX. Dr. Schroeder Noble’s group will crystalize CgoX bound to ‘882. | 6-12 | *S. aureus* CgoX has been purified. | *S. aureus* CgoX crystallization studies are in progress with Dr. Schroeder Noble’s group. |
| Subtask 2: Solve the crystal structure of ‘882 analogs bound to CgoX from other bacteria. Dr. Skaar’s group will purify CgoX from other | 12-36 | | These experiments have not yet begun. |

| | | | |
|---|---|---|---|
| bacteria. Dr. Schroeder Noble's group will crystalize CgoX bound to '882. | | | |
| Milestone(s) Achieved: Crystal structures of CgoX in complex | | This milestone has not yet been achieved. | |
| **Specific Aim 3: Test the therapeutic efficacy of '882 analogs in murine model of *S. aureus* skin infection.** | **Timeline** | **Site 1:** | **Site 2:** |
| **Major Task 1: Test the therapeutic efficacy of '882 analogs in murine model of *S. aureus* infection** | Months | **VUMC** | **WRAIR** |
| Subtask 1: Develop murine model of *S. aureus* skin infection. Develop protocols for administration of new light devices. | 24-48 | Dr. Skaar (40 mice); Developed murine model of superficial *S. aureus* skin infection; '882-PDT decreases bacterial burden *in vivo*. *(see Surdel et. al. Fig 4E-G)* | |
| Subtask 2: Test therapeutic efficacy of optimized '882 analogs in murine model of *S. aureus* skin infection. | 24-48 | Dr. Skaar (150 mice): These experiments have not yet begun and are planned for the current funding year. | |
| Milestone(s) Achieved: Identify therapeutically efficacious '882 analogs for the treatment of staphylococcal skin infections. IACUC and ACURO Approval. | 18 | This milestone has not yet been achieved although we do have IACUC approval for testing. | |
| **Major Task 2: Test the therapeutic efficacy of '882 analogs in murine model of bacterial skin infection.** | Months | **VUMC** | **WRAIR** |
| Subtask 1: Develop murine model of bacterial skin infection caused by CARB priority pathogens. Develop murine model of skin infection using 4 CARB priority pathogens. | 24-48 | Dr. Skaar (250 mice); Developed superficial *P. acnes* skin infection model. *(see Surdel et. al. Fig 4H)* | |

| | | | |
|---|---|---|---|
| Subtask 2: Test therapeutic efficacy of optimized ‘882 analogs in murine model of skin infection. | 24-48 | These experiments have not yet begun. | |
| Milestone(s) Achieved: Identify therapeutically efficacious ‘882 analogs for the treatment of multiple skin infections. IACUC and ACURO approval. | | This milestone has not yet been achieved. | |

- **What was accomplished under these goals?**

  The major goal of this project is to develop small molecule analogs of ‘882 that activate heme synthesis pathways and sensitize bacteria to light-dependent killing. Major activities associated with this project can be divided into three areas: Chemistry, Photonics, and Biology. One exciting development since the last update is that we have isolated and purified monoclonal antibodies that target the iron regulated surface determinant (Isd) of *S. aureus*. We exploited these surface targeting antibodies by conjugating them to photoactivating molecules that absorb red light. These powerful molecules now allow us to kill *S. aureus* using red light, in addition to the blue light killing activity of ‘882 derivatives. Simultaneous administration of mAbs and ‘882 in the presence of both blue and red light leads to complete sterilization of *S. aureus* in culture. This powerful light-based antimicrobial strategy will form the basis for our optimization strategies in the coming year, with the goal of generating a mAb/small molecule cocktail that is efficacious for the treatment of *S. aureus* infection in murine models. Notably, we will continue to develop ‘882 derivatives as antimicrobials against a variety of bacterial pathogens as originally planned.

