

AWARD NUMBER: W81XWH-18-1-0222

TITLE: Probing Mechanisms of *P. falciparum* Artemisinin Resistance Using Single-Cell Transcriptomics

PRINCIPAL INVESTIGATOR: Bjorn F.C. Kafsack, Ph.D.

CONTRACTING ORGANIZATION: Weill Medical College of Cornell University  
New York, NY 10065

REPORT DATE: July 2019

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> July 2019		<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 1 Jul 2018 - 30 Jun 2019	
<b>4. TITLE AND SUBTITLE</b> Probing Mechanisms of <i>P. falciparum</i> Artemisinin Resistance Using Single-Cell Transcriptomics				<b>5a. CONTRACT NUMBER</b>	
				<b>5b. GRANT NUMBER</b> W81XWH-18-1-0222	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Bjorn F.C. Kafsack, PhD  E-Mail: <a href="mailto:bjk2007@med.cornell.edu">bjk2007@med.cornell.edu</a>				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> Weill Medical College of Cornell University Joan & Sanford I Weill Medical College 407 E 61st St, Room 106 1st Floor New York, NY 10065-4805				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> Artemisinin (ART) derivatives are highly potent anti-malarial drugs and are the main treatment option in most malaria-endemic regions across the globe. The emergence and spread of artemisinin-resistant parasites threatens reverse the recent gains in reducing malaria morbidity and mortality. Resistance is associated with mutations in the <i>kelch13</i> gene (K13). K13 mutant ring-stages contain a sub-population able to survive ART exposure for a prolonged period and continues replicating once drug pressure is removed. Our ongoing experiments use single-cell transcriptomics (scRNAseq) to characterize unique patterns of gene expression within this "survivor" population that underlie their resistance phenotype. Subsequent experiments will then evaluate the function of the involved genes in artemisinin-resistance. Over the first 12 months of the project, 1. Ring-stage survival assays to measure ART resistance level of sensitive and K13 resistance mutants were successfully implemented. 2. scRNAseq equipment was successfully transferred from our previous collaborator and established in the lab. 3. Initial round of scRNAseq was carried out. 4. scRNAseq procedures were optimized for <i>P. falciparum</i> ring stages with substantial improvements in transcript capture. Unexpected personnel turnover and reagent quality issues resulted in unanticipated delays and a request for a 12-month no-cost extension was filed and approved.					
<b>15. SUBJECT TERMS</b> Malaria, drug resistance, single cell transcriptomics,					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			<b>19b. TELEPHONE NUMBER (include area code)</b>
Unclassified	Unclassified	Unclassified	Unclassified		

## Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	5
5. Changes/Problems.....	5
6. Products.....	6
7. Participants & Other Collaborating Organizations.....	7
8. Special Reporting Requirements.....	8
9. Appendices.....	8

## 1. INTRODUCTION:

Artemisinin (ART) derivatives are highly potent anti-malarial drugs that make up the backbone of treatment options in most malaria-endemic regions across the globe. The emergence and spread of artemisinin-resistant parasites threatens to halt and reverse the recent gains in reducing malaria morbidity and mortality by the global malaria control and eradication effort. Resistant blood-stage parasites exhibit a prolonged ring-stage that allows a subpopulation of parasites to emerge from ART pressure and associated with mutations in the *kelch13* gene (K13). K13 mutant ring stage parasites contain a subpopulation able to survive ART exposure for a prolonged period and continues replicating once drug pressure is removed. Our ongoing experiments use single-cell transcriptomics to characterize unique patterns of gene expression within this “survivor” population that underlie their resistance phenotype. Subsequent experiments will then evaluate the function of the involved genes in artemisinin-resistance.

## 2. KEYWORDS:

Malaria, Antimicrobial Resistance, artemisinin-resistance, single cell transcriptomics

## 3. ACCOMPLISHMENTS:

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

Planned milestones for this reporting period:

Aim 1: scRNA-seq of ART-S and ART-R parasites (0-12 months)

Milestone #1: Validate reported RSA results. (3 months, 100% complete)

Milestone #2: Optimize RSA scale for scRNA-seq (3 months, 100% complete)

Milestone #3: Sequence >2000 ART-R and >400 ART-S single cell transcriptomes (9 months)

Milestone #4: Identify survivor sub-population and determine specific gene expression (12 months)

Aim 2: Characterize overexpression phenotypes of genes uniquely expressed in the "survivor" subpopulation (9-18 months)

No milestones were scheduled for completion. However, in anticipation of the results from Aim1, backbone constructs for conditional overexpression were generated and are ready for cloning of target coding sequences.

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

Aim 1 Milestones #1 and #2 were successfully completed on time. This involved establishing and validating ring-stage survival assays of ART resistance for wildtype and K13 mutant parasites. This was followed by an initial round of scRNAseq to optimize protocols for scRNAseq of rings-stage, which resulted in substantial improvements in transcript capture. Work towards Milestone #3 is still ongoing but completion was delayed due to unexpected personnel turnover and reagent quality control issues. These problems were resolved and newly optimized methods have been developed. A 12 month no-cost extension was applied for and approved.

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

Nothing to report.

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

Nothing to report.

