AWARD NUMBER: W81XWH-14-2-0169

TITLE: Development of Novel Therapeutics for Neglected Tropical Disease Leishmaniasis

PRINCIPAL INVESTIGATOR: Danett K. Bishop, Ph.D

RECIPIENT: U.S. Naval Medical Research Unit No. 6 (NAMRU-6)
Lima, Peru

REPORT DATE: OCTOBER 2018

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
OCT 2018	Annual	30SEP2017 - 29SEP2018

4. TITLE AND SUBTITLE

Development of Novel Therapeutics for Neglected Tropical Disease Leishmaniasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-2-0169

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Danett K. Bishop, Ph.D

EMAIL: danett.k.bishop.mil@mail.mil

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

US Naval Medical Research Unit No. Six
Venezuela Av. Block 36
Bellavista, Callao-Peru

Asociacion Benefica PRISMA
Calle Carlos Gonzáles N°251 Urbanización Maranga,
Lima 32 - Perú

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Infections caused by protozoan parasites of the genus Leishmania include cutaneous (CL), mucosal (ML) and visceral leishmaniasis (VL). Over 12 million people are currently suffering from leishmaniasis, and approximately 2 million new cases occur annually, making it a major global health problem and WHO designated neglected tropical disease (NTD). Recently, CL has been seen in all branches of the US military and among DOD contractors returning from Leishmania-endemic countries such as Iraq and Afghanistan. Current widely used treatment for all forms of leishmaniasis including CL involves multiple injections of antimonial drugs (GlucantimeTM or PentostamTM) for 20 days or more. Therefore, this treatment has poor compliance, numerous adverse effects including death and is also not approved by the FDA therefore requiring use under IND in the US. Furthermore, in immunocompromised individuals antimonial treatment is associated with relapses. Other antileishmanial treatments currently under development do not offer new alternatives because they are either reformulations or combinations of existing drugs. Hence, there is pressing need for novel drugs for leishmaniasis. Our team is interested in discovering novel drugs to treat leishmaniasis from natural products. Work from our recently completed NIH-funded project has led to the discovery of antileishmanial molecules from the plant Pentalinon andrieuxii, which has been used by Mayan traditional healers for CL for many years. We have identified six sterols, including a novel sterol, pentalinosterol (PEN), with broad-spectrum activity against Leishmania species that cause CL and visceral leishmaniasis (VL). The synthesis of PEN has been established and methods for large scale synthesis of other active molecules are under development (PCT Int. App. WO 2012145734A1). Our preliminary studies show that synthetic PEN (sPEN) is safe and more potent than antimonials (SSG) in the treatment of CL and VL in animal models. We have also found that PEN exhibits immunomodulatory activity and promotes cellular immune responses required for leishmaniasis resolution. These findings indicate that PEN and other bioactive sterols as well as their derivatives could be novel broad-spectrum anti-leishmanial drugs. The goals of this 3 year project are to address the critical developmental need of lead optimization of analogues of two most promising compounds, PEN and DNER in the context of drug potency and specificity. Solubility and stability, key parameters in the development of a useful drug for this disease, will also be considered during the course of lead optimization and determine the mechanism(s) of antiparasitic action of bioactive analogues using a combination biochemical and in silico approaches. Aim 1 will comprise the synthesis of PEN and DNER analogues and screening their antileishmanial activity and toxicity using novel screening assays. Aim 2 will evaluate efficacies of active analogues in prevention and treatment of CL using an animal model. Aim 3 will determine the mechanisms of antiparasitic and/or immunomodulatory activities of active compounds using a combination biochemical and in silico approaches. These data will lay the foundation for advancing PEN and DNER analogues as novel drugs for leishmaniasis in humans.

