

AWARD NUMBER: W81XWH-15-2-0034

TITLE: Identifying New Chemical Entities that Treat and Prevent Relapsing Vivax and Drug-Resistant Falciparum Malaria in U.S. Military Personnel

PRINCIPAL INVESTIGATOR: Dr. Norman Waters

CONTRACTING ORGANIZATION: The Geneva Foundation
Tacoma, WA 98402

REPORT DATE: October 2017

TYPE OF REPORT: Annual

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

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14. ABSTRACT Our project has now nearly completing the screening of 400,000 compounds from the National Center for Advancing Translational Sciences (NCATS) chemical library, for activity against <i>Plasmodium falciparum</i> asexual blood stages. To date we have nearly 2,000 compounds that inhibit growth at low to submicromolar concentrations Nearly 600 of our prioritized hit compounds have been tested against rodent malaria liver stages and we have a set of 43 active compounds that have promising potency and selectivity from which to initiate <i>in vivo</i> assays to assess for cure and prophylaxis. We have also made progress with generating plasmids to engineer <i>Plasmodium cynomolgi</i> parasites that can be used to screen for activity against liver stages in a model of relapsing malaria. We are on track with our goal to identify chemical series that can be tested as candidate medicines to cure and prevent malaria in US Military personnel.					
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1. Introduction:

The goal of this project is to identify novel chemical compounds that are active against the blood and liver stage forms of malaria parasites and that are useful for both prophylaxis and treatment of *Plasmodium vivax* and *Plasmodium falciparum* infections. Malaria has been identified as one of the most significant threats to deployed troops worldwide. This disease is endemic to Southwest Asia including Afghanistan, Southeast Asia, Africa, the Middle East, the Pacific, and both Central and South America. The project combines expertise from Columbia University as the Initiating Institution, the Walter Reed Army Institute of Research (WRAIR) as the Partnering Institution, and the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) as a subsite affiliated with the WRAIR award. These teams combine chemistry, pharmacology and molecular and cellular parasitology to pursue a “hits to lead” program, whose primary objective is to generate and characterize compounds that could be developed into new medicines to treat and prevent malaria in US Military personnel.

2. Keywords:

Malaria, *Plasmodium falciparum*, asexual blood stages, liver stages, high-throughput screen, drug assays, cell culture, prophylaxis, *P. cynomolgi*.

3. Accomplishments:

3.1. Major Goals:

Our accepted statement of work (SOW) listed the following specific aims and tasks as part of our Year 2 work:

Specific Aim 1: Perform a high throughput screen (HTS)-based identification of antimalarial compounds. Our Major Task 1.1 from Year 1 was to confirm initial 2,045 hits from first screen of 250,000 compounds. This was completed on schedule. Our Major Task 1.2 was to implement a HTS with an additional 100,000 compounds and confirm hits. We proposed to achieve this by the end of year 1 and had completed 95% of the work by that time. In the past year, we completed all of these screens, going beyond our original proposal by combining all hits from our two initial screens into a further set of validation assays with a set of 4,300 compounds. Our Milestone for this Aim was to move our second set of confirmed hits into downstream screens, which we have met by testing our hits for activity against mammalian HepG2 cells in order to examine parasite selectivity.

Specific Aim 2: Screen for inhibitors of rodent liver stage parasites *in vitro*. Our Major Task 2.1 was to identify compounds that selectively inhibit *P. berghei* liver stage parasites *in vitro* at submicromolar concentrations. Our first Subtask was to perform *in vitro* liver stage screens. Our second Subtask was to screen out non-selective compounds that inhibit HepG2 cells. Our timeline for this Aim was the first 15 months. We completed this work on time, having already screened 999 blood stage-active compounds against *P. berghei* liver stages cultured *in vitro*. Our completed Milestone for this Aim defined a list of compounds with parasite-specific sub-micromolar *in vitro* liver stage activity. In total, we identified 151 compounds with >50% liver-stage inhibition when tested at 1 μ M.

As part of our statement of work (SOW), we acquired IACUC and ACURO approval in Year 1. Our IACUC protocol at CUMC (AC-AAAN5200) was reviewed and approved for yearly renewal on August 22, 2017. An ACURO document was submitted 10/29/2015 and approved on 12/28/2015, signed by Colonel Bryan Ketzenberger, Director of the Animal Care and Use Review Officer at the US Army.