  **Chemistry**-The chemistry support for this project is being performed by Vanderbilt chemists as well as chemists at WRAIR. The objective of the chemistry teams is to develop small molecule analogs of ‘882 with increased efficacy as photosensitizing antibiotics. The major activities of this group include chemical synthesis to develop analogs. In this regard, the combined chemistry teams at WRAIR and Vanderbilt have synthesized 146 distinct analogs of ‘882.

  **Photonics**-The objective of the photonics team is to create enhanced devices to deliver light for photodynamic therapy-based strategies. The major activities of this group involve purchasing and/or building new lights that can be used by the biology team to test efficacy in antibacterial assays. We have found that, although blue light is highly effective for photodynamic killing of bacteria in culture, the nature of blue light does not enable it to penetrate into skin beyond a few millimeters. To circumvent this issue, the photonics team has been analyzing the biophysical features of coproporphyrin III (CPIII) and found that this molecule has additional peak absorbances in the green-red region of visible light. Light at these wavelengths is not absorbed as

strongly by the skin as blue light and is thus able to penetrate deeper into the skin. In addition, we have recently created monoclonal antibodies that target *S. aureus* and are conjugated to photactivators that are activated by red light. These exciting new molecules are highly inhibitory to *S. aureus* in the presence of red light, and sterilize *S. aureus* cultures when used in combination with '882 analogs. Notably, using red light responsive photoactivators will allow us to penetrate deeper tissue and should be very valuable in murine models of infection. Therefore, the photonics team is now creating lights that simultaneously deliver blue, green, and red light in the hopes of maximizing antibacterial activity while simultaneously enabling deep tissue penetration.

**Biology**-The objectives of the biology team are to test the antibacterial activity of '882 analogs created by the chemistry team, as well as photoactivator conjugated mAbs, using lights created by the photonics team. In addition, the biology team is testing each analog created by the VU and WRAIR chemists for the ability to activate heme synthesis, and hence CPIII accumulation, using reporter assays. To date, the biology team has tested 146 of these molecules and prioritized them based on their activities. This information is then used by the chemistry team in an iterative fashion to drive new molecule synthesis.

Future experiment in the coming year will continue this iterative process in an effort to identify optimized '882 analogs that are highly antibacterial against *Staphylococcus aureus* and efficacious in murine models of bacterial infection. In addition, we will begin to test the toxicity profiles of our most potent '882 analogs to prioritize animal work. Once we have a suite of '882 analogs that are highly antibacterial and exhibit minimal toxicity, we will test them for therapeutic efficacy, alone and in combination with the mAbs described above.

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

  This project has resulted in training and professional development for all individuals involved. Alec Walter is a graduate student in Biomedical Engineering who is working on this project and who is moving between the fields of microbiology and photonics to accomplish the aims of this proposal. In addition to his discipline specific training in photonics, he is becoming proficient in basic microbiological assays. Alec presented his findings at a major Biophotonics conference in January, and we have presented internally at the Vanderbilt Symposium on Infection and Immunity.

Dennis Horvath, Jocelyn Simpson, and Joseph Zackular previously worked on this project and have moved on to new positions. Notably, Joseph is now an Assistant Professor at Pennsylvania University. Others currently involved in this project are Andy Weiss and Andrew Montieth both of whom have had to develop various technical skills in order to achieve the stated Aims. Andrew and Andy are working on optimizing murine models of infection that can be used to test the efficacy of candidate analogs, as well as testing mAbs in various biologic assays. We have recently hired two new research assistants (Anderson Miller and Sydney Drury) who have become proficient in the light killing assay and apply it routinely to test the efficacy of new analogs. Anderson and Sydney both have ambitions to attend graduate school, so they are getting excellent hands-on research experience.

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

  We have presented our work at SPIE Photonics West, a major national photonics conference.