-

15. SUBJECT TERMS

Cutaneous leishmaniasis, antileishmanial drugs, Pentalinonsterol and 6,7-dihydroneridienone analogues

16. SECURITY CLASSIFICATION OF			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Page

Contents

1. INTRODUCTION:

The leishmaniases comprise a number of diseases caused by obligate intracellular parasites of the genus Leishmania. More than 350 million people worldwide are at risk of contracting leishmaniasis. Cutaneous leishmaniasis (CL) is the most common form of infection, which manifests as localized skin lesions that may heal or become chronic, leading to significant tissue destruction and disfigurement. Other forms of infections are diffuse cutaneous leishmaniasis (DCL), mucosal leishmaniasis (ML), or potentially life-threatening visceral leishmaniasis (VL), which is characterized by dissemination of the parasites to the liver, spleen and bone marrow. Several drugs including pentavalent antimonials (Sb), Amphotericin B, miltefosine and paromomycin are used to treat leishmaniasis. However, all these drugs suffer from significant drawbacks, including the need for parenteral routes of administration, poor patient compliance due to long treatment lengths and toxicity, and/or high cost, which limits their use in disease endemic regions. In addition, the emergence of antimonial-resistant strains of VL is rapidly increasing worldwide. Therefore, there is a strong need for new anti-leishmanial drugs that are safe, affordable, and have broad-spectrum activity against different species of Leishmania, including Sb-resistant parasites.

Our team is interested in discovering novel drugs to treat leishmaniasis from natural products. Work on an ongoing NIH-funded project (AI092624; A. Satoskar, PI, A.D. Kinghorn, Co-PI) has led to the discovery of antileishmanial molecules from the plant Pentalinon andrieuxii, which has been used by Mayan traditional healers to successfully treat CL for many years. We have identified six sterols, including 6.7-Dihydroneridienone (DNER) as well as a novel sterol, pentalinosterol (PEN) and its closely related structural analogue cholest-4-en-3-one (C3ONE), with broad-spectrum activity against Leishmania species that cause CL and VL. The synthesis of PEN has been established and methods for large scale synthesis of other active molecules are under development (PCT Int. App. WO 2012145734A1). Our preliminary studies show that synthetic PEN (sPEN) is safe and more potent than antimonials for in the treatment of CL and VL in animal models. We have also found that PEN exhibits immunomodulatory activity and promotes cellular immune responses required for leishmaniasis resolution. These findings indicate that PEN and other bioactive sterols as well as their derivatives could be novel broad-spectrum anti-leishmanial drugs. The goals of this 3 year project are to: 1) synthesize and evaluate analogues of two most promising compounds, PEN and DNER; 2) use in vitro high throughput screening assays and an animal model of visceral leishmaniasis to explore structure-activity relationships and optimize the physicochemical properties of this natural product for advancing these classes as a potential therapeutic agents for the treatment of leishmaniasis; and 3) determine the mechanism(s) of antiparasitic action of bioactive analogues using a combination biochemical and in silico approaches. These studies will address the critical developmental need of lead optimization in the context of drug potency and specificity. Solubility and stability, key parameters in the development of a useful drug for this disease, will also be considered during the course of lead optimization.

2. KEYWORDS:

Cutaneous leishmaniasis, antileishmanial drugs, Pentalinonsterol and 6,7-dihydroneridienone analogues.

3. ACCOMPLISHMENTS:

What are the major goals of the project?

i) To optimize promastigotes and amastigote *in vitro* assays to measure the susceptibility of *Leishmania* clinical isolates to standard anti-leishmanial drugs.

ii) To assess 5 PEN/DNER compounds for anti-leishmania activity using the promastigote assay previously optimized and clinical isolates of *L (Viannia) peruviana* and *L.(V.) braziliensis.*

iii) To assess 5 PEN/DNER compounds for anti-leishmania activity using the amastigote assay previously optimized and clinical isolates of *L.(V.) peruviana* and *L.(V.) braziliensis.*

iv) To amend ongoing IACUC protocols to infect mouse with *L. (V.) peruviana* and *L.(V.) braziliensis* and evaluate 5 PEN/DNER for *in vivo* anti-leishmanial activity.

What was accomplished under these goals?

Execution of this study was delayed one year due to contracting and invoicing issues and activities initiated on November 2015. This report covers activities conducted from October 2015 to September 2018. The major accomplishments during this period are:

