e-ASIA Joint Research Program
Final 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：January 1st, 2017 ～ March 31st, 2019

3. Research Team：

■ **Japan team**（up to 6 people including the Principal Investigator）
Funding period: June 1st, 2015 ～ March 31st, 2019
Total Funded Amount (in Local Currency): 30,423,100 JPY (Direct: 23,402,385 JPY, Indirect: 7,020,715 JPY)

	Name	Position	Affiliation	Role in the project
PI	Kenji Hirayama	Professor	Department of Immunogenetics, Institute of Tropical medicine (NEKKEN), Nagasaki university	PI
Collaborator	Shusaku Mizukami	Associate professor	Department of Immune Regulation, NEKKEN, Nagasaki university	Researcher
Collaborator	Taeko Naruse	Assistant Professor	Department of Immunogenetics, NEKKEN, Nagasaki university	Researcher
Collaborator	Mahamoud Sama Cherif	Assistant Professor	Department of Immunogenetics, NEKKEN, Nagasaki university	Researcher
Collaborator	Chisato Narahara	Assistant Professor	Department of Clinical Product Development, NEKKEN, Nagasaki university	Researcher
Collaborator	Yoshito Fujii	Associate professor	Department of Eco-Epidemiology, NEKKEN, Nagasaki university	Researcher
Total number of participants including students: 10				

■ **Philippines team**（up to 6 people including the Principal Investigator）
Funding period: Jan. 1st, 2017 ～ Dec. 31st, 2019
Total Funded Amount (in Local Currency): PHP8,780,919

	Name	Position	Affiliation	Role in the project
PI	Mario	Head	Immunology Depa	PI

	Antonio Jiz II		rtment, Research Institute for Tropical Medicine (RITM)	
Collaborator	Edelwisa Mercado	Head	Molecular Biology Laboratory, RITM	Researcher
Collaborator	Effie Espino	Head	Parasitology Department, RITM	Researcher
Collaborator	Jennifer Luchavez	Chief Science Research Specialist	Parasitology Department, RITM	Researcher
Collaborator	Eleonor Avenido	Senior Science Research Specialist	Immunology Department, RITM	Researcher
Collaborator				
Total number of participants including students: 9				

■ **Myanmar team**（up to 6 people including the Principal Investigator）
Funding period: in kind collaboration
Total Funded Amount (in Local Currency): None

	Name	Position	Affiliation	Role in the project
PI	Tin Maung Hlaing	Director	Defence Services Medical Research Centre (DSMRC)	PI
Collaborator	Khine Zaw Oo	Molecular and Genetic Research ConsultantHead	Nuclear Medical Research Department, DSMRC	Researcher
Collaborator				
Collaborator				
Collaborator				
Collaborator				
Total number of participants including students: 2				

4. Summary of the joint research

Even with rapid decrease of cases and deaths in 21st century, still malaria is global health problem, and almost the half of the global population is at the risk. The region most suffering from malaria is Africa, but Southeast Asian countries have the second largest number of infections. In addition to *Plasmodium falciparum*, which is known for most severe symptom among malaria, Asian region has *P. vivax*.

Plasmodium has its complicated life cycle as an obstacle, and it has been difficult to develop an effective vaccine so far. A variety of malaria antigens have been reported in the liver stage and blood stage targeted in human, and numerous attempts have been made to develop vaccines targeting these antigens. We have reported PyTAM (*Plasmodium yoelii* GPI8p-transaminase related protein), a GPI anchor protein, is a vaccine candidate for the blood stage in mouse malaria model. The GPI anchor protein is a group of proteins having various functions such as receptors, differentiation antigens and enzymes, and catalase activity of GPI8 is indispensable for cleavage of the signal sequence accompanying the GPI anchor. Institute of Tropical Medicine (NEKKEN), Nagasaki University (Japan), Research Institute for Tropical Medicine (RITM, Philippines) and Defence Services Medical Research Centre (DSMRC, Myanmar) started an collaboration study titled “Antigenicity to humans and gene polymorphism of the new malaria vaccine candidate, TAM (Trans-amidase like molecule) of Asian malaria” for the e-ASIA Joint Research Program.

