No.	K25-3190	
Project Title	Employing molecular and systems approaches for human vaccine and immunotherapy development against cancer and infectious diseases	
Principal Investigator	Peter Katsikis (Erasmus University Medical Center・Professor)	
Project Member(s)	IMSUT Host Researcher	Ken J ISHII (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Peter Katsikis (Erasmus MC, University Medical Center/Immunology・Professor)
	Project Member (s)	Yvonne Müller (Erasmus MC, University Medical Center/Immunology・Assistant Professor)
	Project Member (s)	Christopher Schliehe (Erasmus MC, University Medical Center/Immunology・Assistant Professor)
	Project Member (s)	Kou Hioki (Erasmus MC, University Medical Center/Immunology・Postdoc fellow)
	Project Member (s)	Quentin Sattentau (Oxford University/SWD school of pathology・Professor)
	Project Member (s)	Kouji Kobiyama (Division of Vaccine Science, Department of Microbiology and Immunology, International Vaccine Design Center (vDesC)・Associate Professor)
	Project Member (s)	Burcu Temizoz (Division of Vaccine Science, Department of Microbiology and Immunology, International Vaccine Design Center (vDesC)・Assistant Professor)
	Project Member (s)	Tomoya Hayashi (Division of Vaccine Science, Department of Microbiology and Immunology, International Vaccine Design Center (vDesC)・Assistant Professor)
	Project Member (s)	Hideo Negishi (Institute for Quantitative Biosciences・Lecturer)
	Project Member (s)	Etsushi Kuroda (Hyogo College of Medicine/Immunology・Professor)
	Project Member (s)	Menno van Zelm (Erasmus MC, University Medical Center/Immunology・Professor)

(Form 2)

Principal Investigator
Peter Katsikis / Ken Ishii

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

Name	Institution / Department	Position	Role
Peter Katsikis	Erasmus MC, University Medical Center/Immunology	Professor	Leading the project
Yvonne Müller		Assist. Prof.	Co-leading the project
Christopher Schliehe		Assist. Prof.	Co-leading the project
Kou Hioki		Postdoc. Fellow	Conducting the experiments
Quentin Sattentau	Oxford University/SWD school of pathology	Professor	Co-leading the project
Ken Ishii	Division of Vaccine Science, Department of Microbiology and Immunology, International Vaccine Design Center (vDesC)	Professor	Leading the project
Kouji Kobiyama		Assoc. Prof.	Conducting the experiments
Burcu Temizoz		Assist. Prof.	Conducting the experiments
Tomoya Hayashi		Assist. Prof.	Conducting the experiments
Hideo Negishi	Institute for Quantitative Biosciences	Lecturer	Conducting the experiments
Etsushi Kuroda	Hyogo College of Medicine/Immunology	Professor	Co-leading the project
Menno van Zelm	Erasmus MC, University Medical Center/Immunology	Professor	Analysis of formation, persistence and reversibility of human memory B cells

Research Progress Report

During this reporting period, our collaborative research between the University of Tokyo (IMSUT) and Erasmus MC has further advanced the understanding of innate immune sensing mechanisms and their implications in autoinflammation and immunotherapy, with a particular focus on STING signaling and cyclic dinucleotides (CDNs), and further advanced the study about our neopeptide-based cancer vaccine strategy (Prime-Target (P/T) vaccination), which is a combination of subcutaneous (SQ) and intra-tumor (IT) neopeptide vaccinations.

A major outcome of this collaboration is our recent study published in the *Journal of Autoimmunity* (Shibahara and Temizoz et al., 2025), which demonstrated that microbial dysbiosis contributes to STING-driven autoinflammation through microbiota-derived CDNs (Figure 1). Using SAVI (STING N153S) mouse models and patient samples, we showed that gut microbiota enriched in segmented filamentous bacteria (SFB) leads to increased production of CDNs, which in turn drive systemic inflammation via aberrant STING activation. Importantly, antibiotic-mediated modulation of the microbiota ameliorated disease phenotypes, highlighting the causal role of microbiota-derived CDNs in disease pathogenesis. These findings establish a mechanistic link between dysbiosis and STING-associated autoinflammatory diseases and suggest potential therapeutic strategies targeting either the microbiota or STING signaling.