Performance period from October 2015 – September 2017

- A contract was successfully established between Asociacion Benefica PRISMA in Peru and USAMRAA to support the activities under this project.
- Study personnel was hired and trained on *in vitro* culture of *Leishmania* promastigotes and flow cytometry.
- Procurement of laboratory supplies has been completed for the first aim. This includes a battery of standard anti-Leishmania drugs such as pentavalent and trivalent antimonial, paramomycin, miltefosine and amphotericin B.
- We optimized the promastigote assay and characterized the growth kinetics of a control *L.(V.) braziliensis* strain (MHOM/PER/90/LTB300) and seven clinical *L. (V.) braziliensis* isolates. (**Figure 01**). These results allowed us to proceed with standard drug susceptibility testing and assessment of EC50s.
- We conducted drug susceptibility testing against Potasium antimonyl tartrate trihydrte (SbIII) on the LTB300 *L.(V.) braziliensis* strain. The exposure of *L. (V.) braziliensis* promastigotes to SbIII resulted in significant parasite killing (**Figure 02**). Moreover, 7 independent experiments resulted in similar EC50 values (1.7-2.25ug/ml) (**Figure 03A and B**).
- We performed drug susceptibility testing against SBIII on the 07 clinical *L. (V.) braziliensis* isolates that were previously described (**Table 01**). Only one isolate presented a profile that indicates SBIII resistance.
- We have received the PEN drug from Dr. Abhay Satoskar. This drug is currently been optimized and will be tested against a panel of field isolates from our parasite repository.
- We have requested the U937 CRL-1593.2 cell line (Lymphocyte) and *L. (V.) braziliensis*

(MHOM/BR/75/M2903) strain from the American type culture collection organization (ATCC). These cell lines will be used for the intracellular amastigote assay.

Performance period from October 2017 until September 2018

- We have received the U937 CRL-1593.2 cell line (Monocyte) and *Leishmania (V.) braziliensis* (MHOM/BR/75/M2903) strain from the American type culture collection organization (ATCC).

- We determinate the growth kinetics of a control *L. (V.) braziliensis* strain (MHOM/BR/75/M2903) (**Figure 04**). These results allowed us to proceed with standard drugs susceptibility testing and assessment of EC50s.

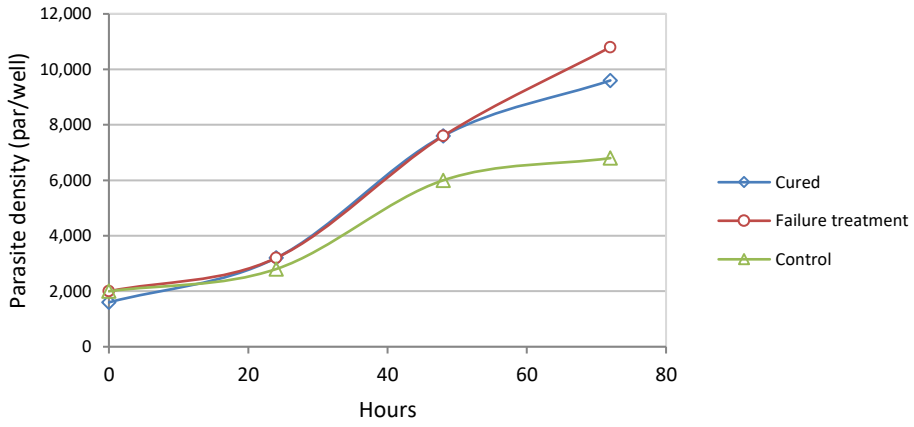
- We optimized the promastigote assay and evaluated drug susceptibility testing against Pentalinosterol drug (PEN) on *L. (V.) braziliensis* control strain (MHOM/BR/75/M2903). The exposure of *L. (V.) braziliensis* promastigotes to PEN resulted in significant parasite killing (**Figure 05**). In 3 independent experiments resulted in similar EC50 values (20.4 – 20.8 ug/ml)

- We standardized the promastigote assay and evaluated drug susceptibility testing against standard drugs, Miltefosine (HePc) and Amphoterin B (AmpB) on *L. (V.) braziliensis* control strain (MHOM/BR/75/M2903).

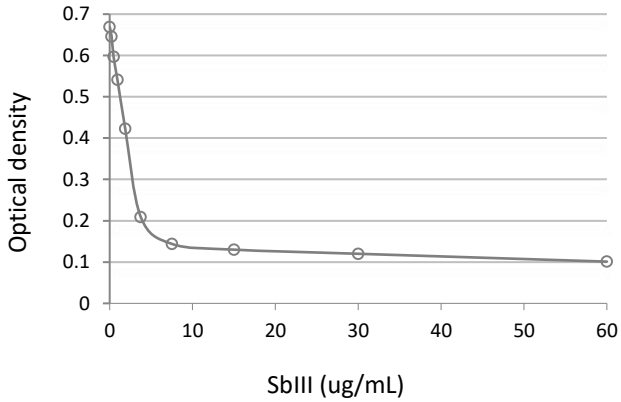
- We evaluated the a metacyclogenesis assay on *Leishmania (V.) braziliensis* (strain MHOM/BR/75/M2903) in order to obtain the most infective phase on the parasite´s life cycle, (**Figure 06**).