Specific Aim 3: Test hits for *in vivo* prophylaxis and blood stage cure in rodents. Our Major Task 3.1 was to triage hits, assess toxicity and metabolism. Our first Subtask was to triage out known metabolic liabilities and toxicophores. Our second Subtask was for the remaining pharmacophores, to assess toxicity and metabolism. Our Milestone for this work was to finalize a list of hits with acceptable pharmacophores, with a stated goal of achieving this by the 18th month. We successfully completed this work, and identified a set of six novel chemical series with dual blood and liver stage activity that merit further chemical investigation (see below). Our Major Task 3.2 was to test hits for *in vivo* activity against *P. berghei* blood stages in mice. In preparation for this, we tested the safety of four compounds in mice: MLS002157637-02, MLS000573359-01, MLS000777921-01, and MLS000770597-01. All were found to be non-toxic at dose. MLS002157637-02 was then tested for activity in mice infected with *P. berghei* blood stages. However, no activity was identified. We then decided that more emphasis needed to be placed on the medicinal chemistry and pharmacological profiling prior to selecting compounds for *in vivo* testing in mice.

Our Major Task 3.3 was to test hits for *in vivo* activity against *P. berghei* liver stages in mice. Our Milestone for this Task was to define hits with evidence of *in vivo* curative and prophylactic activity, which we had estimated could be achieved by month 21. In the second year, we revised our work plan to devote more resources to pursuing medicinal chemistry to optimize our six prioritized series. This involves synthesizing or commercially purchasing analogs, investigating the structure-activity relationships by examining *in vitro* potency against cultured *P. falciparum* asexual blood stage parasites, and measuring metabolic stability, solubility and membrane permeability. As described below, we have made strong progress in this area.

Specific Aim 4: Test down-selected hits for *in vitro* activity against *P. cynomolgi* proliferating and hypnozoite liver stages. Our Major Task 4.1 was to develop *P. cynomolgi* constructs and reporter lines for compound screens. Our first Subtask was to generate plasmids. Our second Subtask was to begin *in vivo* selection studies to obtain a GFP-luciferase *P. cynomolgi* reporter line. We have accomplished our first subtask. However, our second subtask has been delayed because of the challenges of procuring monkeys that can be used for these *in vivo* infections and transfections. Our focus on optimizing our five selected chemical series has repositioned us to tackle Major Task 4.2 in year 3, namely assaying hits against *P. cynomolgi* liver stage parasites *in vitro*.

Specific Aim 5: Optimize hits, evaluate derivatives *in vivo* and *in vitro*. Our Major Task 5.1 was to perform medicinal chemistry-based derivation of analogs, assessment of toxicity and metabolism and Major Task 5.2 was to test analogs *in vitro* for activity against drug-resistant *P. falciparum* blood stages. These tasks were estimated to require continuous work through the end of our three-year project. As described below we have made strong progress in this area. Our Major Tasks 5.3 and 5.4 were to test analogs *in vivo* for activity against *P. berghei* and *in vitro* against *P. cynomolgi* proliferating and hypnozoite liver stages respectively. Work on these tasks will begin once we have made further progress on the medicinal chemistry part of our hit to lead work as parts of Tasks 5.1 and 5.2.

3.2. Accomplishments made under these goals:

3.2.1. Major activities: Our first accomplishment in year 2 was to complete the HTS work of the NCATS collection of 350,000 compounds. First, we examined our set of ~250,000 compounds that had earlier been screened against the Dd2 strain of *P. falciparum* asexual blood stage parasites. Those initial data were generated using a luciferase-based method and yielded 2,045 hits that showed activity. We then retested 1,882 compounds at NCATS. The remaining 163 were not tested because either they represented compounds of known activity, or had undesirable chemical functionality, or could not be sourced. These 1,882 compounds were tested in a first run of 168 compounds in order to re-establish the

conditions for our HTS. The second run tested 1,714 compounds. Parasites were procured through an internal collaborative arrangement between scientists at NCATS and the NIH. Overall, 659 showed an IC₅₀ value < 2 μ M. We also initiated a screen of the additional 100,000 compounds present in the current NCATS collection. Compounds were first tested in a five-point dilution series (with 5-fold dilutions), and active compounds retested in an 11-point 2-fold dilution series. This yielded an additional 2,400 hits. From this, we obtained a set of 4,300 compounds with IC₅₀ values < 2 μ M that were retested in a final set of confirmatory assays. This work yielded 659 and 443 hits from the first and second screens respectively that showed IC₅₀ values < 2 μ M and a selectivity index > 10 (activity against *P. falciparum* vs. HepG2 cells).