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

  During the next reporting period, we will continue with the plan as originally described with adjustments to accommodate the incorporation of our exciting new mAb-based photoactivators. Our proposal included creating and optimizing analogs for the first 24 months of the project, and we plan to extend this in the beginning of the next funding period. Notably, the chemistry team at WRAIR underwent a significant change in personnel which led to a slight delay, but we are now back up to full speed and should have completed analog design and synthesis in this funding period. The chemistry groups at both Vanderbilt and WRAIR will continue to use information provided by the biology group to create new analogs, and all new analogs will be tested by the biology group for activity with the goal of identifying potent molecules that are highly antibacterial in photosensitization assays and exhibit minimal toxicity. In addition, the Biology team will begin combinatorial killing assays to identify the most antimicrobial small molecule/mAb combination. Finally, the biology group will continue to refine assays for testing activity against both Gram negative and Gram positive pathogens in an effort to create broadly acting antimicrobials for the treatment of various infections. Simultaneously, the photonics group will continue to improve light delivery by optimizing lights for power, duration, and wavelength. We anticipate that we will begin to move to animal studies during this next funding period. The

combined total of this effort will lead to new antibacterial strategies for the treatment of antibiotic resistant infections.

- **IMPACT:**
  - **What was the impact on the development of the principal discipline(s) of the project?**

    Photodynamic therapy has been studied and applied by numerous groups for the treatment of microbial infections. This technique exploits the fact that the penultimate product in the heme biosynthetic pathway (protoporphyrin IX or CPIII depending on the organism) is toxic in the presence of light. We are the first group to exploit this fact by creating small molecule activators of the heme biosynthetic pathway with the goal of increasing coproporphyrin levels and maximizing light-dependent killing. In addition, we recently isolated unique mAbs that target the surface of *S. aureus*, and by conjugating these antibodies to red-light responsive photoactivators, we have created a powerful new class of antibacterial. When used in combination with '882-based photoactivators, we are now able to sterilize cultures of *S. aureus* at a level that matches conventional antibiotic therapy. This strategy has the potential to revolutionize photodynamic therapy-based approaches to antibacterial development and provide therapies for the treatment of antibiotic resistant infections.
  - **What was the impact on other disciplines?**

    Although this project has not yet had an impact upon other disciplines it has the potential to lead to new ways to deliver light which could have an impact on the field of photonics in the future.
  - **What was the impact on technology transfer?**

    We have not presented or published our small molecule/mAb combination and we will be sure to file for intellectual property prior to disclosing this work.
  - **What was the impact on society beyond science and technology?**

    Nothing to report

- **CHANGES/PROBLEMS:**
  - **Changes in approach**

    The only change in approach is to include the red-light photoresponsive mAbs into our workflow which lead to increased antibacterial activity and should lead to increased tissue penetration. This change simply involves the addition of a reagent and does not change our proposed research plan in any way.
  - **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

- **Changes that had a significant impact on expenditures**

  Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to report

- **Significant changes in use or care of human subjects**

  Nothing to report

- **Significant changes in use or care of vertebrate animals.**

  Nothing to report

- **Significant changes in use of biohazards and/or select agents**

  Nothing to report

- **PRODUCTS:**

  - **Publications, conference papers, and presentations**

    **Journal publications.**

    Surdel MC, Horvath DJ Jr, Lojek LJ, Fullen AR, Simpson J, Dutter BF, Salleng KJ, Ford JB, Jenkins JL, Nagarajan R, Teixeira PL, Albertolle M, Georgiev IS, Jansen ED, Sulikowski GA, Lacy DB, Dailey HA, Skaar EP. Antibacterial photosensitization through activation of coproporphyrinogen oxidase. *Proc Natl Acad Sci U S A.* 2017 Aug 8;114(32):E6652-E6659.

    - **Books or other non-periodical, one-time publications.**

      Nothing to report

    - **Other publications, conference papers, and presentations.**

      Conference Presentation: Light as a selective antibiotic: a novel approach of antibacterial photosensitization through activation of coproporphyrin III. Presented at the SPIE West which is hosted by the International Society for Optics and Photonics

  - **Website(s) or other Internet site(s)**

    Nothing to report.