- We cultivated the U937 CRL-1593.2 cell line in a RPMI1640 culture medium and suitable parameters and we used Phorbol 12-myristate 13-acetate (PMA) in order to stimulate the differentiation of monocytes cells to macrophages.

<u>Figure 01</u>. Growth kinetics of three representative *Leishmania (V.) braziliensis* isolates: The figure shows the growth rate of a susceptible, resistant and control *L. (V.) braziliensis* strain using an initial concentration of 2,000 promastigotes/well with assessment of parasitemia during 3 days**.**



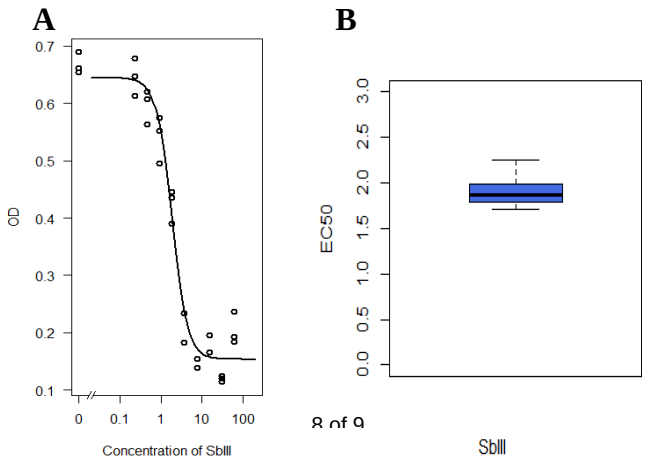
<u>Figure 02</u>. *L. (V.) braziliensis* (LTB300) promastigotes exposed at different concentrations of SbIII at 72h post incubation: The figure shows the effect of different SbIII concentrations (0-60ug/mL) on parasite viability using the MTT assay.



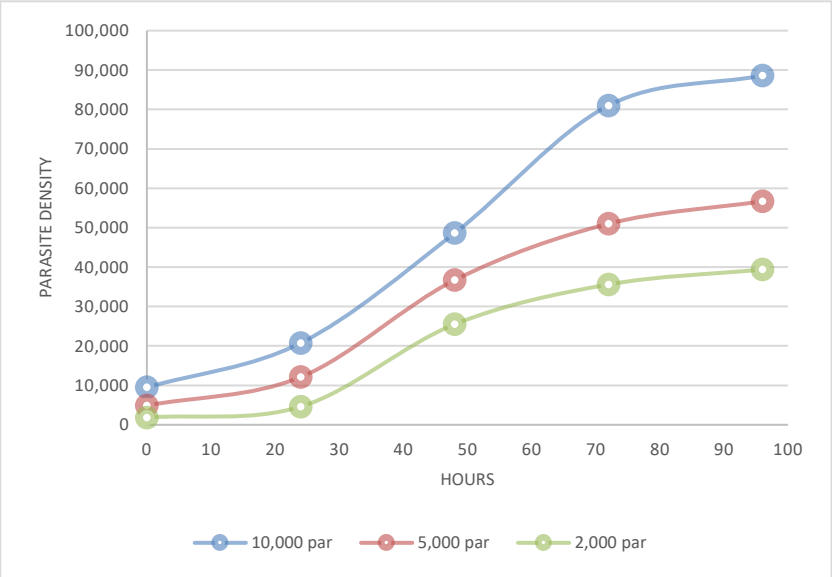
<u>Figure 03</u>. Assessment of EC50 for SbIII on *L. (V.) braziliensis* LTB300 reference strain:

A) 4-parameter regression curve for assessment of SbIII ED50 for the LTB300 strain (1.82ug/mL).