Active compounds were then screened against *P. berghei* liver stages cultured *in vitro*, using a parasite line expressing green fluorescent protein (GFP). GFP signals were examined at 44 hr post-inoculation, corresponding to mature liver-stage parasites. From our first set of 250,000 compounds that yielded 659 active compounds, we tested 565 against *P. berghei* liver stages at single concentrations of 1 and 3 μ M. In parallel, these were tested against HepG2 cells to assay for toxicity against mammalian cells. An example of data collected from our first set of screens is presented in **Table 1**.

Registration ID	Structural Class	<i>P. falciparum</i> IC ₅₀ (μ M)	NCATS HepG2 IC ₅₀ (μ M)	PbLuc IC ₅₀ (μ M)	HepG2 IC ₅₀ (μ M)	Microsomes t _{1/2} (m)	Permeability (PAMPA_Pion_Lipid 1e-6 cm/s)	Solubility (μ g/ml)
MLS000393823-01	benzamide series	0.2512	50.000	3.555	22.47	1.6	70	<1
MLS000850457-01	benzamide series	0.4467	50.000	0.006193	5.739	2.4	<1	1.6
MLS000862045-01	benzamide series	1.4125	50.000	0.006026	1.895	10.3	ND	<1
MLS001099437-01	benzamide series	1.5849	22.387	0.01292	>50	3.4	343.7	>55
MLS001162082-01	benzamide series	1.5849	50.000	0.1425	44.1	>30	117.2	1.8
MLS000041320-01	indole-like series	1.2589	22.387	0.02908	6.755	7.5	1527.3	<1
MLS000557140-01	indole-like series	1.9953	50.000	0.0004029	10.14	2	1183.5	<1
MLS000717833-01	indole-like series	0.3548	50.000	0.4141	0.5773	>30	<1	>46
MLS000770597-01	thiazole amide	0.6310	15.849	0.9377	12.72	>30	289.8	1.1
MLS002157637-01	thiazole amide	0.8913	50.000	0.009346	4.68	4	540.5	7.2
MLS002161290-01	thiazole amide	0.7943	50.000	0.02342	1.585	8.5	656.6	24.3
NCGC00345210-01	thiazole amide	0.8913	50.000	0.2476	1.813	4	540.5	7.2
MLS000074180-01	dihydroquinoline carboxylate series	0.5012	50.000	0.02723	>50	15.9	62.4	4.9
MLS000085191-01	dihydroquinoline carboxylate series	1.5849	50.000	0.009855	>50	20.4	434.3	34
MLS000109039-01	dihydroquinoline carboxylate series	0.6310	50.000	0.01242	24.36	18.7	355.6	1.9
MLS000112473-01	dihydroquinoline carboxylate series	1.9953	50.000	0.01314	10.93	15.9	74.9	3.4
MLS000777921-01	dihydroquinoline carboxylate series	0.2239	39.811	0.2261	>50	>30	12.1	>65
MLS000862522-01	dihydroquinoline carboxylate series	0.8913	50.000	0.1557	>50	17.7	204.6	1.7

Table 1. Potency and pharmacological profiling of a set of antimalarial compounds obtained from our first HTS screen of 250,000 compounds. Activity is graded from green (most favorable) to red (least favorable). Compounds highlighted in yellow show promising liver and asexual blood stage activity.

Based on these results and chemoinformatic analysis, we chose a subset of 44 potent and selective compounds for IC₅₀ determination. Results showed that 43 of these compounds were active against liver stages with IC₅₀ values below 1 μ M. IC₅₀ values were as low as 0.4 nM, indicating exceptional potency. We also completed a screen of 434 hits from our second screen at 1 and 3 μ M, with parallel tests conducted against HepG2 cells. By combining our analysis of both sets of screens, we generated data on 151 compounds with IC₅₀ values below 1 μ M against liver stage parasites. A summary of our overall workflow is presented in **Figure 1**.

