ID No.	K1012
Project Title	Development of a small-size lentiviral vector for efficient vector production and gene marking
Principal Investigator	John F. Tisdale (Chief., NIH)
Project Members IMSUT Host Researcher Members	Naoya Uchida (Associate Prof., IMSUT)

Report

The current hematopoietic stem cell (HSC)-targeted gene therapy results in phenotypic correction in patients with β-hemoglobinopathies including SCD and β-thalassemia, as well as various genetic diseases in hematopoietic diseases and metabolic disorders; however, therapeutic effects vary due to individual variations of lentiviral gene marking, and the cost is too high. Recently, leukemia development (n=2) was reported in our gene therapy trial (Kanter J, Tisdale JF. N Engl J Med. 2022), likely due to a predominant selection of pre-existing leukemic clones, suggesting that maintenance of polyclonal hematopoiesis is important for the safety of HSC gene therapy (Yeri A. N Engl J Med. 2022). Therefore, development of a new globin-expressing lentiviral vector is crucial for efficient vector production, high-level polyclonal gene marking in HSCs, and robust globin expression in erythroid cells. We have recently developed a forward-oriented globin-expressing vector, resulting in efficient vector production and high-level gene marking in HSCs (Uchida N, Tisdale JF. Nat Commun. 2019). In this project, we will further optimize our forward-oriented globin vector for both more efficient vector production as well as higher-level transduction in CD34+ HSCs, along with robust globin expression in erythroid cells. It would allow for lower costs of vector production (due to more efficient vector production) as well as independence of the individual variance of polyclonal gene marking (due to higher-level gene marking), allowing for widely applicable gene therapy.

We performed initial experiments for decreasing sizes of lentiviral vectors by using enhanced green fluorescent protein (GFP) under the control of a general promoter (murine stem cell virus promoter). We demonstrated that lentiviral titers were gradually decreased by partial deletion of each essential fragment, including long terminal repeats, packaging signal, Rev response element, and central polypurine tract. These data suggest that some essential DNA sequence for transduction was removed by deletion of lentiviral vector backbones. We then designed shorter versions (2.6-2.9kb) of lentiviral vectors to include

potentially essential sequences and evaluated vector titers as compared with an original control (3.6kb). We observed similar vector titers in all shorter vectors (not significant in one-way ANOVA), compared with the original control, demonstrating ~25% reduction of vector size with equivalent titers. We are planning to design a shorter β-globin vector using this compact lentiviral backbone.

In this study, we have developed a compact lentiviral vector backbone, which can allow for a smaller-size globin vector construct, possibly resulting in more efficient vector production (titers) and higher-level gene-transfer (transduction) in HSCs. Also, it can allow inclusion of additional thEpoR (truncated human erythropoietin receptor) sequence to enhance β-globin expression in erythroid cells (Uchida N, Tisdale JF. Sci Transl Med. 2021). These results should improve efficiency of lentiviral HSC gene therapy and thus allow to develop more widely-applicable gene therapy. These results should enable us to evaluate lentiviral globin expression in a non-human primate model and improve efficiency and safety of lentiviral HSC gene therapy. Therefore, the results from this project would be useful for widely applicable gene therapy.