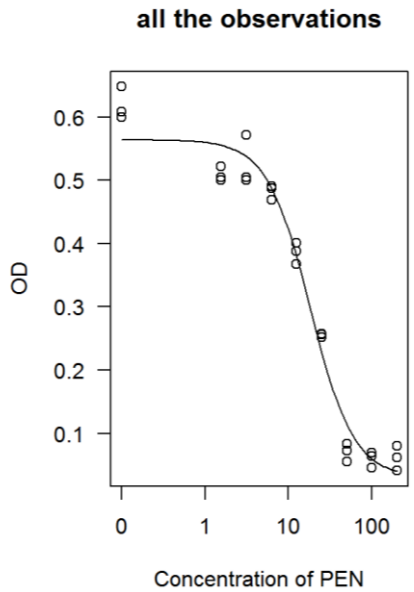
B) EC50 values obtained in 7 independent experiments for LTB300 (1.7-2.2ug/mL).



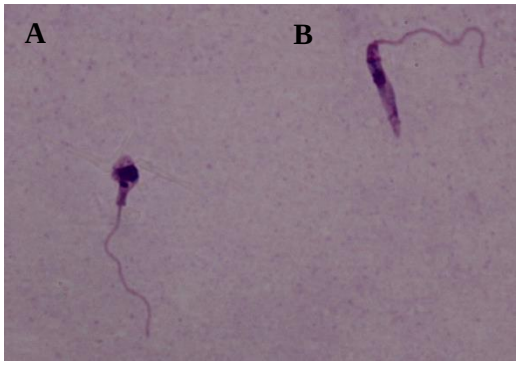
<u>Figure 04</u>. Growth kinetics of *Leishmania (V.) braziliensis* (MHOM/BR/75/M2903): The figure shows the growth rate of *L. (V.) braziliensis* strain using different initial concentration of promastigotes during 4 days**.**



<u>Figure 05</u>. *L. (V.) braziliensis* (MHOM/BR/75/M2903) promastigotes exposed at different concentrations of Pentalinosterol (PEN) at 72h post incubation: The figure shows the effect of different PEN concentrations (0-200ug/mL) on parasite viability using the MTT assay.



<u>Figure 06</u>. Metacyclogenesis assay of *L. (V.) braziliensis* (MHOM/BR/75/M2903) promastigotes: The figure shows the promastigote in late log phase after 15 days old. A suspension of parasites was stained with Giemsa (10%) and analyze by optical microscope 1000X.



<u>Table 01</u>. EC50 values for 07 strains of *Leishmania (V.) braziliensis* promastigote and control strain. The conditions described in Figure 01 were applied for 07 *Leishmania (V.) braziliensis* isolates. R= failure in treatment; S =cured; C=control.

Isolate	Clinical Phenotype	IC50	SEM
1	R	3.87	2.10
2	R	0.97	0.38
3	R	1.38	0.59
4	S	1.51	0.36
5	S	1.06	0.04
6	S	0.40	0.00
7	S	0.40	0.10
LTB300	C	1.82	0.11

Specific Aim	Timeline	%Completion
Aim 1: Synthesize and screen PEN and DNER analogues for their antiparasitic activity and toxicity against different Leishmania species that cause CL, including patient isolates from s endemic regions.		
Kickoff Coordination Meeting of participating institutions	1	100%
Major Task 1.1: Synthesis of PEN and DNER analogues		
Subtask 1.1.1: Synthesis of PEN analogues will be carried out through modification of two key functional groups (10-15 analogs to be tested) Participating teams: • Team A (Drs. Fuchs and Kinghorn Labs will oversee component design) • Team B (Site 1 core facility; fee for service):	1-5	100%, Dr. Satoskar
Subtask 1.1.2: Synthesis of DNER analogues will be carried out through modification of C17 side chain (8 analogs) Participating teams: • Team A (Drs. Fuchs and Kinghorn Labs will oversee component design) • Team B (Site 1 core facility; fee for service):	1-4	100%, Dr. Satoskar
Subtask 1.1.3: Generation of hybrid sterols. • First step Fuchs/Kinghorn Labs • Second step Fuchs/Kinghorn Labs	5-6	100%, Dr. Satoskar
Milestone #1: Library of 20 PEN and DNER analogue compounds to be tested in vitro.	5	100%, Dr. Satoskar
Major Task 1.2: Evaluation of microbicidal activity using standard laboratory strains		
Subtask 1.2.1: Determination of IC50 of compounds using *Leishmania* promastigote cultures	7-9	100%, Dr. Satoskar
Subtask 1.2.2: Determination of IC50 of compounds using *Leishmania* amastigote cultures	7-9	100%, Dr. Satoskar
Subtask 1.2.3: Determination of toxicity of compounds using eukaryotic cell lines	8	100%, Dr. Satoskar
Milestone #2: Library of 5-10 PEN and DNER analogue compounds with data on in vitro activity against standard laboratory strains of Leishmania	5	100%, Dr. Satoskar

