Vector Oligonucleotide Conjugates (VOCs)

A platform for targeted oligonucleotide delivery with redosable viral capsids

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Abstract

Oligonucleotide therapeutics hold potential to target a vast array of disease-associated genes, but tissue-specific delivery remains the key challenge with current therapeutics. Antibody-Oligonucleotide Conjugates (AOCs) have seen recent success in delivery of siRNA to muscle tissue, but this technology has limitations. For example, current AOC platforms can only deliver one siRNA molecule per antibody, restricting AOC use to highly expressed receptors (such as the ubiquitously expressed TfR1) and tissue-specific siRNA targets. Here, we describe Vector Oligonucleotide Carriers (VOCs) - a novel platform that combines vector-mediated delivery with the manufacturability of recombinant proteins. VOCs utilize viral capsid proteins derived from viruses with a long history of safe human exposure to deliver oligonucleotides directly to target cells. Such a platform has the potential for revolutionary manufacturing and redosability capabilities. By delivering many siRNA molecules per capsid, we expect our VOCs to address critical limitations characteristic of other siRNA delivery technologies.

1. **VOCs are manufactured via direct loading of synthetic siRNA molecules onto recombinant capsid proteins (CP) that are then assembled into capsids.**
2. **VOCs can be modified with targeting ligands or antibodies that facilitate functional siRNA payload delivery to target cells.**
3. **VOCs offer unique advantages to existing siRNA delivery platforms and hold the potential to unlock both new targets and new tissues within the space.**

Background

What if the ideal vector is already inside us?

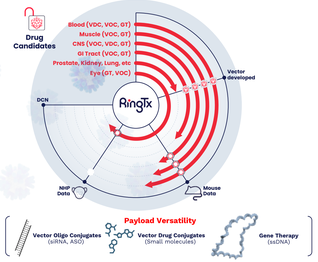
Healthy Human Subjects

Anellovirus
Anelloviridae family
~30 nm in diameter

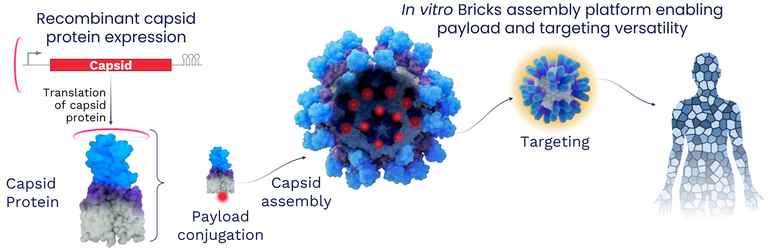
Ubiquitous, commensal DNA viruses with high genetic diversity that have coevolved with mammalian hosts, including humans, for millennia

Bromovirus
Bromoviridae family
~26 nm in diameter

Plant RNA viruses that are regularly ingested as part of a human diet and are found in a host of human tissues in and outside of the digestive tract

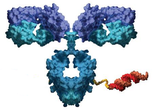


Ring is pioneering a new class of modular targeted medicines that deliver more

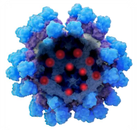


Vector Oligonucleotide Conjugates (VOC)

Conjugation of siRNA to the interior of viral capsids



AOCs (DAR of 1)
- Limited target options (only TfR currently)
- Limited to rare genetic disorders



VOCs (DVR 10-100x)
- Delivery up to 100x the siRNA per internalization event
- Expand targeted options to low expressing receptors
- Expand addressable indication space

VOC Production and QC

Payload Conjugation → **Capsid Assembly** → **Targeting**

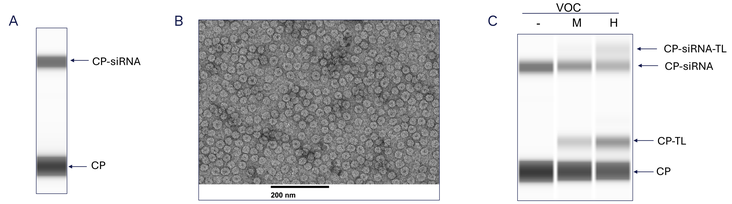


Figure 1: Production and QC of targeted VOC bearing a conjugated siRNA payload. (A) Gel electrophoresis (Jess Automated Western Blot System) of capsid protein (CP) conjugated to siRNA payload. (B) TEM micrograph of VOC assembled from siRNA-conjugated CP. (C) Gel electrophoresis (Jess Automated Western Blot System) of VOC conjugated with medium (M) or high (H) amount of a targeting ligand.

