

No.	K22-3063	
研究課題名	Human Systems Immunology of vaccine and immunotherapy	
研究代表者	Katsikis Peter ( Erasmus University Medical Center ・教授 )	
研究組織	受入教員	石井 健 ( 東京大学医科学研究所 ・教授 )
	分担者	Ken Ishii ( (2) Division of Vaccine Science, Department of Microbiology and Immunology, International Vaccine Design Center (vDesC) ・Professor )

**IMSUT International Joint Usage/Research Center Project <International>****Joint Research Report (Annual/Project Completion)**

<b>Annual Report</b>			
<b>Report</b>			
<b>Project Members</b>			
Name	Institution/ Department	Title	Role
Peter Katsikis		Professor	Leading the project
Melisa D. Castro Eiro	Erasmus MC, University Medical Center/ Dep. of Immunology	Postdoc fellow	Conducting the exp
Kou Hioki		Postdoc fellow	Conducting the exp
Quentin Sattentau		Oxford University/ SWD school of pathology	Professor
Ken Ishii	U Tokyo/ IMS, Dep. Infection and Immunology	Professor	Co-leading the proj.
Kouji Kobiyama		Assoc. Prof.	Conducting the exp
Hideo Negishi		Senior Assist.Prof.	Conducting the exp
Burcu Temizoz		Assist. Prof.	Conducting the exp
Tomoya Hayashi		Postdoc. Fellow	Conducting the exp
Etsushi Kuroda	Hyogo College of Medicine, Dep. Immunology	Professor	Co-leading the proj.

**Research Progress Report**

Current collaborative research focusing on the systems immunology for developing better vaccines and immunotherapies led to various findings, especially about the modes of action of novel vaccine adjuvant combinations as not only vaccine adjuvants but also as cancer immunotherapeutics. In the Peter Katsikis's lab, by using our original adjuvant K3-SPG along with the additional adjuvants sent from Ken Ishii lab, they found that some adjuvants show robust anti-tumor effect, especially when used in combination. They are further conducting these cancer immunotherapy studies in mice to find out the mechanisms of action of those adjuvants. Moreover, based on our discussions about their and our scientific findings about the anti-tumor effect of those adjuvants, we are exploring ways to make reagents GMP quality and perform toxicity testing in order to be able to proceed to clinical trials.

In the Ken Ishii's Lab, studies by Temizoz et al. showed that TLR9 and STING agonists can synergistically induce anti-tumor immunity in mice via the mechanisms regulated at the transcriptional levels. Our further clinical studies by using the TLR9 agonist CpG ODN in the lung cancer patients revealed that CpG ODN can trigger Th1-type immune response and enhance cytotoxic activity in advanced lung cancer patients. Moreover, another phase 1 clinical trial showed that hydroxypropyl- $\beta$ -cyclodextrin shows adjuvant activity by enhancing the immunogenicity of the quadrivalent seasonal influenza vaccine without significant side effects.

Nevertheless, this collaboration led to the finding that potent T cell immunity against tumor neoantigens could be elicited by adjuvant combinations. Studies using neoantigen vaccination SQ have shown potent T cell immunity and protection against subcutaneous melanoma tumors. Initial experiments have suggested that intra-

tumor vaccination augments any protective effect of SQ vaccination but also shows promise when intra-tumor vaccination is used alone. Such intra-tumor vaccinations result in large tumor necrosis and delay in tumor growth. Continuation of these studies will examine the extent of protection in 3 different tumor models (melanoma, mesothelioma and pancreatic cancer).

The manuscript below describing the adjuvant combination used together with neoantigen synthetic long peptides has been submitted:

**Melisa D. Castro Eiro, Kou Hioki**, Ling Li, Merel Wilmsen, Caoimhe H. Kiernan, IngeBrouwers–Haspels, Marjan van Meurs, Manzhi Zhao, Harm de Wit, Dwin G. B. Grashof, Harmen J. G. van de Werken, Yvonne M. Mueller, Christopher Schliehe, **Burcu Temizoz, Kouji Kobiyama, Ken J. Ishii, Peter D. Katsikis**: “TLR9 plus STING agonist adjuvant combination induces potent neopeptide T cell immunity and improves immune checkpoint blockade efficacy in tumor models” (In Revision).

**Peter D. Katsikis, Ken J. Ishii**, Christopher Schliehe: “Challenges in developing personalized neoantigen cancer vaccines” (Submitted)

By using a machine learning-assisted screening on the herbal medicine extracts that could potentially have adjuvant activity, Hioki et al. found novel parameters, such as human G-CSF and mouse RANTES, that could be used as adjuvanticity biomarkers for adjuvant screening by using the data obtained from in vitro human studies and in vitro and in vivo mice studies. Moreover, Yoshioka et al.’s studies in Ken Ishii’s lab demonstrated that the novel adjuvant A-910823 is capable of robustly inducing Tfh cell and humoral immune responses, even when used as a booster dose. And the adjuvant activity of A-910823 was dependent on  $\alpha$ -tocopherol component of this novel adjuvant.

Therefore, we believe that our studies may lead to identification of novel adjuvants or biomarkers that could be used to measure adjuvanticity, as well as mode of action of adjuvants both in human and mice, which could subsequently aid in development of better vaccines or immunotherapies for human.

