IMSUT International Joint Usage/Research Center
International Project-completion Report (FY2022 ver.)

Date of submission: **4** / **28** / **2023**

Principal Investigator	Position, Institution: Vice-Chair, Professor, Department of Surgery, University of Minnesota Name: Masato Yamamoto, M.D., Ph.D.
IMSUT Host Researcher	Division: Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT Name: Takashi Okada, M.D., Ph.D.
Project Title	Development of a CD133-targeted adenoviral vector system
Duration	From 4/1/2022 to 3/31/2023

Project Members

Name	Position, Institution
Masato Yamamoto, M.D., Ph.D.	Vice-Chair, Professor, Department of Surgery, University of Minnesota
Takashi Okada, M.D., Ph.D.	Professor, Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT
Naoya Uchida, M.D., Ph.D.	Leader, Gene Therapy Group, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute, National Institutes of Health
Mikako Wada, Ph.D.	Post-doc fellow, Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT

Project-completion Report on achievements/progress through the entire project period

Hematopoietic stem cell (HSC)-targeted gene therapy is curative for various genetic diseases, including primary immunodeficiencies, hemoglobin disorders, and metabolic diseases. In the current *ex vivo* HSC gene therapy, patient CD34+ HSCs are collected and cultured for lentiviral gene addition, followed by autologous transplantation. However, a cell processing center is required for *ex vivo* HSC culture at the GMP level, and the complexity and cost limit the access of HSC gene therapy. To overcome these issues, proof-of-principle experiments of *in vivo* HSC gene therapy recently demonstrated transposon-mediated integration followed by serial drug selections after systemic injection of adenoviral vectors. However, these adenoviral vectors cannot specifically deliver a therapeutic gene to HSCs, therefore resulting in lower accumulation in the target organ and distribution to unwanted organs. In this project, we sought to develop an HSC-targeted adenoviral vector system, allowing for a systemic injection for *in vivo* HSC gene therapy.

Our group had previously developed a human CD133-targeted oncolytic adenovirus, demonstrating a specific infection to CD133+ cells *in vitro* and anti-tumor effects of CD133+ cancer cells in immunodeficient mice (Sato-Dahlman M, et al.. Oncotarget. 2017). CD133 expresses in various progenitor cells including CD34+ HSCs, and thus, we hypothesized that the CD133-targeted adenovirus allows a specific delivery of a therapeutic gene (or genome editing tools) into CD34+ cells. In this study, we deleted an essential component of self-competent adenovirus (adenoviral E1 gene) and generate an E1-dependent adenoviral vector system targeting CD133. We detected targeted delivery in a CD133-positive cell line by using an enhanced green fluorescent gene (*EGFP*).

This CD133-targeted adenoviral vector system can allow for the development of *in vivo* delivery of a therapeutic gene to CD34+ HSCs. This HSC-targeted *in vivo* gene delivery system would expand HSC gene therapy application to various hospitals due to no requirement for *ex vivo* HSC culture.

Research Results from the Project during FY2022

<Publications>

None

<Patent Applications>

None

Days of visits to IMSUT during FY2022

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
Masato Yamamoto	Vice-Chair, Professor, Department of Surgery, University of Minnesota	Male	**40 or older**	Presentation for gene therapy research, including this project (1 day)
Naoya Uchida	Leader, Gene Therapy Group, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute, National Institutes of Health	Male	**40 or older**	Discuss various research projects in gene therapy (1 day)
Name	**Position, Institution**	**Sex**	**Age**	**Online Meetings (Days)**
N/A				

Name	Position, Institution	Sex	Age	Discussions via E-mail, Slack, etc. (Days)
N/A				

Usage of Facilities/Equipment during FY2022			
Name of Facility	**Equipment**	**Number of Use (Times)**	**Usage time (Hours)**
FACS Core Laboratory	e.g.) FACS Aria (BD)	0	0
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	0	0
Imaging Core Laboratory	e.g.) Zeiss Multiphoton Microscopy（LSM710NLO)	0	0
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	0	0
Human Genome Center	Supercomputer	0	0
Amami Laboratory of Injurious Animals	Experimental lab	0	0
Other		0	0
Usage of Scientific Resources			
Name of Scientific Resource			**Number of Samples/Lines**
Serum (BioBank Japan)			0
DNA (BioBank Japan)			0
Knockout mouse			0
Pathogenic bacteria			0
Other			0
Usage of Database			
Name of Database			**Number of Use (Times)**
N/A			0