qHTS Screening Assay Workflow

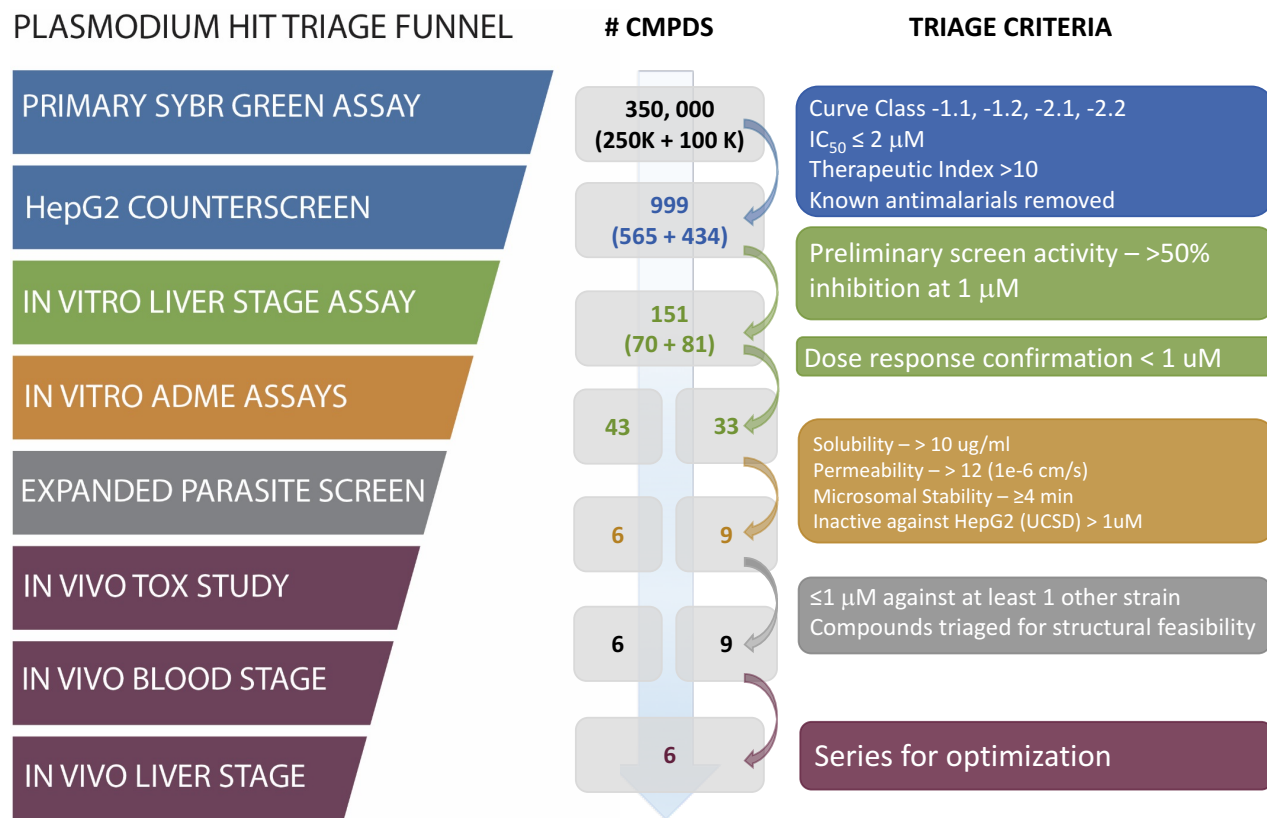


Figure 1. Workflow of compounds from initial HTS campaigns to *in vivo* screens. This workflow has led from screening 350,000 compounds for activity against *P. falciparum* asexual blood stages to 999 tested against *P. berghei* liver stages and HepG2 cells. 151 were found to be active and selective and were then subjected to dose-response assays to determine IC₅₀ values. From these two screens, we now have 6 chemotypes from the two screens that are being currently prioritized for compound analoging and definition of pharmacological properties and structure-activity relationships.

Our major progress in year 2 has been the full-scale profiling of our 1,000 hits from our initial screens through *P. falciparum* asexual blood stage screens, *P. berghei* liver stage screens, and pharmacological profiling for metabolic stability, solubility and membrane permeability. From this work, we have defined six chemical series to pursue in a hit to lead campaign. These are thiadiazines, 8-hydroxyquinolines, pyrimidine azepines, diazaspiro-octanes, amino-indenones and triazoloquinazolines.