  - **Technologies or techniques**

    We are currently in the process of creating new lights for use in photodynamic therapy assays. The majority of the techniques used by the biology and chemistry teams are conventional. All

techniques or technologies developed as a result of this work will be described in peer reviewed publications and shared upon request.

- **Inventions, patent applications, and/or licenses**

  No new patent applications have been filed since the start of this award.

- **Other Products**

  The "products" created as a result of this project include the analogs of '882 that have been developed by our chemistry team. These molecules are being tested for their activity and active molecules may represent useful tool compounds or candidate therapeutics. In addition, we have conjugated red-light responsive photoactivators to mAbs that target *S. aureus*. In the future, we hope that we will develop new lights for use in photodynamic therapy applications.

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

| | |
|---|---|
| Name: | Eric Skaar |
| Project Role: | Principle Investigator |
| Research Identifier (e.g. ORCID ID): | 0000-0001-5094-8105 |
| Nearest person month worked: | 1 |
| Contribution to project: | Dr. Skaar assisted with experimental design /interpretation and supervision of personnel. |
| Funding Support: | |

| | |
|---|---|
| Name: | Duco Jansen |
| Project Role: | Co-Investigator |
| Research Identifier (e.g. ORCID ID): | 0000-0002-1778-6180 |
| Nearest person month worked: | 1 |
| Contribution to project: | Dr. Jansen assisted with experimental design/interpretation and supervision of personnel. |
| Funding Support: | |

| | |
|---|---|
| Name: | Dennis Horvath, Jr. |
| Project Role: | Research technician |
| Research Identifier (e.g. ORCID ID): | 0000-0001-9988-7091 |
| Nearest person month worked: | 9 |
| Contribution to project: | Dr. Horvath tested '882 analogs for *hrtAB* activation and developed murine model of *S. aureus* and *P. acnes* skin infections. |
| Funding Support: | |

| | |
|---|---|
| Name: | Nichole Maloney |
| Project Role: | Research technician |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 1 |
| Contribution to project: | Ms. Maloney assisted with the optimization of murine models of infection and screened for resistance to ‘882. |
| Funding Support: | |

| | |
|---|---|
| Name: | Andrew Montieth |
| Project Role: | Postdoctoral fellow |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 2 |
| Contribution to project: | Dr. Montieth developed *in vitro* neutrophil killing assays in an effort to test the combined effect of PDT with immune mediated killing. |
| Funding Support: | |

| | |
|---|---|
| Name: | Jocelyn Simpson |
| Project Role: | Research technician |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 11 |
| Contribution to project: | Ms. Simpson has tested ‘882 analogs for light-based antimicrobial activity. |
| Funding Support: | |

| | |
|---|---|
| Name: | Alec Walter |
| Project Role: | Graduate student in Biomedical Engineering |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 3 |
| Contribution to project: | Mr. Walter is responsible for optimizing light-based killing strategies by combining lights of various wavelengths. |
| Funding Support: | |

| | |
|---|---|
| Name: | Andy Weiss |
| Project Role: | Postdoctoral fellow |
| Research Identifier (e.g. ORCID ID): | 0000-0002-5221-1032 |
| Nearest person month worked: | 1 |
| Contribution to project: | Dr. Weiss assisted with the optimization of murine models of skin infection. |
| Funding Support: | |

| | |
|---|---|
| Name: | Joseph Zackular |
| Project Role: | Postdoctoral fellow |
| Research Identifier (e.g. ORCID ID): | 0000-0002-3228-3055 |
| Nearest person month worked: | 3 |
| Contribution to project: | Joseph Zackular helped define the broad spectrum utility of small molecule PDT against additional organisms. |
| Funding Support: | |

| | |
|---|---|
| Name: | Sydney Drury |
| Project Role: | Research Technician |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 1 |
| Contribution to project: | Sydney Drury is testing the combined activity of '882 analogs and mAbs against *S. aureus*in PDT assays. |
| Funding Support: | |

| | |
|---|---|
| Name: | Anderson Miller |
| Project Role: | Research Technician |
| Research Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 1 |
| Contribution to project: | Anderson Miller is testing '882 analogs for *hrtAB* activating potential using the XylE assay. |
| Funding Support: | |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