This collaborative research tried to develop effective malaria vaccine, and the major aims of the project were as below. 1. Analyze polymorphism of malaria vaccine candidate antigen including TAM. (Genetic analysis) 2. Establish an *ex vivo* experimental system which is an indicator of residents' antigen-specific immune response. (Immunological analysis) 3. Establish a research cooperation network between the three institutes. (Collaboration network)

After we started this project, due to the several difficulties on the approval for malaria patient blood sample collection, we started a pilot study using stocked filter blood samples instead of collecting samples. To observe the genetic variation of nominal vaccine candidate antigens in a small area of Philippines, we determined genomic sequences of the genes which isolated from peripheral blood of 82 patients with symptomatic malaria in Palawan island by using next generation sequencing (NGS). Each DNA extracted from individuals was amplified by using primer sets which were optimized to 8 of vaccine candidate genes of Plasmodium falciparum, TAM, AMA-1, MSP-1, MSP-2, PF10_0355, GLURP and pfdhfr, respectively. The purified and mixed PCR products were applied to the next generation sequencer, MiSeq (Illumina). After NGS, 82 individuals showed reasonable quality for the analysis, ranged from 11,536 to 188,248 reads per gene per sample. Several tools were involved in the process which include; cut adapt for cleaning, BWA for alignment, GATK for variant calling, samtools, bcftools and picard for data manipulation among others. After trimming them using Genetyx 8.0 software and PlasmoDB, highly probable allele sequences were obtained from each sample's reads. When we estimated levels of polymorphism in each gene, in TAM, 6 alleles were assigned in the population, and 1 allele were newly classified. Likewise, 11 alleles in AMA1, 10 alleles in

MSP-1 and 13 alleles in MSP-2 were identified, so far. Interestingly, a number of samples showed multiple alleles in one individual, suggesting multiple infection . And, between NEKKEN, RITM and DSMRC, tight relationship was established in these years. Especially between NEKKEN and RITM, in addition to the relationship on papers like memorandum of Understanding (MOU) and material transfer agreement (MTA), we had much human exchange with sharing knowledge and technique for the development of this project. Besides, we opened a joint laboratory for this collaboration in Palawan island, the malaria endemic area in the Philippines. I'm sure that these achievements on this e-ASIA collaboration project enable us to continue and progress our collaboration for malaria and other infectious diseases research.
In 2019 (September to December), finally blood sample collection was done in area around Rizal (southwest part of Palawan island). Rapid diagnostic test (RDT) for 1,522 participants and blood sample collection from 178 participants (14 RDT positive and 164 RDT negative) were completed. After that PBMC from 178 samples were stimulated with recombinant proteins of malaria antigens including TAM (and controls), then IFN-gamma producing cells were detected by ELISPOT assay. Our joint laboratory was used for those experiments. Now we are under counting of spot numbers, and results will be summarized. In addition, those blood samples will be used also for our genetic analysis, and relation between immunological and genetic information will be examined.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

During this Joint Research Program (JRP) period, in total 16 persons were exchanged for 69 days, for discussion, training, and sample collection. In addition, we held three meeting and seminars, named "e-ASIA Joint Research Program Kick -Off Meeting (@RITM)", "e-ASIA Joint Research Program Memorandum of Understanding Seminar (@DSMRC)", "e-ASIA Joint Research Program Progress Meeting and Seminar (@NEKKEN)". On these events, we shared plan and progress, and discussed for further development.
Unfortunately, so far, we don't have publication and oral presentation, but after the completion of data analysis, our finding will be shared by those activity.

5-2 Synergistic effects of the international joint research

Unfortunately, because of the lack of close cooperation with DSMRC, the synergistic effect of the Joint Research Program (JRP) between the three institutions was not optimized. Even though, at the beginning of JRP period, all the participants were gathered for the kick-off meeting (DSMRC participated in Skype), and indispensable information about malaria endemic area was shared. And it told us importance of collaboration with researcher in endemic area.
In the sense of mutual benefit of JRP, MOU exchanging was a great point as a platform to know each other and for further collaboration. Between NEKKEN and RITM, not only the preparation of documents such as MOU and MTA, but also establishment of a joint laboratory, sharing experimental know-how, and implementation of joint experiment, were great deals for both of us.
In addition, in total 16 persons were exchanged for 69 days during the JRP

period, and we were able to deepen our relationship more than ever. In particular, Avenido, a young scientist from RITM visited NEKKEN three times to exchange opinions and learn experimental techniques. Also, when the researchers of NEKKEN visited RITM and the joint laboratory in Palawan, we exchanged opinions with Avenido and other young researchers and confirmed their experimental procedures. Human exchanges in the JRP contributed to the development of young researchers.