For the thiadiazines we have made 55 analogs, which are being tested for their absorption, distribution, metabolism, and excretion (ADME) properties. We are also examining their potency against *P. falciparum* asexual blood stage parasites (Dd2 strain, in 1536-well format with SYBR Green I to stain parasites and derive dose-response curves and IC₅₀ values). Additional compounds of this chemotype will be synthesized to develop a thorough structure-activity relationship. From this we intend to select the most potent compounds for entry into *P. berghei* *in vitro* liver stage assays.

For the 8-hydroxyquinolines we have already screened close to 400 compounds. Some of these were found to have moderate *in vitro* activity against *P. falciparum* asexual blood stages (with IC₅₀ values <

μM against the D6 strain) or against *P. berghei* liver stages (also with some revealing IC₅₀ values <1μM). However, there is some concern about off-target activity and this is currently a lower priority series while we explore other chemotypes.

For the diazaspiro-octanes our most promising hit has been procured at the 100 mg scale. This will then be subjected to chiral separation to then examine potency of the individual enantiomers. We have identified close to 260 analogs that we can then pursue as we examine structure-activity relationships (SARs).

Work on the other three series will begin once we have completed more SAR studies with our three series listed above.

If needed, we will move in year 3 to our additional back up “lower priority” series that include thiazole amides, pyridyl pyrimidines, and imidazolopyridazines.

3.2.2. Specific objectives: We have met our specific objective to screen a library of 350,000 compounds and identify hits that are active against *P. falciparum* asexual blood stage parasites and *P. berghei* liver stage parasites. We now have 150 compounds that have promising dual-stage activity and have prioritized several chemotypes for further potency and pharmacological investigation now deeply into the phase of optimizing our hits. We have also achieved our objective of generating a DNA plasmid for later work to screen against *P. cynomolgi* liver stage parasites, which contain both actively replicating forms as well as hypnozoites.

3.2.3. Significant results and key outcomes: These are described above.

3.2.4. Other achievements: Nothing to report.

3.3. Training and professional development opportunities: Nothing to report.

3.4. Dissemination of results to communities of interest: We presented this project to the JPC-2 MIDRP In-Progress Review (IPR) on 4 April 2017, at Fort Detrick, MD. Review officers included Colonel Michael P. Kozar, Ph.D, MT(ASCP), Director, Military Infectious Diseases Research Program Chair, Joint Program Committee-2 Military Infectious Diseases Assistant Corp Chief for Medical Allied Sciences, US Army Medical Service Corp, and CAPT David J. Bacon, Ph.D., MSC, US Navy Liaison Officer. I gave the presentation, with my partnering PI LTC(P) Waters in attendance as well as key personnel from WRAIR (Drs. Mark Hickman and Rick Sciotti) and NCATS (Dr. Bryan Mott) in attendance.

3.5. Plans during next reporting period to accomplish goals: Our major objective is to pursue an in-depth hit to lead campaign with our prioritized list of chemotypes, with the goal of identifying compounds that are potent *in vitro* against *P. falciparum* asexual blood stages and *P. berghei* liver stages. Our experiments will investigate structure-activity relationships in these series and also identify pharmacological properties, which will be considered as we pursue chemical optimization. Our goal is to produce and characterize several compounds that will enter *in vivo* testing in blood and liver stage *P. berghei* models. Our efforts will center on trying to generate lead compounds from one or two chemical series. Evidence of *in vivo* activity will then lead to further rounds of chemical optimization in order to test compounds for activity against *P. cynomolgi* parasites *in vitro*. We will also pursue our *P. cynomolgi* transfection objectives.

4. Impact:

4.1. Impact on development of principal discipline of the project: Nothing to report.

4.2. Impact on other disciplines: Nothing to report.

4.3. Impact on technology transfer: Nothing to report.

4.4. Impact on society beyond science and technology: Nothing to report.

5. Changes/Problems:

5.1. Changes in approach and reasons for change: Nothing to report.

5.2. Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report. We are on track with our statement of work, timeline and milestones.

5.3. Changes that had a significant impact on expenditures: (needs updating once numbers come back from Geneva) No major changes.

5.4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

6. Products:

6.1. Publications, conference papers and presentations: Nothing to report.

6.2. Websites: Nothing to report.

6.3. Technologies or techniques: This project has enabled NCATS to optimize their quantitative HTS studies with cultured *P. falciparum* asexual blood stage parasites that allows them to derive IC₅₀ values for hundreds of thousands of compounds in a period of several months. These data provide this project with an outstanding set of novel compounds to drive our malaria drug discovery program.

