In Vivo Gene Transduction via Lentiviral Retargeting System
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Introduction
• There is a plethora of chronic diseases such as coronary artery disease (CAD) and other neurological & physiological diseases that have high mortality rates, yet there are few therapeutic options or targets to lessen the burden of such devastating diseases.
• In the early 2000s, Harold Varmus and colleagues designed a mouse expressing the avian receptor TVA and demonstrated effective and specific transduction in vivo using avian retroviruses, but because these retroviruses can only infect actively dividing cells, this model had limited applicability model.
• Lentiviral vectors have become attractive candidates for gene delivery within the last two decades as they are capable of efficiently delivering genes in both dividing and nondividing cells.
• Several groups have demonstrated that lentivirus can be successfully pseudotyped with a ‘blinded’ Sindbis virus envelope protein and have shown successful re-targeting of these pseudotyped particles by attaching various cell-specific binding proteins to an external loop of the Sindbis envelope protein.

Abstract
• Construction of a stable diphtheria toxin receptor (DTR) expressing HEK 293T cell line
• Pseudotype lentivirus with Sindbis-DT-RBD fusion envelope proteins
• Transduce DTR-expressing cells with Sindbis-DT-RBD pseudotyped lentivirus

Materials and Methods
• Construction of a stable diphtheria toxin receptor (DTR)-expressing HEK 293T cell line
• Pseudotype lentivirus with pMD2.G-VSVG-SpyTag fusion envelope proteins
• Transduce DTR-SpyCatcher expressing cells with pMD2.G-VSVG-SpyTag pseudotyped lentivirus

Results

Conclusions
• The data suggests the proposed lentiviral constructs and the interaction between SpyCatcher and SpyTag were insufficient in mediating a stronger infection than the traditional VSV-G pseudotyped lentivirus positive control.
• The proposed envelope protein iterations are not viable for in vivo experimentation.
• The data; however, suggests the design of the viral envelope protein is significant and influences the mediation of infection.
• Continued work is needed to design other iterations of the viral envelope protein capable of mediating gene transfer in a cell-type specific manner

References

Figure 1. Experimental lentiviral constructs insufficient in robustly mediating the transduction of control and experimental cell lines. a & c are GFP-expressing cells and d & f are DTR-expressing cells. a & d were infected with Sindbis-DT-RBD virus; b & e were infected with Sindbis-DT-RBD-JXTag-Tag with polylysine lentivirus. c & f were infected with traditional VSV-G pseudotyped lentivirus.