

# Targeted ASO Delivery To Mouse Lower Limb by Exosome Carrying A Muscle Targeting Moiety



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Minghao Sun, Yujia Li, SunMafalda Cacciottolo, Mahrou Sadri, Michael LeClaire, David Tran, Kristi Elliott, Linda Marbán

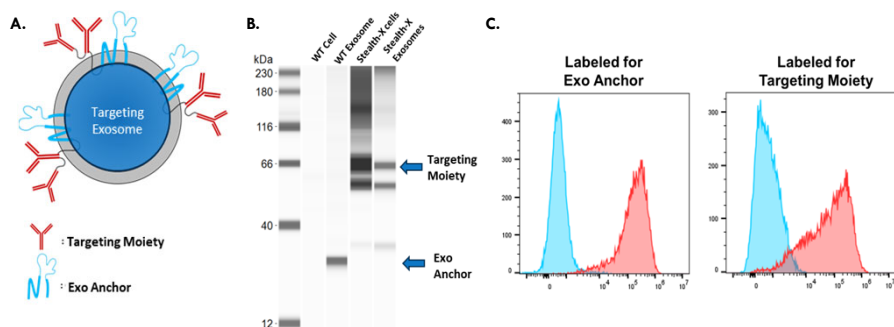
Capricor Therapeutics, Inc. (Nasdaq: CAPR) 10865 Road to Cure, Suite 150, San Diego, CA 92121



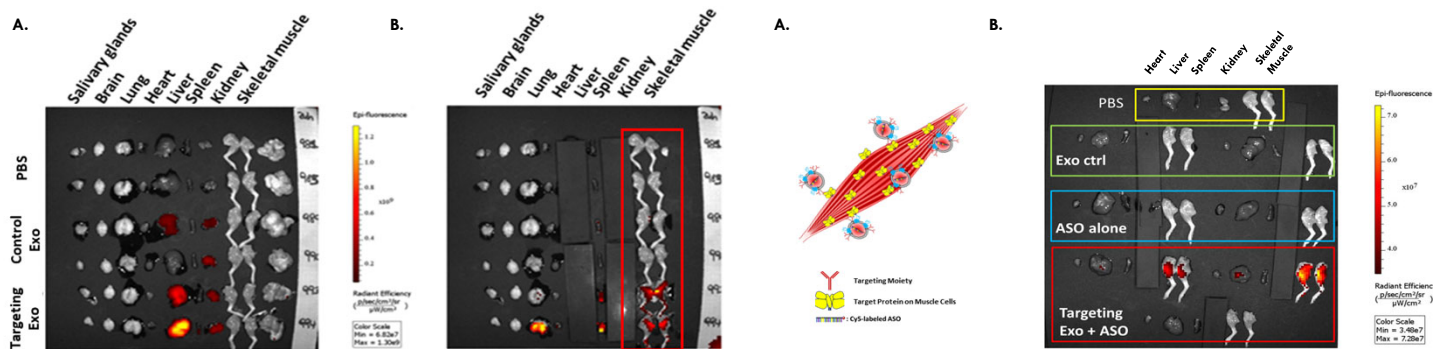
## ABSTRACT

Duchenne muscular dystrophy (DMD) is a severe, progressive, X-linked disease affecting both skeletal and cardiac muscle with severely reduced life expectancy. The predominant strategy for treating DMD is employment of antisense oligonucleotides (ASOs) to exclude exons resulting in DMD proteins with partially restored function. In the last 6 years, 4 exon skippers have been approved by FDA. The main challenge of antisense drugs is their limited efficacy due to poor delivery to target tissues. It has been estimated that <1% of the ASO reaches the correct cellular compartment therefore limiting restoration of function. To overcome the targeting limitation, a muscle targeting moiety was engineered on the surface of exosomes using StealthX™ technology. Initially, the exosomes carrying the targeting moiety were stained with a far-red fluorescence dye and I.V. injected into wild-type Balb/c female mice with results showing that labeled exosomes were detected in the lower limbs 24 hours post-injection. Notably, the exosomes carrying the muscle targeting moiety were not detected in any other tissues except for the expected clearance pathway (kidney and liver). To further evaluate the possibility of using exosomes as a targeted delivery tool, a fluorescence labeled ASO was loaded into the exosomes carrying the muscle targeting moiety and I.V. injected into mice. Intriguingly, the labeled ASO was also detected in the lower limbs. The initial data collected here strongly suggests that StealthX™ technology developed at Capricor could potentially pair with current ASO therapies for efficient delivery to muscle, improving restoration of function.

## RESULTS



**Figure 1. In vitro validation of the exosomes engineered with the targeting moiety.** 293F cells were engineered using Capricor' StealthX™ exosome platform to express the targeting moiety on the surface of both cells and exosomes. **A.** Schematic representation of exosome carrying the targeting moiety. **B.** The expression of the targeting moiety on the cells and exosomes was confirmed by Jess Automated Western Blot. Non engineered cells and exosomes were used as controls. **C.** Expression of the targeting moiety on the surface of exosomes was confirmed by Flow Cytometry assay. Blue: control exosomes. Red: Engineered exosomes.



**Figure 2. In vivo validation of muscle targeting.** Targeting exosomes were fluorescence labeled and injected into mice by I.V. administration. 24 hours post injection, tissues were collected and imaged by IVIS imaging system. **A.** At 24 hours post injection, as expected, the exosomes were localized in the expected clearance pathway (kidney and liver). **B.** Notably, the addition of the targeting moiety on the exosome surface alters the localization of the targeting exosomes compared to the control exosomes, with increased localization to the skeletal muscle (lower limbs, red rectangle). The muscle signal was only observed for the exosome carrying the targeting moiety but not the control exosomes, confirming the specificity of the signal.

**Figure 3. In vivo delivery of ASO to the muscle by exosome carrying targeting moiety.** A fluorescence labeled ASO was loaded into the exosomes carrying the muscle targeting moiety and I.V. injected into mice. 24 hours post injection, tissues were collected and imaged by IVIS imaging system. Control exosomes and ASO alone were used as controls. **A.** Schematic representation of the mechanism of action of exosome carrying the targeting moiety. **B.** 24 hours post injection, the labeled ASO was detected in the lower limb when loaded in the exosome carrying the targeting moiety, but not when injected by itself, suggesting the possibility of using exosomes as a targeted delivery tools.

## CONCLUSIONS

Capricor' StealthX exosome platform was used to engineer a muscle targeting moiety on the surface of exosomes. Our data showed that:

1. Capricor' StealthX exosome platform can be used to engineer moieties of interest on the surface of exosomes
2. The muscle targeting moiety was correctly engineered on the surface of both cells and enriched on the surface of exosomes
3. The targeting exosomes were able to target the desired tissues, in vivo after intravenous injection, with clear localization to the muscle
4. The targeting exosomes were able to deliver a therapeutic (i.e. ASO) to the desired tissues (i.e muscle), increasing its availability compared to the therapeutic alone (i.e ASO alone) or the control exosomes (i.e. exosome lacking the targeting moiety).

All together, the data support the possibility of using the StealthX™ exosome platform for a tissue targeted therapy tool.

## REFERENCE

Delivery is key: lessons learnt from developing splice switching antisense therapies. (2017) EMBO Mol. Med., 9, 545–557

## DISCLOSURE

StealthX™ is proprietary of Capricor Therapeutics, Inc. (NASDAQ: CAPR)