Major Task 1.3: Evaluation of microbicidal activity using clinical isolates		
Subtask 1.3.1: Determination of IC50 of compounds using *L (V.) peruviana* and *L (V.) braziliensis* promastigote cultures from clinical isolates • Selection of 20 geographically diverse isolates of *L. (V.) peruviana* and *L. (V.) braziliensis* with high and low IC50 to antimonial drugs (sodium stibogluconate) • Determine IC50 on 20 clinical isolates for each compound (n=10)	8-12	80%, LCDR. Bishop
Subtask 1.3.2: Determination of IC50 of compounds using *L (V.) peruviana* and *L (V.) braziliensis* amastigote cultures from clinical isolates • Selection of 20 geographically diverse isolates of *L. (V.) peruviana* and *L. (V.) braziliensis* with high and low IC50 to antimonial drugs (sodium stibogluconate) • Determine IC50 on 20 clinical isolates for each compound *(n=10)*	8-12	0%, LCDR. Bishop
Milestone #3: Library of 5-6 PEN and DNER analogue compounds with accepted microbicidal activity in promastigote and amastigote model on 20 different clinical isolates	12	15%, LCDR. Bishop
Aim 2: Evaluate the efficacy of active PEN and DNER analogues for the treatment of leishmaniasis using an animal model of CL.		
Major Task 2.1: Development of topical formulations of PEN (PEN-A) and DNER (DNER-A) analogues		
Subtask 2.1.1: • First step Topical formulation of PEN-As • Second step Topical formulation of PEN-As	12-15	0%, Dr. Satoskar
Major Task 2.2: Evaluate the efficacies of PEN and DNER analogues in preventing development of CL using *L. major* and *L. mexicana* models		
Subtask 2.2.1: • First step: Efficacy of PEN-A in prevention of CL • Second step: Efficacy of DNER-A in treatment of CL	16-24	0%, Dr. Satoskar
Major Task 2.3: Evaluation of efficacies of PEN and DNER analogues in the treatment of CL using L. major and L. Mexicana models		
Subtask 2.3.1: • First step: Efficacy of PEN-A in treatment of CL • Second step: Efficacy of DNER-A in treatment of CL	17-24	0%, Dr. Satoskar
Major Task 2.4: Evaluation of efficacies of PEN and DNER analogues in the treatment of CL using *L(V.) peruviana* and *L braziliensis* mouse model in Peru		
Subtask 2.4.1: Amend ongoing IACUC protocol to infect mouse with *L(V.) peruviana* and *L braziliensis*	12-24	0%, LDCR. Bishop
Subtask 2.4.2: Efficacy screening of 5-6 PEN and DNER	12-24	0%, LDCR. Bishop

analogue compounds **(Total mice needed= 130)**		
Milestone #4: Library of 1-4 PEN and DNER analogue compounds with in vivo efficacy data on old and new world Leishmania species causing CL	24	0%, Dr. Satoskar 0%, LCDR. Bishop

4. IMPACT:

Nothing to report

5. CHANGES AND PROBLEMS:

- This project was delayed due to contractual and invoicing issues. The award notification was issued in early April 2014. After 6 months of negotiation, the contract between USAMRAA and Asociacion Benefica PRISMA was signed. There were additional delays related to release of funding and working out the invoicing system. PRISMA received funds for the first year of the study in November 2015.

- We have experienced difficulties and delays for purchases of supplies and reagents due to importation issues. These supplies need to be purchased in the United States and the Peruvian government requires importation permits for each of these items which can take up to 90 days. We have now a mechanism to import reagents for this project with a time-around of approximately 40-60 days.

- We had a delay in the estimated time of training at the Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM) in Colombia due to delays in synthesis of the compound PEN .