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

Now that the newly optimized scRNAseq protocols are fully operational in our lab, we anticipate completion of Milestones #3 and #4 in the next six months. This will allow us to initiate the Aim 2 experiments characterizing genes specifically expressed in the “survivor” subpopulation.

#### 4. IMPACT:

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

Through optimization of microfluidic flow and cell lysis buffer composition we were able to dramatically improve the single-cell RNA capture efficiency for *Plasmodium falciparum* ring stages.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Nothing to report.

#### 5. CHANGES/PROBLEMS:

- a. **Changes in approach and reasons for change**

Nothing to report.

- b. **Actual or anticipated problems or delays and actions or plans to resolve them**

While we were able to complete Milestones #1 and #2 for the single-cell RNA sequencing aspect of the project (Major Task 1) within the projected time frame, Milestone #3 was substantially delayed due to a combination of personnel turnover and reagent quality issues beyond our control.

- I. Unexpected Personnel Turnover.*

- The technician (Gabriela Funez-dePagnier) who was hired and trained specifically for this project in August 2019, left the lab after only five months to pursue a career outside of science. This required retraining of new personnel to perform the ring-stage survival assays.
- Our main collaborator for single-cell RNA sequencing (Dr. Olivier Elemento) stopped performing these experiments when the operator of the equipment left his lab last fall. As a result, we had to acquire and install the equipment for single-cell capture in our laboratory, followed by the necessary operator training and performance validation.

Both of these issues have been resolved. We acquired the necessary equipment to carry out the single-cell capture in our laboratory without using funds allocated to this project and fully trained a graduate student operate it.

- II. Reagent quality issues.*

This spring, two consecutive batches of RNA capture beads, a critical reagent for single cell RNA sequencing, failed our rigorous quality control process and had to be replaced by the vendor. Unfortunately, Chemgenes Corporation is the sole-source supplier of this reagent and only a single batch is synthesized each month.

To resolve these supply issues, we acquired a large amount of RNA capture beads from the last batch that passed quality control. These quantities will be more than enough to complete the proposed experiments.

**c. Changes that had a significant impact on expenditures**

The delays described in section 5b meant that a substantial fraction of allocated funds for the first 12 months remained unspent. As a result, we requested a 12-month no-cost extension for this project earlier in July 2019. The single cell capture equipment was acquired without additional cost to this project.

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

Nothing to report.

**e. Significant changes in use or care of human subjects**

Nothing to report.

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

Nothing to report.

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

Nothing to report.

**6. PRODUCTS:**

**a. Publications, conference papers, and presentations**

**i. Journal publications.**

Nothing to Report.

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

Nothing to Report.

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

Nothing to Report.

**b. Website(s) or other Internet site(s)**

Nothing to Report.

**c. Technologies or techniques**

Substantially improved techniques for single cell RNA sequencing of malaria parasite ring stages. Techniques will be shared through future publication, the laboratory website, and personal communications.

**d. Inventions, patent applications, and/or licenses**

Nothing to Report.

**e. Other Products**

Nothing to Report.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### a. What individuals have worked on the project?

Name:	Björn Kafsack, PhD
Project Role:	Principle Investigator
Researcher Identifier	ORCID 0000-0003-0089-1096
Person month worked:	2
Contribution to Project:	Project supervision, experiment design, bioinformatics, data analysis
Funding Support:	NIAID R01 AI138499 Mechanisms of environmental sensing and responses by malaria parasites Role: Co-Investigator, PI: Kirk Deitsch  NIAID R01 AI141965. Regulation of Sexual Differentiation in Malaria Parasites Role: PI

Name:	Laura Kirkman, MD
Project Role:	Co-investigator
Researcher Identifier	ORCID 0000-0003-4085-554
Person month worked:	1
Contribution to Project:	Dr. Kirkman helped with the design and training for the ring-stage survival assays.
Funding Support:	NIAID R21 AI12379402 Novel inhibitors of malaria proteasome. Role: Co-investigator, PI: Gang Lin  NIAID R01 AI14615301 Role of translesional polymerases in genome diversification of the malaria parasite. Role: PI

Name:	Gabriella Funez-Pagnier
Project Role:	Technician
Researcher Identifier	Not available
Person month worked:	5
Contribution to Project:	Ms. Pagnier was hired & trained to carry out the ring-stage survival assays. She left the lab after 5 months.
Funding Support:	none

Name:	Christopher Noetzel
Project Role:	Graduate Student
Researcher Identifier	ORCID 0000-0001-6065-889X
Person month worked:	4
Contribution to Project:	Designs, executes, analyzes ring-stage survival assays and single cell sequencing experiments.
Funding Support:	Jacques Cohenca Predoctoral Fellowship

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

*New Active Support for Björn Kafsack:*

NIAID R01 AI141965      Jun 2019 – May 2024  
NIAID R01 AI138499      Apr 2018 – Mar 2023

No change in effort.

*New Active Support for Laura Kirkman:*

NIAID R21 AI12379402 Feb 2018 – Jan 2020  
NIAID R01 AI14615301 Jun 2019 – May 2024

No change in effort.

- c. **What other organizations were involved as partners?**

Nothing to report.

## **8. SPECIAL REPORTING REQUIREMENTS**

- a. **COLLABORATIVE AWARDS:**

Nothing to report

- b. **QUAD CHARTS:**

Nothing to report

## **9. APPENDICES:**

No appendices.