

*e-ASIA Joint Research Program
Progress Report*

1. Project Title: Genetic and Molecular Basis of Drug Resistance and Drug Action in Vivax Malaria

2. Joint Research Period: 1 Feb, 2018 to 1 Aug, 2019

3. Principal Investigators:

- NZ: Bruce Russell, Associate Prof. University of Otago
 - Planned Funding Period: 1 Feb, 2018 to 1 Aug, 2019
- Vietnam: , Hien Tran Trinh Prof, Oxford University Clinical Research Unit
 - Planned Funding Period: In-kind
- Cambodia: Didier Menard, Prof, Institut Pasteur
 - Planned Funding Period: In-kind

4. Summary of the Progress of the Joint Research:

Plasmodium vivax infects ~20 million people each year. Most of these vivax malaria cases occurring the Asia-Pacific region where more than 2 billion people are at risk of infection. Unfortunately, resistance to the first line treatment for vivax malaria; (chloroquine) is rapidly spreading though SE Asia. Tools to reliably detect the spread of P. vivax chloroquine resistance (CQR) and determine its sensitivity to new therapeutics are urgently needed. In this proposal we seek to identify the genetic/molecular basis of P. vivax drug resistance in Vietnam and Cambodia. In doing so, we hope to provide novel markers for the rapid detection of CQR P. vivax and new therapies targeted at reversing CQR.

To this end we have collected over 160 clinical isolates of *P. vivax* from SE Asian patients failing treatment with CQ and tested their ex vivo drug sensitivity profile and correlated these with mutations found in genes coding for membrane transporter pumps, such as PvMDR1. While we have not yet discovered any conclusive molecular marker of CQR in the P. vivax isolates tested to date, we do note that many of the currently accepted markers such as SNPs in pvMDR1 do not seem to show any clear predictive value. Therefore, we have focused on other novel methods to investigate the basis of CQR.

The most important breakthrough funded by the eASIA program is the reinvigoration of the *in vitro* culture of *P. cynomolgi* model for *P. vivax* (Chua *et al* 2019 Nature Communications). Unlike *P. falciparum* we cannot continuously culture *P. vivax*, leading to considerable roadblocks in our understanding of this important parasite. *P. cynomolgi* is the sister species of *P. vivax*, both sharing many important genetic and phenotypic characteristics. Our *P. cynomolgi* culture has not only allowed us to screen a range of novel drugs which may be

useful alternatives to CQ for treating vivax malaria; but it has also allowed us to genetically manipulate orthologous transporter genes such as PcMDR1 to help confirm or disprove the role of these genes in CQR P.vivax.

Another important outcome of the eASIA grant is the work conducted by a PhD student funded by eASIA (Peter Christensen) who has developed a high sensitivity qrtPCR method for detecting both ultralow infections of P.vivax (detecting 1000s of copies mRNA per parasite rather than 4 copies of the DNA). This method will be critical for use in future attempts to conduct in vivo CQ drug efficacy trials. This important work (Reverse transcription quantitative PCR to detect low density malaria infections has been submitted to Journal of Clinical Microbiology June 2020)

We gave an update on our eASIA vivax malaria drug resistance program at 2nd Plasmodium cynomolgi meeting at Le site CEA de Fontenay-aux-Roses, 25 June 2019 (French Nuclear Agency, Paris). I was also invited (Full sponsorship) to speak at the 7th International Conference on Plasmodium vivax research June 26th to 28th 2019 at Institut Pasteur in Paris. My talk focused in on using the P. cynomolgi model for drug discovery targeting CQR vivax malaria

We held an initial training meeting in Thailand 2-5 May 2018 (for Vietnamese and Cambodian participants 8 Pax) this was followed up by a workshop in Vietnam at the *Department of Malaria, Oxford University of Clinical Research Unit, HCM City* on 28 Mar 2019. Our final meeting at the Singapore malaria network meeting 2020 was postponed due to the COVID19 pandemic.

5. Scientific Achievements and Implemented Activities (Research Exchange, Workshop, Publication, etc. if any):

**For this item, please fill in the attached Excel file.*

See attached File

6. Future Goals and Plan of Activities within and after the project period:

1. Utilize the CRISPrCAS9 molecular tool to investigate the relationship between mutations in important transporters with changes to antimalarial drug sensitivity.
2. Conduct a final meeting at the Singapore Malaria Network meeting which will be held in early 2021.

7. Recommendations and Comments to the Program (if any): NA

(ex. Any support to request from the Program in order to achieve item 6.)