- See below:

**Active Support**

**(December 18, 2018)**

**SKAAR, ERIC P.**

**CURRENT**

**(Previously Pending Now Active)**

***Title:*** "Investigating type IV secretion in *Acinetobacter baumannii* and its interplay with antibiotic resistance," VUMC66687 1R01 AI125363-01 (Skaar, Co-Investigator; Feldman PI)
*Time commitments:* Skaar (4%)
*Support agency:* Washington University/NIH
*Procuring Contracting/Grants Officer:*
Elizabeth R. Sihombing
National Institute of Allery and Infectious Diseases

*Performance period:* 6/01/2018 - 5/31/2019
*Level of funding:* current direct cost, entire period direct cost *Goal:*

We propose that differentiation of A. *Baumannii* cells into bacterial killers involves multiple phenotypic and metabolic changes and that the fitness costs associated with MDR and T6SS phenotypes are the driving forces for this differentiation.

*Specific Aims:*

1.) Test the virulence capacity of $Ab_{04}$ plasmid$^{+}$T6SS$^{-}$, plasmid$^{+}$T6SS$^{+}$, plasmid$^{-}$T6SS$^{+}$, and plasmid$^{-}$T6SS$^{-}$.
2.) The virulence of strains harboring the WT $Ab_{17978}$ plasmid$^{+}$T6SS$^{-}$ will be compared to the ΔACX60_11480, ΔACX60_17565, and the strains overexpressing lactate or arginine metabolic operons. We will also test the lactate overexpressing strain with the ΔACX60_11480 mutation.

**(Previously Pending Now Active)**

***Title:*** "Molecular mapping of microbial communities at the host-pathogen interface by multi-modal 3-dimensional imaging mass spectrometry," 1R01 AI138581-01A1 (Skaar/ Spraggins Co-PIs,)
*Time commitments:* Skaar (11.5%)
*Support agency:* NIH/NIAID
*Procuring Contracting/Grants Officer:*
Bora Uzima Mpinha
National Institute of Allery and Infectious Diseases

*Performance period:* 9/19/2018 - 8/31/2023
*Level of funding* current direct cost, 0 entire period direct cost *Goal:*

This proposal will enable detailed views of the molecular components of infectious disease with unprecedented resolution through the development of a multimodal, 3-dimensional imaging platform. The proposed technologies will improve throughput and molecular specificity, enable automated high-precision and high-accuracy image alignment, and allow for descriptions of molecular signals in 3-D through the fusion of multi-modal imaging data.

*Specific Aims:*

1.) Define the heterogeneous microbial subpopulations throughout the 3-D volume of a *S. aureus* community
2.) Uncover the host molecules that form the abscess and accumulate to restrict microbial growth in murine models
3.) Elucidate molecular markers that differentiate *in vivo* biofilms at the host-pathogen interface, between abscesses at various stages of progression, and under distinct degrees of nutrient stress.

**(Previously Active, Effort ended)**

***Title***: "The role of dietary metals and calprotectin in the pathogenesis of asthma," 15-0056 (Skaar, PI)
*Time commitments:* Skaar (5%, 0.6 calendar months)
*Support agency:* American Asthma Foundation
*Procuring Contracting/Grants Officer:*
Valerie Dougherty, Program Manager
American Asthma Foundation
University of California, San Francisco
500 Parnassus Avenue, Room MU-W-416
San Francisco, CA 94143-0509

*Performance period:* 7/1/2015-6/30/2017
*Level of funding:* entire period direct cost *Goal:*

