Conference: 27th ASGCT Annual Meeting 2024

Title: Rational Design of a Detargeted Vesiculovirus Fusogen to Enable Targeted *In Vivo* Gene Delivery

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Current *ex vivo* autologous and allogenic CAR T cell approaches utilize wild type (WT) VSV-G pseudotyped lentiviral vectors to achieve high efficiency transduction of patient or donor cells. *In vivo* generation of CAR T cells is meant to circumvent complications with *ex vivo* approaches, such as limited accessibility and lengthy manipulation processes that can negatively affect the outcome of therapy; however, WT VSV-G cannot be used for *in vivo* approaches due to a broad tropism that is not limited to T cells. Our goal was to rationally design a fusogenic molecule to enable targeted *in vivo* delivery of a gene of interest.

Previous work has shown that binding and fusion mediated by VSV-G are uncoupled processes[1]; thus, we reasoned the tropism of VSV-G pseudotyped vectors could be redefined by reducing the affinity of VSV-G for its native receptor (LDL-R) while simultaneously redirecting vector binding to a target molecule expressed by cells of interest. Using the available crystal structure of VSV-G bound to LDL-R[1], we visually scanned for residues in VSV-G whose side chains protrude toward the negatively charged contact residues in LDL-R. We then introduced negatively charged side chains at the identified sites in VSV-G to destabilize critical ionic interactions with its receptor.

Substitution of isoleucine at position 182 with either glutamic acid or aspartic acid was sufficient to abolish the transduction by vector pseudotyped with the mutant glycoproteins. Providing a membrane-anchored scFv redirecting vector to target cells restored titers to within a log of WT VSV-G, indicating these mutations only impacted binding to LDL-R, but not fusogenic activity. Importantly, substitution of alanine at residue 182 did not have this effect, suggesting the mechanism is dependent on charge as designed. Cells transfected with the I182E mutant were not labeled by a soluble form of LDL-R (sLDL-R), confirming the reduced affinity to the native receptor. As a concern with using VSV-G based vectors is the reported sensitivity to complement, we also incorporated substitutions in VSV-G that have been shown to improve stability in human serum[2]. Reflecting the iterative nature of this fusogen's design, we dubbed this version Generation 2.1 or "Gen 2.1" for short.

To further demonstrate specific retargeting, lentiviral vectors pseudotyped with the Gen 2.1 fusogen were produced and used to transduce CD7+ cells. As expected, vector pseudotyped with the Gen 2.1 fusogen alone did not effectively mediate transduction. When a plasmid encoding a binder specific to CD7 was included during vector production, infectivity titers were restored on

CD7+ SupT1 cells, but not on the same cells in which CD7 was knocked out or a panel of CD7- B cell lines. Additionally, vectors pseudotyped with the Gen 2.1 fusogen were resistant to neutralization by sLDL-R, confirming the retargeting via the CD7 binder. These data suggest that the Gen 2.1 fusogen has potential to be used as a platform for targeted *in vivo* delivery. Leveraging industry standard manufacturing processes for WT VSV-G based lentivirus production, we have successfully manufactured Phase 1 clinical supplies of a CAR20 vector product (INT2104) made with Gen 2.1 fusogen and a CD7 binder to support a FIH study in oncology starting in 4Q2024.

1. Nikolic, J., et al. "Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein." *Nature Communications* 9.1 (2018): 1029.
2. Hwang, B-Y., and D. V. Schaffer. "Engineering a serum-resistant and thermostable vesicular stomatitis virus G glycoprotein for pseudotyping retroviral and lentiviral vectors." *Gene Therapy* 20.8 (2013): 807-815.