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TITLE: Preclinical Development of an Antimalarial Agent with a New Mechanism-of-Action

PRINCIPAL INVESTIGATOR: Stuart L. Schreiber

CONTRACTING ORGANIZATION: Broad Institute
Cambridge, MA 02142

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Broad Institute, Inc.
415 Main Street
Cambridge, MA 02142

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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14. ABSTRACT
We developed a series of compounds with potent activity against all human stages of the malaria parasite and excellent *in vivo* efficacy in mouse models of infection. We demonstrated that these compounds act *via* a novel mechanism-of-action (inhibition of *P. falciparum* cytosolic phenylalanyl tRNA synthetase, *Pf*cPheRS) and as such this program represents an important development to combat antimalarial resistance. A lead compound, BRD5018, was identified that had potent *in vivo* efficacy and an improved safety profile relative to early lead compounds. In biochemical aminoacylation assays, BRD5018 strongly inhibited *Pf*cPheRS, with high selectivity over human cPheRS. We have completed the manufacturing of GLP materials and the corresponding impurity identification studies. We also initiated preliminary dose form studies, physicochemical characterization, analytical development and salt selection studies on BRD5018. Contracts with CROs for IND-enabling preclinical safety and DMPK studies have been signed and await final approvals to initiate work. Our goals are to complete Investigational New Drug (IND)-enabling GLP studies with BRD5018 and subsequently nominate this compound as a clinical candidate for Phase I studies.

15. SUBJECT TERMS
Malaria, phenylalanyl tRNA synthetase, novel mechanism-of-action, bicyclic azetidine, preclinical development.

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
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

U.S. service members deployed in endemic areas should be considered at high risk for malaria infection. The emergence of resistance to front-line antimalarial therapies has created an urgent need for drugs with new mechanisms of action (MoA). Additionally, while most antimalarials target symptomatic asexual blood-stage parasites, drugs targeting the liver and transmission stages are essential to protect populations in endemic regions, including deployed military personnel. The Broad Institute, in collaboration with Eisai Inc., has developed a series of compounds with potent *in vitro* activity against blood-, liver- and transmission-stage parasites and excellent *in vivo* antimalarial efficacy, including single-dose cures in *P. berghei* and *P. falciparum* mouse models. We have demonstrated that these compounds act *via* a novel mechanism of action (inhibition of *P. falciparum* cytosolic phenylalanyl tRNA synthetase, *Pf*cPheRS)— as such, this program represents an important development to combat antimalarial resistance. Optimization studies produced a bicyclic azetidine candidate, BRD5018, that had good *in vitro* and *in vivo* efficacy and suitable ADME, PK and *in vitro* safety parameters for progression towards advanced studies. Early toxicity assessments in rodents suggests primary toxicities are monitorable and reversible. The purpose of this proposal is to complete Investigational New Drug (IND)-enabling GLP studies with BRD5018 and subsequently nominate this compound as a clinical candidate for Phase I studies. We will produce sufficient quantities of the Active Pharmaceutical Ingredient (API) to support the appropriate IND-enabling DMPK and nonclinical animal studies.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Malaria, novel mechanism-of-action, phenylalanyl tRNA synthetase, bicyclic azetidine, preclinical development, IND enabling studies, antimalarial resistance.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific aim 1. Complete salt selection, physicochemical characterization and impurity identification studies on BRD5018.

1. Salt formation studies and polymorph screening of BRD5018 **(70% complete)**

2. IND-enabling studies for physicochemical characterization and elucidation of the structure of the API **(10% complete)**

3. Impurity identification study prior to manufacturing a batch for toxicology studies **(100% complete)**

Specific aim 2. Prepare GLP material for nonclinical animal studies.

1. Manufacturing of GLP material for reference standard and nonclinical studies **(100% complete)**

Specific aim 3: Preliminary development of dosage form.

1. Complete formulation development study **(60% complete)**
2. Complete analytical method development study **(50% complete)**
3. Complete stability study **(60% complete)**

Specific Aim 4: IND enabling Nonclinical Safety Studies.

1. Perform rodent PK study-Mouse and Rat **(10% complete)**
2. Perform non-rodent PK study-Dog **(10% complete)**
3. Determine plasma protein binding and Kb/p ratio **(10% complete)**
4. Determine CYP Induction, CYP Inhibition / TDI **(10% complete)**
5. Perform Reaction Phenotyping **(10% complete)**
6. Prepare deuterated Internal standard **(100% complete)**
7. LC/MS/MS method validation for rat and dog plasma **(10% complete)**
8. Complete rat GLP 3-day repeat-dose toxicity study with 28-day recovery **(10% complete)**
9. Complete dog GLP 3-day repeat-dose toxicity study with 28-day recovery **(10% complete)**
10. Complete dog GLP Cardiovascular safety study **(10% complete)**
11. Complete Rat GLP CNS and respiratory safety study **(10% complete)**
12. Complete GLP hERG **(0% complete)**
13. Complete GLP Ames and in-vitro micronucleus assay **(0% complete)**

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results

achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) major activities

Major activities include completion of manufacturing of GLP materials and impurity identification studies. We also initiated preliminary dose form studies, physicochemical characterization, analytical development and salt selection studies on BRD5018. Study contracts with CROs for IND-enabling preclinical safety and DMPK studies have been signed and awaiting final approvals to initiate.

