

*e-ASIA Joint Research Program
Progress Report*

1. Project Title: 「Antigenicity to humans and gene polymorphism of the new malaria vaccine candidate, TAM (Trans-amidase like molecule) of Asian malaria.」

2. Joint Research Period: Jun. 1st, 2015 ~ May 31st, 2018

3. Principal Investigators:

Japan: Kenji Hirayama, Professor, Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), Nagasaki University

Planned Funding Period: Jun. 1st, 2015 ~ Mar. 31st, 2019

Philippines: Mario Jiz, Head, Immunology Department, Research Institute for Tropical Medicine (RITM)

Planned Funding Period: Jan. 1st, 2017 ~ Dec. 31st, 2019

(from Department of Science and Technology (DOST), Philippines)

Myanmar: Tin Maung Hlaing, Hlaing, Director, Defence Services Medical Research Centre (DSMRC)

4. Summary of the Progress of the Joint Research:

1) Overview: Following a genome-wide search for a blood stage malaria DNA-based vaccine using web-based bioinformatics tools, research group of Prof. Hirayama (NEKKEN) identified *Plasmodium yoelii* GPI8p-transamidase related protein (PyTAM) as protein containing GPI-anchor motif with protective immune response.

Then, his group launched the joint research project with two research institutes, RITM (Philippines) and DSMRC (Myanmar) in malaria endemic area, for these objectives. i) Establish *ex vivo* assay system to examine antigen specific human immune response that can kill the parasite (Immunological analysis part). ii) Collect information of genetic polymorphism of *P. falciparum* (Pf) TAM, other nominal vaccine antigens, and host factors that may affect to immune response (Genetic analysis part). iii) To establish an effective collaborative network between three institutes for human vaccine development (Collaborative network part). For each objective, preparations and experiments described as below were done by the present.

2) Immunological analysis part: ELISPOT and ELISA were selected as method to observe immune response. After the selection of antigens,

recombinant Pf merozoite surface protein 1 (MSP-1), circumsporozoite protein (CSP) and TAM were prepared for *in vitro* assay. After that, experimental condition for ELISPOT and ELISA were optimized with those proteins and other controls.

3) Genetic analysis part: Next generation sequencing (NGS) was selected to be utilized to observe genetic variation of host and malaria factors. And, as the first step, target malaria and host genes for NGS were listed up. Then, primer setting and PCR condition were optimized. For this analysis, peripheral blood DNA samples of malaria patient in Philippines were provided by RITM for NEKKEN.

4) Collaborative network part: Prior to kick-off meeting at RITM, several teleconferences and Skype meetings were held to draw the framework of the collaborative research. In the kick-off meeting, researchers from all three institutes discussed for research protocol and agreement on the collaboration. After that, protocol was submitted to the ethics committee in each institute, and MOU R/D and MTA were prepared. Research group of NEKKEN visited RITM and DSMRC to see the present status of their preparation. Research group of RITM visited NEKKEN to learn technique for ELISPOT, ELISA and NGS. And for sample collection and experiment in this collaboration, NEKKEN-RITM Joint Laboratory in Palawan Island (Philippines) is under preparation.

5. Outstanding Results and Achievements (Training, Workshop, Publication, etc, if any):

5-1. Training

- 1) Training for immunological analysis (Mar. 14th ~ 17th, 2017, Nagasaki university): Research group of RITM visited NEKKEN, and learned basic technique of ELISPOT and ELISA.
- 2) Training for genetic analysis (Mar. 14th ~ 16th, 2017, Nagasaki university): Research group of RITM visited NEKKEN, and learned basic technique of genetic analysis for NGS.

5-2. Workshop

- 1) Kick-off meeting (Jan. 25th, 2016, RITM): Research group of NEKKEN visited RITM, and discussed about research plan for this e-ASIA Joint Research Program. Dr. Khine Zaw (DSMRC) also join by Skype.
- 2) Joint seminar (Mar. 2nd, 2017, DSMRC): Research group of NEKKEN visited DSMRC, and exchanged the information of research in each institute.
- 3) Joint seminar (Mar. 14th, 2017, NEKKEN): Research group of RITM visited NEKKEN, and exchanged the information of e-ASIA research progress in each institute and discuss about plan for remaining period.

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

1) Over view: After all the approval and establishment of joint laboratory in Palawan Island, fresh malaria patient blood samples will be collected. In this collection, samples from symptomatic, asymptomatic malaria patient and healthy donor will be the donor and utilized for Immunological and Genetic analysis. From each analysis, achievement indicated as below will be expected.

2) Immunological analysis part: Fresh Peripheral Blood Mononuclear Cells (PBMC) from malaria patient will be cultured with recombinant malaria antigen proteins, and immune response will be measured by ELISPOT and ELISA. Cytokine production and antibody titer will be the indicators of response. From these results and clinical information of patient, antigenicity of each antigen will be evaluated.

3) Genetic analysis part: Existing and freshly isolated DNA sample in Philippines will be utilized for NGS. As a result, polymorphism in parasite and host genes will be analyzed. In addition to these sample, DNA sample collected in Myanmar will be involved in our study. Moreover, genetic difference between asymptomatic and symptomatic malaria may possible to be clear in this study.

4) Collaborative network part: To achieve our goal, we will make our relationship tighter. Staff and student exchange will be increased for training and mutual understanding, then knowledge and skill will be shared by all three institutes. After data analysis, symposium as a summary of this joint research will be held in Nagasaki. Finally, acceleration of the collaboration even after the e-ASIA Joint Research Program period will be expected.

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

(ex. Any support to request from the Program in order to achieve item 6.)
None.