6.4. Inventions, patent applications and/or licenses: Nothing to report.

6.5. Other products: Our project shares and regularly updates a Master file that lists compound structures and names, blood and liver stage activity, IC₅₀ values for parasites and HepG2 cells, and pharmacological properties (metabolic stability, permeability and solubility). We also have compound potency data for four *P. falciparum* strains: D2, W2, C235 and C2B, which include mechanisms of resistance to chloroquine, atovaquone, pyrimethamine and mefloquine. We have generated DNA plasmids that are ready to generate recombinant *P. cynomolgi* parasites for assessment of compound activity against proliferating or hypnozoite liver stage parasites.

7. Participants & Other collaborating organizations:

7.1. Individuals that have worked on the project:

Name	David Fidock (CUMC). ORCID 0000-0001-	Name	Santha K. Tiruppadiripuliyur (CUMC)
Project role	Initiating PI	Project role	Postdoctoral Scientist
Nearest person month worked	2	Nearest person month worked	9
Contribution to project	Led project, organized monthly teleconference calls and distributed	Contribution to project	Worked on <i>in vitro</i> parasite studies with compounds
Funding support	CDMRP, NIH, Bill & Melinda Gates	Funding support	CDMRP, NIH
Name	Kathryn Wicht	Name	Manu Vanaerschot
Project role	Postdoctoral Scientist	Project role	Postdoctoral Scientist
Nearest person month worked	3	Nearest person month worked	6
Contribution to project	<i>In vitro</i> parasite studies with compounds and assisted with compound analysis	Contribution to project	<i>In vitro</i> parasite studies with compounds
Funding support	CDMRP, NIH	Funding support	CDMRP, NIH
Name	Judith Straimer	Name	LTC Norman Waters (WRAIR)
Project role	Postdoctoral Scientist	Project role	Partnering PI
Nearest person month worked	1	Nearest person month worked	1
Contribution to project	Molecular biology on <i>P. cynomolgi</i> plasmids	Contribution to project	Managed WRAIR contribution to pharmacology and compound testing
Funding support	CDMRP, NIH	Funding support	CDMRP and WOC
Name	Dr. Robert Campbell	Name	Dr. Richard Sciotti (WRAIR)
Project role	Senior Medicinal Chemist	Project role	Medicinal Chemist
Nearest person month worked	12	Nearest person month worked	2
Contribution to project	Performed pharmacologic and efficacy studies with active compounds	Contribution to project	Medicinal chemistry of promising hits
Funding support	WRAIR	Funding support	CDMRP and WOC
Name	Juan Marugan (NCATS)	Name	Wenwei Huang (NCATS). ORCID: 0000-
Project role	Project manager at NCATS subsite	Project role	Partnering PI and Chemistry Lead at
Nearest person month worked	1	Nearest person month worked	1
Contribution to project	Managed project resources including personnel and lab operations	Contribution to project	Managed project resources and performed data analysis
Funding support	NIH/NCATS	Funding support	NIH/NCATS
Name	Daniel Jansen	Name	George Djorbal
Project role	Staff chemist	Project role	Staff Biologist
Nearest person month worked	12	Nearest person month worked	2
Contribution to project	Performed compound synthesis, managed compound procurement and	Contribution to project	Conducted high-throughput screens
Funding support	NIH/NCATS	Funding support	NIH/NCATS
Name	Richard T. Eastman	Name	Alexey Zakharov
Project role	Postdoctoral Scientist	Project role	Informatics
Nearest person month worked	2	Nearest person month worked	1
Contribution to project	Tested compound efficacy against	Contribution to project	Analyzed screen data
Funding support	NIH/NCATS	Funding support	NIH/NCATS
Name	Katie Pohida		
Project role	Post Baccalaureate fellow		
Nearest person month worked	6		
Contribution to project	Performed compound synthesis		
Funding support	NIH/NCATS		

7.2. Change in active other support of the PD/PI or senior/key personnel since the last reporting period:

There are no changes in other support for either Drs. Fidock or Waters since the time of the last annual report.