- Another challenge has been the development of the amastigote assay. Most amastigote assays developed to date are conducted with standard parasites strains which have predictable growth rate and high macrophage infection rates. Very few investigators have used clinical isolates collected from the field to develop drug susceptibility assays. The reason is that clinical isolates have different phenotypic features (eg. different growth rates, resetting formation, variable infection rates in host cells, etc) that makes standardization difficult. Some researchers have used complex preconditioning of promastigotes to increased infection rates of field isolates to macrophages (da Luz *et al*, 2009). A research team from CIDEIM in Colombia has established a robust assay to estimate the susceptibility of *Leishmania spp* parasites to various drugs used in humans for the treatment of cutaneous leishmaniasis (Fernández O et al, 2012; Fernández OL et al, 2014). We are currently discussing to establish a research collaboration agreement to be able to implement this system in NAMRU-6.

6. PRODUCTS:

Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

The following individuals have worked on this project:

Name:	Danett K. Bishop, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	not available
Nearest person month worked:	1
Contribution to Project:	Danett K. Bishop is currently overseing all administrative aspect of the project, including award negotiation, budget allocation and contracting issues.
Funding Support:	Danett K. Bishop is an active duty U.S. military
Name:	Carmen Lucas, ScM
Project Role:	Research Manager
Researcher Identifier (e.g. ORCID ID):	not available
Nearest person month worked:	1
Contribution to Project:	Mrs. Lucas has so far provided logistical support for laboratory research including placing orders, tracking packages, coordinating custom liberation of imported items. Additionally, she manages the laboratory of Parasitology coordinating all research activities
Funding Support:	Mrs. Lucas is a U.S. government employee.
Name:	Lucy Espinoza, BS.
Project Role:	Research technician
Researcher Identifier (e.g. ORCID ID):	not available
Nearest person month worked:	12
Contribution to Project:	Ms. Espinoza has so far received training on all laboratory procedures needed to complete this project. Additionally, she is in charge of keeping updated the inventory of all materials and supplies

	that is being purchased for this project
Funding Support:	Ms. Espinoza is a PRISMA full time employee who was hired to complete this project
Name:	Maxy De los Santos, Ph.D
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID): not available	
Nearest person month worked:	0.5
Contribution to Project:	Dr De los Santos received his doctoral degree from Universidad de Madrid in Spain. He has extensive research experience in molecular biology and parasitological techniques and has over 15 years of experience working with Leishmania. He is our consultant for all experiments involving parasite manipulation during this project
Funding Support:	Dr. De los Santos is a U.S. government employee. No salary is provided for him under this project

The following organizations are partners for this project:

Asociacion Benefica PRISMA

Calle Carlos Gonzáles N°251 Urbanización Maranga, Lima 32 - Perú

Ohio State University

281 W. Lane Ave.

Columbus, Ohio 43210

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES: SOW

The following SOW describes the breakdown of the proposed work as well as the key personnel involved and the location of the study sites. This study involves two study sites:

Site 1: The Ohio State University, 320 W 10th Ave, Columbus, OH 43210.

Ohio State is the primary organization conducting this study. Dr Satoskar, the initiating PI for this application, and his research team will oversee all activities related to the synthesis of PEN and DNER analogues as well as initial screening using standard laboratory strains. Dr. Satoskar's team will be in charge of selecting the most promising analogues and ship them to NAMRU-6 for *in vitro* testing with clinical *Leishmania* isolates. In addition, Dr. Satoskar team will conduct in vivo studies to test the efficacy of a selected subset of compounds using the mouse model of cutaneous leishmaniasis. Finally, Dr. Satoskar's team will conduct a series of mechanistic experiments to elucidate the mode of actions of the leading drug candidates identified in the previous experiments.

Site 2: Naval Medical Research Unit No. Six (NAMRU-6), Venezuela Avenue block 36, Callao 2, Peru.

NAMRU-6 is the collaborative institution for this study. Dr. Danett K. Bishop, the partnering PI, and his team will oversee all aspects of *in vitro* testing of selected PEN and DNER analogues using a variety of *L. (V.) peruviana* and *L (V.) brazilienesis* clinical isolates collected from endemic areas. In addition, NAMRU-6 will conduct in-vivo testing of selected products.