2) specific objectives

Our goals are to complete Investigational New Drug (IND)-enabling GLP studies with BRD5018 and subsequently nominate this compound as a clinical candidate for Phase I studies. Specifically, we will (1) complete salt selection, physicochemical characterization and impurity identification studies, (2) prepare GLP material for nonclinical animal studies, (3) complete formulation development and analytical method development and preliminary stability studies, (4) perform IND-enabling nonclinical studies on BRD5018.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

1) *Completed IND-enabling physicochemical characterization and elucidation of the structure of the API, impurity identification study, salt formation studies and polymorph screening on BRD5018.*

Based on the salt formation study, we have selected a free form of BRD5018 for further development. The polymorph screening study is on-going, comprising both *in silico* calculations and wet screening at XtalPi Inc. Additionally, we have started IND enabling studies for physicochemical characterization and elucidation of the structure of the API. Finally, we completed an impurity identification study for the preparation of the toxicology batch; the structures of seven impurities have been identified.

2) *Prepared GLP material of BRD5018 for nonclinical animal studies*

A 720-g batch of BRD5018 was manufactured for toxicology studies and batch release: 45 g were used for analysis, DMPK analytical method development and validation, and as control sample; 675 g are ready to ship to study site based on study plan. Of note, improvements in process chemistry permitted the manufacturing of a quantity of BRD5018 80% greater than expected (720 g vs. 400 g). A related reference standard to support the release of the toxicology batch was also manufactured and qualified.

3) *Completed formulation development, analytical method development, and stability studies on BRD5018.*

For the feasibility studies for first-in-human (FIH) formulation, the compatibility of drug substance with excipients was evaluated using material prepared via the preliminary synthesis route. In addition, dosage forms (Tablet, Granule in capsule and API in capsule) were manufactured as part

of the pre-formulation study, a preliminary short-term stability study (60°C/75%RH for 2 weeks) was conducted, and analytical methods of related substances and dissolution were developed to evaluate the prototype dosage forms in the stability study.

4) Initiated IND enabling nonclinical safety studies

We have completed the preparation of a deuterated internal standard (BRD5018-d_6). Study contracts were signed for all the in vitro DMPK studies needed for IND filing. The protocols for the *in vitro* studies are under review at this time. The studies will initiate in August and are expected to be completed by October 2019.

Contract for all *in vivo* safety studies (rat and dog 3-day repeat dose toxicity studies, including respiratory and CNS assessment in rat and dog telemetry cardiovascular study) with CiTox Lab (now part of Charles River Laboratories). Protocols are finalized and under ACURO review.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

During the next reporting period, we will complete polymorph screening studies and IND enabling studies for physicochemical characterization and elucidation of structure of the API. Additionally, we will continue our efforts towards completion of initial formulation and analytical method development studies. Specifically, we will focus on: 1) a stability-indicating assay, 2) a comprehensive related substances test, 3) an appropriate dissolution method, and 4) a drug product uniformity analysis. We will also continue to evaluate prototype drug products prepared on a small scale for essential properties such as related substances and dissolution. Simultaneously, we will complete all proposed IND-enabling preclinical safety and DMPK studies. As required, we will coordinate with our CRO for IACUC approvals and submit for ACURO reviews prior to the start of any studies involving animals.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Long ACURO review period pushed back the timeline to start in vivo PK, toxicity and safety pharmacology studies at CRO.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This project has established that inhibition of the *P. falciparum* enzyme cytosolic phenylalanyl tRNA synthetase (cPheRS) is an important new mechanism of action with promising potential for the treatment of malaria. We have discovered BRD5018, a small molecule that inhibits *P. falciparum* cPheRS potently and selectively, reduces parasite burden *in vivo*, and presents good pharmacokinetic and safety profiles. We are now establishing the developability of BRD5018 in a pre-clinical setting. Collectively, our findings have increased interest for cPheRS in the malaria field as well as for the discovery of other aminoacyl tRNA synthetase inhibitors for the treatment of malaria.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We have discovered that inhibition of parasite cytosolic phenylalanyl tRNA synthetase is applicable to other disease areas. Specifically, we discovered that compounds from this program have in vitro potency against *C. parvum*, *L. donovani*, *T. cruzi* and *T. gondii*, with *in vivo* efficacy in *C. parvum* and *L. donovani* mouse models. We are following up on these results— for instance, a program has been initiated to develop analogues from this series with CNS penetration for the treatment of *T. gondii*. As a result, our advances in the pre-clinical development of BRD5018 directly inform efforts in these therapeutic areas.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