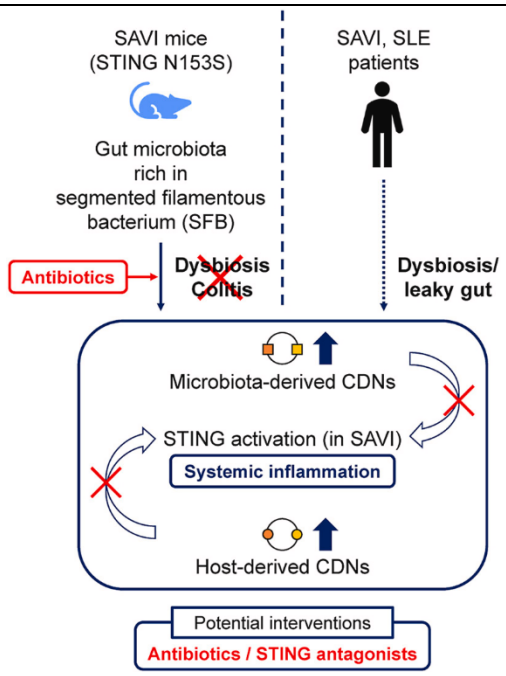

Figure 1: Schematic representation of how microbial dysbiosis promotes STING-dependent systemic inflammation via microbiota-derived cyclic dinucleotides in SAVI models and patients.

In addition, we recently published a study in *Nature Communications* (Negishi et al., 2026), which uncovered a novel mechanism of innate immune activation through nucleocytosis. In this work, we demonstrated that during cell death, nuclear DNA is actively extracted and transferred to neighboring cells via macrophage-mediated processes, leading to activation of the cGAS–STING pathway and induction of type I interferon responses. Mechanistically, antiviral cationic amphiphilic drugs (CADs) were shown to induce lysosomal dysfunction, nuclear calreticulin exposure, and subsequent nucleocytosis, thereby amplifying innate immune signaling (Figure 2). This study provides new insights into how endogenous DNA can be mobilized and sensed by the immune system, offering a previously unrecognized pathway linking cell death to inflammation.

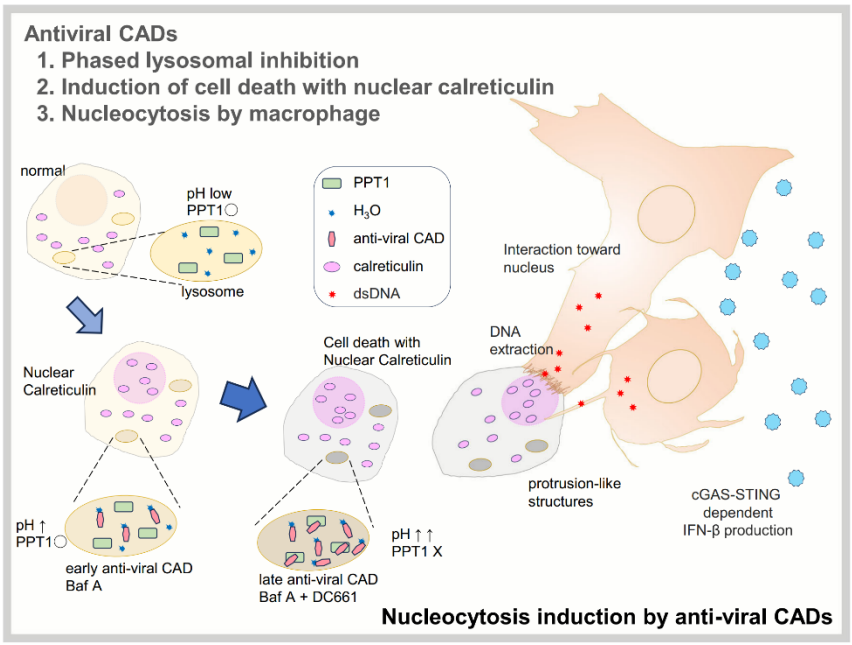

Figure 2: Mechanism of nucleocytosis: nuclear DNA released from dying cells is transferred to neighboring cells, activating the cGAS-STING pathway and inducing type I interferon responses.

