

**P016** Vectorization of morpholino oligomers by the (R-Ahx-R)<sub>4</sub> peptide allows efficient splicing correction in the absence of endosomolytic agents

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The efficient and non-toxic nuclear delivery of steric-block oligonucleotides (ON) is a prerequisite for therapeutic strategies involving splice-correction or exon skipping. Cationic cell penetrating peptides (CPPs) have given rise to much interest for the intracellular delivery of biomolecules, but their efficiency in promoting cytoplasmic or nuclear delivery of oligonucleotides has been hampered by endocytic sequestration and subsequent degradation of most internalized material in endocytic compartments. In the present study, we compared the splice correction activity of three different CPPs conjugated to PMO705, a steric-block ON targeted against the mutated splicing site of human  $\beta$ -globin pre-mRNA in the HeLa pLuc705 splice-correction model. In contrast to Tat48-60 (Tat) and oligoarginine (R9F2) PMO705 conjugates, the 6-aminohexanoic spaced oligoarginine (R-Ahx-R)<sub>4</sub>-PMO705 conjugate was able to promote an efficient splice correction in the absence of endosomolytic agents. Our mechanistic investigations about its uptake mechanisms lead to the conclusion that these three vectors are internalized using the same endocytic route involving proteoglycans, but that the (R-Ahx-R)<sub>4</sub>-PMO705 conjugate has the unique ability to escape from lysosomal fate and to access to the nuclear compartment. This vector, which displays an extremely low cytotoxicity, the ability to function without chloroquine adjunction and in the presence of serum proteins. It thus offers a promising lead for the development of vectors able to enhance the delivery of therapeutic stericblock ON in clinically relevant models