There are no significant changes in the project or its direction.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Long ACURO review period pushed back the timeline to start in vivo PK, toxicity and safety pharmacology studies at CRO.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

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

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

A provisional patent application was filed in January 2019 entitled: METHOD FOR SYNTHESIS OF DIAZABICYCLO[6.2.0]DECANE RELATED COMPOUNDS

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Stuart Schreiber
Project Role:	PD/PI

Nearest person month worked: 1
Contribution to Project: Scientific and administrative oversight
Funding Support: DOD

Name: Eamon Comer
Project Role: Medicinal Chemist
Nearest person month worked: 2
Contribution to Project: Project management
Funding Support: DoD

Name: Bruno Melillo
Project Role: Medicinal Chemist
Nearest person month worked: 2
Contribution to Project: Project management
Funding Support: Gates

Name: Cindy Hon
Project Role: Research Strategy and Operations
Nearest person month worked: 1
Contribution to Project: Alliance Management
Funding Support: GHIT

Name: Fabian Gusovsky
Project Role: Co-PI
Nearest person month worked: 1
Contribution to Project: Scientific and administrative oversight
Funding Support: Eisai

Name: YVONNE A. VAN GESSEL
Project Role: Eisai
Nearest person month worked: 2
Contribution to Project: Oversight of *in vivo* safety studies
Funding Support: Eisai

Name: Vaishali Dixit
Project Role: Eisai
Nearest person month worked: 3
Contribution to Project: DMPK studies
Funding Support: Eisai

Name: Branko Mitasev
Project Role: Eisai
Nearest person month worked: 6
Contribution to Project: Route optimization and CMC studies
Funding Support: Eisai

Name:	Jiong Yang
Project Role:	Eisai
Nearest person month worked:	6
Contribution to Project:	Synthetic route development
Funding Support:	DoD

Name:	Vijay Gupte
Project Role:	Eisai
Nearest person month worked:	6
Contribution to Project:	Alliance Management
Funding Support:	Eisai

Name:	B Venkata Sasidhar
Project Role:	Eisai
Nearest person month worked:	6
Contribution to Project:	Route optimization, scale up studies, Identification & evaluation of CMO (Contract manufacturing organization), and technology transfer to CMO. Manufacturing and supply of BRD5018 for pre-clinical safety studies, physicochemical studies and salt studies etc.
Funding Support:	Eisai

Name:	Anil Khile
Project Role:	Eisai
Nearest person month worked:	3
Contribution to Project:	Synthetic process optimization
Funding Support:	Eisai

Name:	Masaharu Gotoda
Project Role:	Eisai
Nearest person month worked:	2
Contribution to Project:	Structural analysis/salt form selection
Funding Support:	Eisai

Name:	Mie Kamoto
Project Role:	Eisai
Nearest person month worked:	2
Contribution to Project:	Physico-chemical analysis/salt form selection
Funding Support:	Eisai

Name:	Dev Kant Shandilya
Project Role:	Eisai
Nearest person month worked:	6
Contribution to Project:	Drug substance analytical development
Funding Support:	Eisai

Name:	Hiroharu Kojima
Project Role:	Eisai
Nearest person month worked:	2
Contribution to Project:	Drug Product dosage form development
Funding Support:	Eisai

Name:	Jonathan French
Project Role:	Eisai
Nearest person month worked:	2
Contribution to Project:	CMC-Coordination
Funding Support:	Eisai

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

The Broad institute (non-profit) and Eisai (industrial) are the principal organizations in this project.
CiToxLAB (commercial) in Canada for in vivo safety studies (rat and dog 3 day repeat dose toxicity studies including respiratory and CNS assessment in rat and dog telemetry cardiovascular study).
Corning (commercial) in USA for DMPK studies.
XtalPi, Inc. (commercial) in USA for polymorph screening study.

Organization Name: Eisai
Location of Organization: Andover, MA; Tsukuba, Japan; Parawada, India; Kakamigahara, Japan
Partner's contribution to the project: In-kind support; Facilities; Collaboration; Personnel exchanges.

Organization Name: CiToxLAB
Location of Organization: Quebec, Canada
Partner's contribution to the project: Facilities

Organization Name: Corning
Location of Organization: Massachusetts, USA
Partner's contribution to the project: Facilities

Organization Name: XtalPi, Inc.
Location of Organization: Massachusetts, USA
Partner's contribution to the project: Facilities

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

N/A