A growing body of evidence has linked alterations in dietary zinc levels to the incidence of asthma and the protective effects of zinc are modeled to be due to its antioxidant properties. We have previously discovered that calprotectin is one of the most abundant proteins during lung inflammation, and calprotectin exhibits potent zinc binding activities suggesting that this protein is a key contributor to zinc distribution during inflammation. Notably, oxidized calprotectin has been identified at high levels in the lungs of asthmatic patients, and calprotectin has been modeled to have a protective role as an oxygen scavenger. Based on these observations, we hypothesize that calprotectin has a dual function as an antioxidant in the lung whereby its zinc binding properties contribute to airway zinc distribution while its oxygen scavenging properties protect against oxidative damage. Further, we hypothesize that the protective role of calprotectin during asthma is affected by dietary zinc levels. This

model will be tested through a series of three integrated specific aims (below). Taken together, the results of these experiments will reveal the importance of dietary zinc and calprotectin on the pathogenesis of asthma and lay the groundwork for the development of therapeutics and interventions for the treatment of asthma.

*Specific Aims:*

1) We will elucidate the impact of altered dietary zinc on asthma pathogenesis.
2) Studies will determine the contribution of calprotectin to metal distribution during asthma.
3) Define the abundance and distribution of oxidized calprotectin in the asthmatic lung.

**(Previously Active; Ended)**

***Title***: "Therapeutically modified gut bacteria for treatment of obesity," 1 R01 AT007830-01-05 (Davies, PI; Skaar, Co-Investigator)

*Time commitments:* Skaar (0.05%, 0.06 calendar months)

*Support agency:* NIH/National Center for Complementary & Alternative Medicine

*Procuring Contracting/Grants Officer:*
Leslie D. Boggs, Grants Management Specialist
National Center for Complementary & Alternative Medicine

*Performance period:* 5/1/2013-4/30/2018

*Level of funding:* entire period direct cost *Goal:*

About 1/3 of Americans are obese, which greatly increases their chances of having heart attacks, diabetes, or many other diseases. The purpose of the proposed studies is to determine if genetically modified probiotic bacteria can be used to treat obesity.

*Specific Aims:*

1) Determine if colonization of gut with pNAPE-EcN sustainably prevents and reverses obesity in mice induced by diet and genetics.
2) Determine the overarching neuroendocrine mechanisms by which pNAPE-EcN modulates food intake and energy expenditure to reduce obesity.
3) Determine the specific molecular mechanisms of pNAPE-EcN action in the intestine.

**Active Support**

**(December 18, 2018)**

**JANSEN, DUCO**

**No changes to report**

- **What other organizations were involved as partners?**
    - **Organization Name:** Vanderbilt University
    - **Location of Organization:** Nashville, TN
    - **Partner's contribution to the projec**t *(identify one or more)*
        - **Facilities***:*
            - Build and characterize light sources
            - Fluorescent imaging systems to explore near IR fluorescence of CPIII
            - MANTIS system for two-photon photodynamic therapy
            - Spectrophotometer for CPIII absorption characteristics and exploring optical clearing for mouse skin
        - **Collaboration**
            - Jeremy Ford, Biomedical Engineering Graduate student
            - Logan Jenkins, Biomedical Engineering Graduate student
        - **Personnel exchanges**
            - Alec Walter, Biomedical Engineering Graduate student works in both Dr. Jansen (VU) and Dr. Skaar's laboratories (VUMC)

- **SPECIAL REPORTING REQUIREMENTS**
    - **COLLABORATIVE AWARDS:**
    - **QUAD CHARTS:** With Attachments
- **APPENDICES:**
    - Surdel MC, Horvath DJ Jr, Lojek LJ, Fullen AR, Simpson J, Dutter BF, Salleng KJ, Ford JB, Jenkins JL, Nagarajan R, Teixeira PL, Albertolle M, Georgiev IS, Jansen ED, Sulikowski GA, Lacy DB, Dailey HA, Skaar EP. Antibacterial photosensitization through activation of coproporphyrinogen oxidase. *Proc Natl Acad Sci U S A.* 2017 Aug 8;114(32):E6652-E6659. PUBLISHED