5-3 Broader impacts including contribution to society

In our original proposal, we aimed to collect malaria patient blood samples in the Philippines and Myanmar, and complete genetic and immunological analysis. It was planned to obtain data related with the correlation between gene polymorphism, expression and clinical symptoms, and how malaria vaccine candidate antigens such as TAM activate immune cells of infected individuals. In addition, by comparing the data between the Philippines and Myanmar, we thought it would be possible to examine the specific characteristics of malaria in each area. Those results would be important, and contribute to control malaria, which is still a global problem in East and Southeast Asia. Unfortunately, due to the delay of sample collection, actual data could not be obtained within the initial research period, but many were obtained, such as the research plan, the joint research laboratory, and the establishment of tight relationship of institution in Japan and malaria endemic countries. Recently, sample collection, immunological analysis by ELISPOT using patient PBMC (data analysis is still undergoing), and genetic analysis using existing samples have been completed. In near future, we will also complete data analysis of ELISPOT and genetic analysis using newly collected blood samples. When each analysis is completed, the above-mentioned initial plan will be done, and the results will contribute to the prevention and treatment of malaria.
In addition, the technology transfers of ELISPOT and next generation sequencing from NEKKEN to RITM, which were planned to be used for our analysis, has already been completed. In particular, even RITM, the leading institute in the Philippines, didn’t have next generation sequencer, and it was introduced during this JRP period. This equipment is useful not only for RITM, an institute in malaria endemic area, but also for researchers in Japan like us, who will conduct joint research in this area. In addition, the technologies and knowledge which were shared in the JRP can be applied to various research in the future, not limited to malaria. We would like to develop our collaboration by sustainable technical guidance, support and frequent exchanging.

5-4 Development and sustainability of the cooperation

Although initial period of the Joint Research Program (JRP) ended in March 2019, we would like to continue the collaboration for malaria by the three institutes, NEKKEN, RITM, and DSMRC.
First, considering collaboration with RITM, finally we were able to collect samples and carry out various originally planned analyzes in 2019FY. In many steps (e.g. approval of the original proposal and start of budget supply from DOST, equipment transfer, and approval to start sample collection), RITM had

to wait more than expected. It made delay, and result in incompletion of original schedule. In order to avoid a similar situation, we believe that more frequent contact (not teleconference, but mutual visits) will be necessary for more fruitful collaboration in the future.
Next, regarding collaboration with DSMRC, we consider their insufficient human resource, hardware and budget are major reasons which made our collaboration difficult. Malaria in Myanmar is still a big problem compared to that in the Philippines, and it is necessary to continue research. For the progress of study, international collaborators like us are indispensable for them, and they are also indispensable for us to examine samples in endemic area. Therefore, to make our collaboration robust, we will have a restructure of the framework by involving other institutions such as universities in Myanmar. In addition, in order to achieve financial stability, it is necessary to obtain budget for our collaboration from funding agency.

6. Future Goals and Plan of Activities after the project period
In the collaboration with RITM, finally we were able to collect samples in FY2019. As a result, the immunological analysis leaves only the counting and analysis. And, since the preparation of the experimental setting has been completed for the genetic analysis, the results also can be obtained in near future. In the Philippines, infectious diseases other than malaria such as dengue and schistosomiasis are still recognized. Our members from RITM have a deep knowledge of these diseases, and NEKKEN are also conducting collaboration with them. Therefore, after achieving the goal regarding malaria, by utilizing our joint laboratory in Palawan and tight relationship which ware established in this JRP, we will expand the target, and achieve the similar genetic/immunological analysis. At that time, in order to make the collaboration stable and solid, we will jointly apply for grant. I am convinced experiences gained in this e-ASIA JRP will be utilized in this further collaboration. Regarding collaboration with DSMRC, we must rebuild that by following the methods which was used with RITM.

7. Scientific Achievements and Implemented Activities (Publication, Research Exchange, Workshop, etc.)
For this item, please fill in the attached Excel file.

8. Recommendations and Comments to the Program
One of the problems in our research group was the time lag of member countries initiation of the research. Maybe it would be better to adjust the research period to coordinate the collaboration.

9. Others (agenda of workshop, photos of research teams, meetings, and etc.)

Philippines team
Mario Jiz, Edelwisa Mercado

Myanmar team
Tin Maung Hlaing, Saw, Khine Zaw Oo

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.)