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)
Chua, A.C.Y. Ong, J.J.Y., Malleret, B., Suwanarusk, R., Kosaisavee, V. Zeeman, A.-, Cooper, C.A., Tan, K.S.W., Zhang, R.d, Tan, B.H., Abas, S.N., Yip, A., Elliot, A., Joyner, C.J., Cho, J.S, Breyer, K., Baran, S. Lange, A. Maher, S.P. Nosten, F., Bodenreider, C., Yeung, B.K.S., Mazier, D., Galinski, M.R., Dereuddre-Bosquet, N., Le Grand, R., Kocken, C.H.M.f, Rénia, L. Kyle, D.E.g, Diagana, T.T.c, Snounou, G. Russell, B. Robust continuous in vitro culture of the Plasmodium cynomolgi erythrocytic stages. Nature Communications 2019 volume 10, Issue 1, 1 December 2019, Article number 3635	DOI: 10.1038/s41467-019-11332-4	published	Top Tier Publication Impact factor 11.9

1 Total

1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Christensen Peter, Bozdech Zbynek, Watthanaworawit Wanitda, Renia Laurent, Malleret Benoit, Ling Clare Nosten Francois Bruce Russell, Reverse transcription quantitative PCR to detect low density malaria infections; journal of clinical microbiology		in press		NZ

1 Total

Lists of Achievements and Implemented Activities

2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
June 27, 2019	Guest/Invited Speaker	Bruce Russell Plasmodium Cynomolgi Continuous Culture 7th International Conference on Plasmodium vivax Research (ICPvR), Paris, June 26-28, 2019
June 25, 2020	Guest/Invited Speaker	Bruce Russell ASIA vivax malaria drug resistance program at 2nd Plasmodium cynomolgi meeting at Le site CEA de Fontenay-aux-Roses, 25 June 2019 (French Nuclear Agency, Paris).
October 17, 2019	Guest/Invited Speaker	Bruce Russell Using P.cynomolgi to uncover molecular markers of P.vivax CQR 2nd Malaria Workshop: Current Tools to Combat Malaria- with a special focus on Plasmodium knowlesi Faculty of Medicine, University of Malaya, Malaysia, 14-18 October 2019 (Invitation fully sponsored by host)

3 Total

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team

0 Total

Lists of Achievements and Implemented Activities

3. Workshops, Seminars, Symposia and Other Events (Organized by the Project)

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
2-5 May 2018	Bruce Russell	Inaugural eASIA Vivax malaria drug resistance meeting	Thailand, Bangkok	8	<ol style="list-style-type: none"> 1. Discuss the objectives of the EASIA Grant 2. Specific roles of each lab as they relate to the goals of the grant 3. Update on the continuous in vitro culture P. cynomolgi model for P. vivax drug susceptibility 4. Strategies for better resolving Ex vivo/In vivo and molecular markers with regards to vivax malaria response to antimalarials 5. Discuss funding needs 6. Future directions
28-Mar-19	Bruce Russell	2nd eASIA Vivax malaria drug resistance meeting	Vietnam, HCM	10	<p>Our expected work agenda on 28 Mar 2019 as follow:</p> <ol style="list-style-type: none"> 1. 9:00 – 11:30 <ol style="list-style-type: none"> a. Meeting on EASIA project. b. Participants: Dr. Bruce, malaria group and CTU. c. Place: meeting room of Dep. of HTD Laboratory, ground floor, OUCRU 2. 11:30 – 12:30 <ol style="list-style-type: none"> a. Lunch b. Place: coffee room, 3rd floor, OUCRU 3. 13:00 – 14:00 <ol style="list-style-type: none"> a. Presentation and discussion b. Participants: # 20 persons c. Place: room 307, 3rd floor, OUCRU 4. 18:00 – 20:00: <ol style="list-style-type: none"> a. Dinner b. Participants: Dr. Bruce, malaria group and CTU. c. Place: HCM Hospital
14th to the 26th of August 2019	Bruce Russell & Peter Christensen	1st eASIA Vivax Malaria Training Workshop	Thailand, Mae Sod	4	An extensive hands on training program conducted at the Shoklo Malaria Research Unit focused on the exvivotesting of P.vivax isolates collected from parients on the Thai Myanmar Border

3 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination (Country, City, Research Organization etc)	Description of Exchange Content/Purpose	Duration of Exchange (autocompleted)
								0
								0
								0
								0

Total (Person)

Total (Person-day)

Lists of Achievements and Implemented Activities

5. Patent Applications

5.1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team

Total (Number of Application)

Total (Number of Registration)

5.2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)

Total (Number of Application)

Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team

Total