7.3. Other organizations involved as partners: Nothing to report.

8: Special reporting requirements:

8.1. Collaborative Awards: The Initiating PI Dr. David Fidock and the Partnering PI LTC Norman Waters are providing independent annual reports for this project (W81XWH-15-2-003 and W81XWH-15-2-0034 respectively). Each report has its own separate cover page, SF298 and quad chart.

8.2. Quad Chart: Please see next page. The quad chart for LTC Waters is provided in his separate report.

9: Appendices: None.

Identifying New Chemical Entities that Treat and Prevent Relapsing vivax and Drug-Resistant falciparum Malaria in U.S. Military Personnel

PR140137P1



PI: LTC Norman Waters **Organization:** The Geneva Foundation/WRAIR

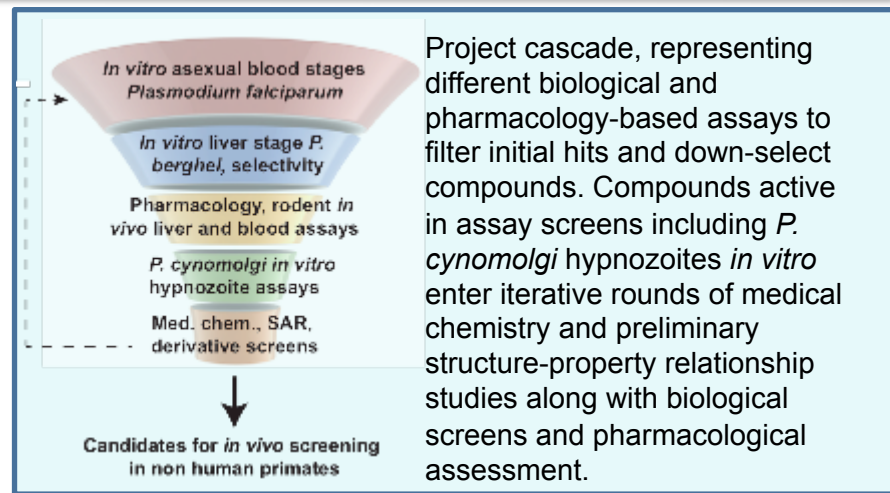
Award Amount: \$1,345,850

Study/Product Aim(s)

- Aim 1: Identify antimalarials using a high-throughput screen (HTS) against *Plasmodium falciparum* asexual blood stages.
- Aim 2: Screen for selective inhibitors of rodent liver stage parasites *in vitro*.
- Aim 3: Test hits for suitable pharmacological properties and *in vivo* efficacy as prophylactic and curative agents in rodent malaria models.
- Aim 4: Identify inhibitors of *P. cynomolgi* liver stages *in vitro*.
- Aim 5: Optimize hits, evaluate derivatives *in vivo* and *in vitro*.

Approach

Our project, involving Columbia University, the Walter Reed Army Institute of Research, and the National Center for Advancing Translational Sciences, is designed to discover new chemical agents that can be developed into prophylactic and curative medicines to protect US Military personnel exposed to malaria.



Accomplishment: Identification of lead compounds to prevent and cure vivax and falciparum malaria.

Timeline and Cost

Activities	CY	15	16	17	18
Aim 1 (milestone: HTS)					
Aim 2 (milestone: liver stages)					
Aims 3,4 (milestone: <i>in vivo</i> cure)					
Aim 5 (milestone: <i>P. cynomolgi</i>)					
Budget (1,345,850) in \$		128,424	496,504	430,503	290,419

Goals/Milestones

CY15 Goal – Initiate analysis of current hit compounds

X Confirm initial hits active against *P. falciparum* blood stages

CY16 Goals – Identify new hit compounds

X Screen additional 150K compounds against blood stages

☐ Identify liver stage-active inhibitors

CY17 Goals - Define *in vivo* active compounds

☐ Identify inhibitors active in mice, test against *P. cynomolgi* *in vitro*

CY18 Goal – Optimize hits, liver and blood stage efficacy

☐ Medicinal chemistry, ADMET/toxicity

☐ Test inhibitor activity in mice, *P. cynomolgi* liver stages *in vitro*

Comments/Challenges/Issues/Concerns

• None.

Budget Expenditure to Date (WRAIR and NCATS)

Projected Expenditure: \$866,727.88 (began 30 September 2015)

Actual Expenditure: \$418,111.21 (as of 26 October 2017)

Updated: Tacoma, WA, October 26, 2017