VOC deliver cargo to target cells

Conjugation of a targeting ligand (TL) to VOC surface drives target cell internalization

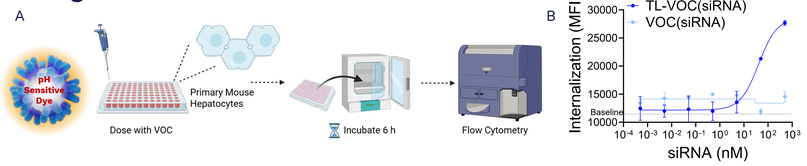


Figure 2: Targeting ligand (TL) dependent internalization of VOC *in vitro*. (A) VOC were labeled with a pH-sensitive dye to quantify internalization into acidic endolysosomal compartments and applied to primary mouse hepatocytes at the indicated doses. After 2-6 hours of incubation, cells were processed for flow cytometry to quantify pH-sensitive dye intensity. VOC conjugated with a targeting ligand (TL) showed strong internalization and untargeted VOC showed no detectable internalization at this timepoint.

Conjugation of mAb to VOC surface drives target cell internalization

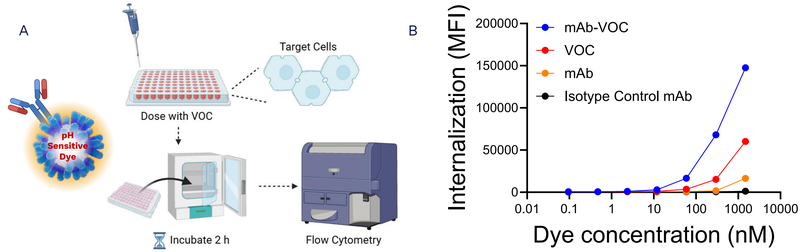


Figure 3: Targeting mAb dependent internalization of VOC *in vitro*. (A) Targeting mAb was conjugated to surface of VOC. VOC were labeled with a pH-sensitive dye to quantify internalization into acidic endolysosomal compartments and applied to target cells at the indicated doses. After 2-6 hours of incubation, cells were processed for flow cytometry to quantify pH-sensitive dye intensity. VOC conjugated with a targeting mAb showed stronger internalization compared to both untargeted VOC and mAb alone.

VOC trigger gene knockdown in target cells

TL-conjugated VOC deliver functional siRNA *in vitro*

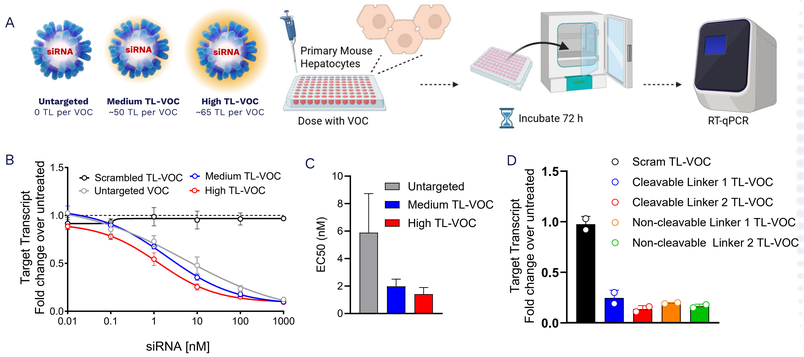


Figure 4: VOC deliver functional siRNA payloads to target cells and conjugation of VOC with targeting ligand (TL) enhances functional potency. (A) VOC carrying active siRNA or scrambled siRNA were functionalized with targeting ligand at two different levels (medium and high) and compared with untargeted VOC. Primary mouse hepatocytes were treated with VOC at the indicated concentrations and gene expression was assessed after 72 hours via RT-qPCR. (B-C) Dose response curves of VOC test articles. VOC delivering scrambled siRNA payload do not affect target gene expression. Functionalization of VOC with TL enhances potency ~5-fold compared to untargeted VOC. In B, error bars denote SD. In C, error bars denote SEM. (D) TL-VOC were generated with various linkers attaching the siRNA to the capsid protein. Similar to reports of AOC linker formats, all linker chemistries (including both enzyme-cleavable and non-cleavable linkers) performed similarly, suggesting that payload release is likely dependent on complete proteolysis of the VOC.