#### Published Papers

Yoshioka Y, **Kobiyama K**, Hayashi T, Onishi M, Yanagida Y, Nakagawa T, Hashimoto M, Nishinaka A, Hirose J, Asaoka Y, Tajiri M, Hayata A, Ishida S, Omoto S, Nagira M, **Ishii KJ**. A-910823, a squalene-based emulsion adjuvant, induces T follicular helper cells and humoral immune responses via  $\alpha$ -tocopherol component. *Front Immunol.* 2023 Feb 20;14:1116238. doi: 10.3389/fimmu.2023.1116238. PMID: 36891311; PMCID: PMC9986537.

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## Patent Applications

Collaborative effort has resulted in a patent application that was filed 28th of April 2022 (European patent application No. 22386021.4 Erasmus University Medical Center Rotterdam and The University of Tokyo; Inventors: Peter D. Katsikis and Ken Ishii).

<b>On-site Meetings</b>			
<b>Name</b>	<b>Position, Institution</b>	<b>Total Days of Visits to IMSUT</b>	<b>Date of Visit</b>
Peter Katsikis	Professor, Erasmus University	3	February 20 <sup>th</sup> 2023 February 21 <sup>st</sup> 2023 February 24 <sup>th</sup> 2023

On February 20<sup>th</sup> 2023, Professor Peter Katsikis visited IMSUT for scientific discussions between Professor Ken Ishii.

On February 21<sup>st</sup> 2023, Professor Peter Katsikis visited IMSUT for scientific discussions between Professor Ken Ishii, Dr. Kouji Kobiyama, Dr. Hideo Negishi, Dr. Burcu Temizoz and Dr. Tomoya Hayashi during a lunch meeting.

International Joint Usage/Research Center Seminar titled "Overcoming the obstacles for developing effective tumor neoantigen vaccines" was given by Professor Peter Katsikis on February 21<sup>st</sup> 2023 in IMSUT, Japan.

On February 24<sup>th</sup> 2023, Professor Peter Katsikis visited IMSUT for scientific discussions between Professor Ken Ishii and Dr. Burcu Temizoz.

### **Online Meetings**

Throughout 2022 monthly 1½ hour joint online meeting were held between the Katsikis and Ishii laboratories. Postdoctoral fellows and students from both laboratories present their scientific research and share their latest findings while receive input and suggestions from both teams.

<b>Name</b>	<b>Position, Institution</b>	<b>Date of the Online Meetings</b>
Peter Katsikis	Professor, Erasmus University	February 15 <sup>th</sup> 2023
Ken Ishii	Professor, IMSUT	February 15 <sup>th</sup> 2023
Kouji Kobiyama	Assoc. Prof., IMSUT	February 15 <sup>th</sup> 2023
Burcu Temizoz	Assist. Prof., IMSUT	February 15 <sup>th</sup> 2023
Tomoya Hayashi	Postdoc, IMSUT	February 15 <sup>th</sup> 2023
Kou Hioki	Postdoc, Erasmus University	February 15 <sup>th</sup> 2023
Yvonne Mueller	Assist. Prof., Erasmus University	February 15 <sup>th</sup> 2023
Peter Katsikis	Professor, Erasmus University	December 5 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	December 5 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	December 5 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	December 5 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	December 5 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	December 5 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	December 5 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	November 7 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	November 7 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	November 7 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	November 7 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	November 7 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	November 7 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	November 7 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	October 7 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	October 7 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	October 7 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	October 7 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	October 7 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	October 7 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	October 7 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	September 5 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	September 5 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	September 5 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	September 5 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	September 5 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	September 5 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	September 5 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	August 10 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	August 10 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	August 10 <sup>th</sup> 2022

Burcu Temizoz	Assist. Prof., IMSUT	August 10 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	August 10 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	August 10 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	August 10 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	July 13 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	July 13 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	July 13 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	July 13 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	July 13 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	July 13 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	July 13 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	June 1 <sup>st</sup> 2022
Ken Ishii	Professor, IMSUT	June 1 <sup>st</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	June 1 <sup>st</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	June 1 <sup>st</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	June 1 <sup>st</sup> 2022
Kou Hioki	Postdoc, Erasmus University	June 1 <sup>st</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	June 1 <sup>st</sup> 2022
Peter Katsikis	Professor, Erasmus University	May 10 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	May 10 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	May 10 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	May 10 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	May 10 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	May 10 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	May 10 <sup>th</sup> 2022
Peter Katsikis	Professor, Erasmus University	April 6 <sup>th</sup> 2022
Ken Ishii	Professor, IMSUT	April 6 <sup>th</sup> 2022
Kouji Kobiyama	Assoc. Prof., IMSUT	April 6 <sup>th</sup> 2022
Burcu Temizoz	Assist. Prof., IMSUT	April 6 <sup>th</sup> 2022
Tomoya Hayashi	Postdoc, IMSUT	April 6 <sup>th</sup> 2022
Kou Hioki	Postdoc, Erasmus University	April 6 <sup>th</sup> 2022
Yvonne Mueller	Assist. Prof., Erasmus University	April 6 <sup>th</sup> 2022