# Photosensitization of Bacterial Pathogens through Small Molecule Activators of Heme Biosynthesis

**BA-160565**

WX81WH-17-2-0003

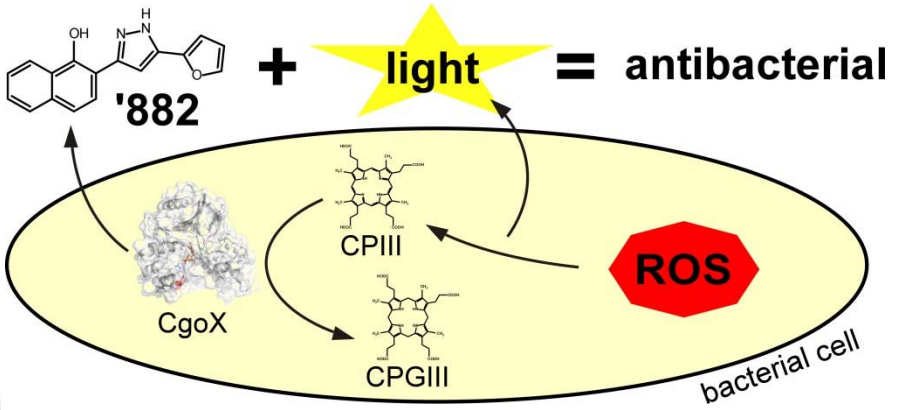
**PI:** Eric Skaar **Org:** Vanderbilt University Medical Center **Award Amount: $1,907,932**

## Study/Product Aim(s)

- Synthesize and test analogs of '882 for CgoX activating potential.
- Define the mechanism by which '882 activates CgoX and use this information to design improved analogs.
- Test the therapeutic efficacy of '882 analogs in murine models of skin infections.

## Approach

It is imperative to develop new therapies for post-traumatic infection that are effective against antibiotic-resistant *S. aureus.* The objective of this proposal is to develop new photoactivatable antibiotics for the treatment of *S. aureus* infections. One promising area of research focuses on *S. aureus* heme synthesis which is vital to survival within the host. Studies proposed in this application will lead to the design of molecules that disrupt heme homeostasis and inhibit staphylococcal disease as well as diseases caused by a variety of other important pathogens.




Accomplishments:

- Combined chemistry teams (VU and WRAIR) have synthesized 45 distinct analogs of '882.
- Biology team has tested 31 analogs for *hrtAB* activating potential.
- Surdel *et. al.* PNAS 2017 Aug 8; 114(32):E6652-E6659. Published.

## Timeline and Cost

| Activities / CY | 17 | 18 | 19 | 20 |
|---|---|---|---|---|
| Synthesize '882 analogs | | | | |
| Test '882 analogs for *hrtAB* activating potential | | | | |
| Test ability of '882 analogs to photosensitize *S. aureus* to light killing | | | | |
| Test therapeutic efficacy of '882 analogs in murine models of skin infections | | | | |
| **Est. Budget ($K) Direct Costs** | **$332K** | **$318K** | **$340K** | **$344K** |

**Updated:** (26 December 2018 )

**Goals/Milestones (Example)**

**CY17 Goal** – Synthesize '882 analogs

- Combined efforts of VU Chemical Synthesis Core and WRAIR synthesized 45 '882 analogs

**CY18 Goals** – Determine activity of '882 analogs against live *S. aureus*

- Tested 31 '882 analogs for *hrtAB* activating activity

**CY19 Goal** – Determine activity of '882 analogs against live bacteria

- Tested 24 '882 analogs for ability to photosensitize *S. aureus* and *P. acnes* to light-based killing

**CY20 Goal** – Test efficacy of '882 analogs in murine skin infection models

- Demonstrated 882-PDT decreases bacterial burdens *in vivo*

**Comments/Challenges/Issues/Concerns**

- N/A

**Budget Expenditure to Date**

Projected Expenditure: $650,000

Actual Expenditure: $1,123,351