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

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

Principal Investigator	Position, Institution: Chief, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute, National Institutes of Health
	Name: John F. Tisdale, M.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 small-size β-globin vector for efficient vector production and gene marking
Duration	From 4/1/2022 to 3/31/2023
Project Members	
Name	**Position, Institution**
John F. Tisdale, M.D.	Chief, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute, National Institutes of Health
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
Toru Uchiyama, M.D., Ph.D.	Chief, Division of Molecular Pathogenesis, Department of Human Genetics, National Center for Child Health and Development
Takashi Okada, M.D., Ph.D.	Professor, Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT
Project-completion Report on achievements/progress through the entire project period	

The efficacy and safety of hematopoietic stem cell (HSC)-targeted gene therapy have been proven in various genetic diseases, including sickle cell disease (SCD); however, therapeutic effects vary among patients due to the variances of transduction efficiency in HSCs, and the current cost of gene therapy is too high. Recently, leukemia development was reported in a gene therapy trial for SCD (Tisdale JF, et al.. N Engl J Med. 2022), likely due to a dominant selection of pre-existing leukemic clones. Therefore, maintenance of polyclonal hematopoiesis should improve the safety of HSC gene therapy, and the further development of globin-expressing vectors remains crucial for high-level polyclonal gene marking. We have developed a forward-oriented globin-expressing vector, enabling efficient vector production and high-level gene marking in HSCs (Uchida N, Tisdale JF, et al.. Nat Commun. 2019). In this project, we will further optimize the forward-oriented globin vector by decreasing the vector backbone size without the reduction of lentiviral titers. It should allow for the inclusion of an additional sequence to enhance therapeutic effects. Additionally, the size reduction might allow for more efficient vector production as well as higher-level transduction in HSCs.

In a preliminary study using enhanced green fluorescent protein (*EGFP*) under the control of a murine stem cell virus promoter, we simply deleted backbone sequences of the lentiviral vector except for the known essential sequences. However, lentiviral titers were gradually decreased by more deletion of the vector backbone. It suggests that unknown essential fragments are included in the deleted sequences. Therefore, we more precisely designed several versions of short lentiviral vectors to include potentially essential sequences. We obtained similar vector titers in the shorter vectors (up to ~25% smaller), compared with the original control. This compact lentiviral backbone allowed for a ~10% smaller size of the forward-oriented β-globin vector without the reduction of lentiviral titers.

In summary, we have developed a compact lentiviral vector system, without the reduction of lentiviral titers. It could allow for the inclusion of additional sequences to enhance therapeutic effects in target cells. These results should improve the efficiency of lentiviral vectors, allowing for more widely-applicable HSC gene therapy.

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)
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, including this project (1 day)
Name	**Position, Institution**	**Sex**	**Age**	**Online Meetings (Days)**
John F. Tisdale	Chief, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute,	Male	**40 or older**	The one-to-one meeting for 1 hour (~20 times)

	National Institutes of Health			
Name	**Position, Institution**	**Sex**	**Age**	**Discussions via E-mail, Slack, etc. (Days)**
John F. Tisdale	Chief, Cellular and Molecular Therapeutics Branch, National Heart, Lung, and Blood Institute, National Institutes of Health	Male	**40 or older**	Discussion via email constantly (~25 times)

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