0 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

0 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.

0 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
January 25, 2016	Kenji Hirayama	e-ASIA Joint Research Program Kick -Off Meeting	Philippines, Manila, RITM	7	At the start of this joint research program, the kick-off meeting was held.
March 2, 2017	Tin Maung Hlaing	e-ASIA Joint Research Program Memorandum of Understanding Seminar	Myanmar, Naypyidaw, DSMRC	20	Members from Nagasaki university and DSMRC shared the information of their recent study, and discussed for further collaboration.
March 14, 2017	Kenji Hirayama	e-ASIA Joint Research Program Progress Meeting and Seminar	Japan, Nagasaki, Nagasaki University	8	Members from Nagasaki university and RITM shared their progress and discussed the plan of this joint research.

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)
January 24, 2016	January 26, 2016	Kenji Hirayama	Japan	Nagasaki University	Professor	Philippines, Manila, RITM	e-ASIA Joint Research Program Kick -Off Meeting	3
January 24, 2016	January 26, 2016	Shusaku Mizukami	Japan	Nagasaki University	Assistant Professor	Philippines, Manila, RITM	e-ASIA Joint Research Program Kick -Off Meeting	3
January 24, 2016	January 26, 2016	Mahamoud Sama Cherif	Japan	Nagasaki University	Assistant Professor	Philippines, Manila, RITM	e-ASIA Joint Research Program Kick -Off Meeting	3
March 1, 2017	March 4, 2017	Kenji Hirayama	Japan	Nagasaki University	Professor	Myanmar, Naypyidaw, DSMRC	e-ASIA Joint Research Program Memorandum of Understanding Seminar	4
March 1, 2017	March 4, 2017	Shusaku Mizukami	Japan	Nagasaki University	Assistant Professor	Myanmar, Naypyidaw, DSMRC	e-ASIA Joint Research Program Memorandum of Understanding Seminar	4
March 6, 2017	March 10, 2017	Shusaku Mizukami	Japan	Nagasaki University	Assistant Professor	Philippines, Puerto Princesa, PHO	Sample collection site and laboratory visit	5
March 13, 2017	March 17, 2017	Mario Jiz II	Philippines	RITM	Head	Japan, Nagasaki, Nagasaki University	e-ASIA Joint Research Program Progress Meeting and Seminar	5
March 13, 2017	March 17, 2017	Edelwisa Mercado	Philippines	RITM	Head	Japan, Nagasaki, Nagasaki University	e-ASIA Joint Research Program Progress Meeting and Seminar	5
March 13, 2017	March 17, 2017	Jennifer Luchavez	Philippines	RITM	Chief Science Research Specialist	Japan, Nagasaki, Nagasaki University	e-ASIA Joint Research Program Progress Meeting and Seminar	5
March 13, 2017	March 17, 2017	Eleonor Avenido	Philippines	RITM	Senior Science Research Specialist	Japan, Nagasaki, Nagasaki University	e-ASIA Joint Research Program Progress Meeting and Seminar	5
June 18, 2018	June 19, 2018	Eleonor Avenido	Philippines	RITM	Senior Science Research Specialist	Japan, Nagasaki, Nagasaki University	Meeting for the project, and learning experimental procedure	2
July 7, 2018	July 12, 2018	Shusaku Mizukami	Japan	Nagasaki University	Assistant Professor	Philippines, Puerto Princesa, PHO	Sample collection site and laboratory visit	6
October 2, 2019	October 5, 2019	Shusaku Mizukami	Japan	Nagasaki University	Associate Professor	Philippines, Manila, RITM	Discussion for sample collection and analysis	4
October 8, 2019	October 12, 2019	Kenji Hirayama	Japan	Nagasaki University	Professor	Philippines, Puerto Princesa, PHO,	Visit sample collection site, and discussion	5
October 8, 2019	October 12, 2019	Shusaku Mizukami	Japan	Nagasaki University	Associate Professor	Philippines, Rizal,	Visit sample collection site, and discussion	5
November 16, 2019	November 20, 2019	Eleonor Avenido	Philippines	RITM	Senior Science Research Specialist	Japan, Nagasaki, Nagasaki University	Discussion for sample collection and analysis	5
					Total (Person)	16	Total (Persond-day)	69

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

0 Total (Number of Application)

0 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)

0 Total (Number of Application)

0 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

0 Total