Together, these studies highlight complementary mechanisms by which both microbiota-derived and host-derived nucleic acids activate the cGAS-STING pathway, contributing to systemic inflammation and immune regulation. Our collaborative efforts continue to bridge mechanistic insights from murine models to human disease, with the goal of identifying novel therapeutic targets and optimizing vaccine and immunotherapy strategies.

In addition to mechanistic studies, our collaboration has contributed to the development of a systematic platform for adjuvant evaluation, as demonstrated in our recent publication in *Cell Chemical Biology* (Natsume-Kitatani et al., 2025). In this study, we established a comprehensive Adjuvant Database (ADB), integrating transcriptomic profiles of 25 representative adjuvants across multiple species, organs, doses, and time points using standardized experimental protocols. By aligning this dataset with the widely used toxicogenomics resource Open TG-GATEs, we enabled cross-platform analyses for simultaneous prediction of both adjuvanticity and toxicity. Using machine learning approaches, we demonstrated that gene expression signatures can robustly distinguish adjuvants and predict their biological effects independent of species or tissue context. Importantly, this framework allowed the identification of novel properties, such as previously unrecognized adjuvant activity of colchicine and hepatotoxicity of FK565, which were subsequently validated experimentally (Figure 3). This work provides a scalable, data-driven strategy for the rational design and safety assessment of vaccine adjuvants and immunotherapeutics, complementing our mechanistic studies on innate immune activation.

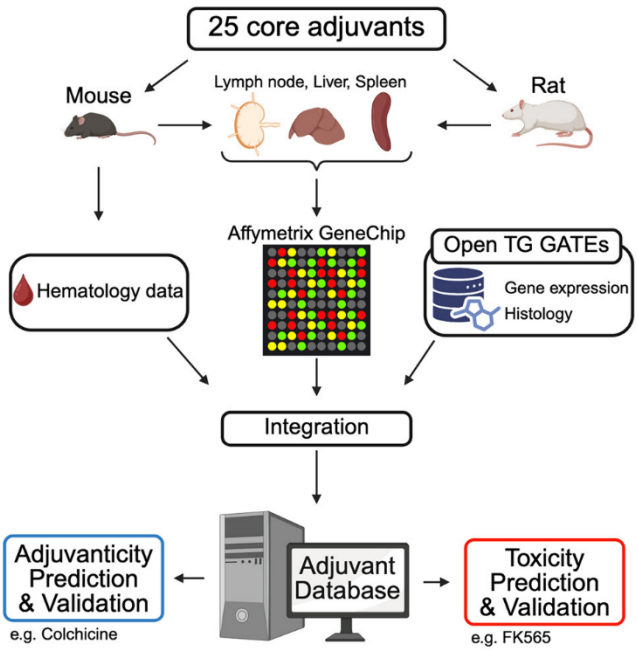

Figure 3: Overview of the Adjuvant Database (ADB), integrating multi-species transcriptomic data to predict adjuvant efficacy and toxicity using machine learning approaches.

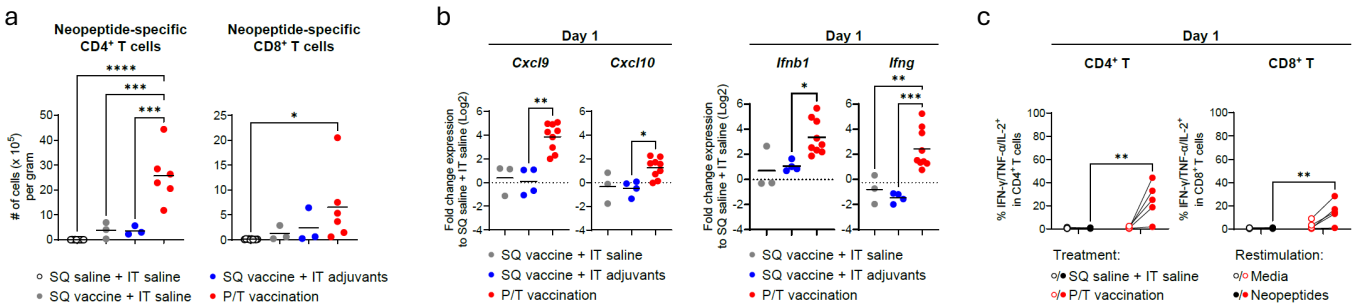

Figure 4: P/T vaccination induces rapid T cell recruitment and TME remodeling. (a) Neopeptide-specific T cells in tumors at day 7 post-IT vaccination analyzed by flow cytometry. (b) Expression of Cxcl9, Cxcl10, Ifnb1, and Ifng in tumors at day 1 post-IT, analyzed by qPCR. (c) Neopeptide-specific T cells in tumors at day 1 post-IT vaccination, analyzed by flow cytometry.

The other major outcome of this collaboration is a study investigating the novel P/T vaccination strategy, which is currently available as a preprint (Hioki et al. bioRxiv (2026)). In this study, we demonstrated that SQ vaccination alone is insufficient to recruit vaccine-induced T cells into tumors. In contrast, P/T vaccination effectively drives the recruitment of SQ vaccine-induced T cells into the tumor microenvironment (TME), with a peak observed at 7 days after a single IT vaccination (Figure 4a). These findings indicate that the P/T vaccination strategy overcomes the T cell exclusion phenotype characteristic of immunosuppressive solid tumors. We further investigated early changes in the TME following P/T vaccination by analyzing tumors at day 1 post-IT vaccination. At this early time point, P/T-vaccinated tumors showed significant upregulation of T cell–recruiting chemokines (Cxcl9 and Cxcl10) and interferons (Ifnb1 and Ifng), which are key features of a T cell–inflamed TME (Figure 4b). Consistent with these molecular changes, SQ vaccine-induced T cells were already detectable within tumors at day 1 (Figure 4c). Together, these results demonstrate that P/T vaccination rapidly remodels the TME toward a T cell–inflamed state within one day of a single IT dose in SQ-primed animals. A full manuscript describing this work is currently in preparation.

Published Papers

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On-site meeting

Name	Position, Institution	Days of Visits to Erasmus University	Date of Visit
Ken Ishii	Prof., U Tokyo/IMS	1	6th February, 2026

On 6th February, Prof. Ken Ishii visited Erasmus University Medical Center to give a talk at 2nd Joint Research Conference of IMSUT - Dept. of Immunology, Erasmus MC. Prof. Peter Katsikis, Dr. Kou Hioki, and Dr. Yvonne Müller also gave talks. Dr. Hideo Negishi, Dr. Burcu Temizoz, and Dr. Tommoya Hayashi joined the conference online and gave talks.

<Titles of the talks>
"Off-target of vaccine adjuvant towards neo-immunotherapeutics for cancer, allergy and senescence" by Prof. Ken Ishii

"Multiomic approach to classify and understand Still disease" by Prof. Peter Katsikis

"Functional characterization of extracellular particles during viral infection by high-resolution single-particle mapping and sorting" by Dr. Tomoya Hayashi

"cGAS-IFN-I responses by extracting nuclear DNA from dying cells via nucleocytosis" by Dr. Hideo Negishi

"Prime-Target neoantigen vaccination drives robust intratumoral T cell immunity in immunologically "cold" tumors" by Dr. Kou Hioki

"Memory Inflation in COVID-19" by Dr. Yvonne Müller

Online meetings

Throughout FY2025, bi-monthily 1 hour joint online meetings were held between the Katsikis and Ishii labs. Researchers and students from both labs presented their scientific research and shared their latest findings while receiving input and suggestions from both teams.