Enhance Nuclear Delivery for Genomic DNA- Targeted Therapeutics

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Purpose
Focusing on the genetic blueprint of disease and achieving long-lasting, curative effects depends on advancing technology that improves stability and facilitates nuclear delivery. Various delivery systems for nucleic acid therapeutics have been developed over the past few decades and rely on chemical modifications. Translocation of therapeutic scaffolds across the nucleus membrane of Eukaryotic cells is challenging for nucleic acid therapeutics. Taking advantage of the nucleus imports machinery that translocates the synthesized proteins from the nucleus to the cytoplasm. The Nucleus Localization Signal (NLS) of Simian Virus 40 (SV 40), a short peptide rich in basic amino acids is identified to translocate proteins effectively. We aimed to develop a delivery system by maintaining the stability and enhancing the nucleus transfection without altering the therapeutic efficacy of synthetically developed next generation NLS for in vivo application.

Methods
SV40 NLS is composed of Seven Amino acids Pro-Lys-Lys-Lys-Arg-Lys-Val (PKKKRKV). L and D Conformational amino acids are used in the synthesis of NLS by solid phase synthesis and tested for cellular uptake by performing microscopic and flow cytometry studies. Non-specific interactions of NLS with RNA and DNA are evaluated by using Agarose and Page electrophoresis. We have tested the biodistribution of L-NLS and D-NLS in the Xenograft mice model. The stability of L and D amino acids is determined by incubating with Human serum. To further evaluate the effectiveness and stability when conjugated with Peptide Nucleic acid (PNA), we have synthesized c-Myc PNA conjugating with L and D NLS by solid phase synthesis. PNA-L NLS and PNA D-NLS are tested for cellular uptake and binding efficiency. We quantified the c-Myc levels and their downstream targets using gene expression assays and western blots. Stability studies of PNA L NLS & PNA D NLS are carried out by incubating PNA with Human serum, and aliquots collected at different time points are analyzed using RP-HPLC

Results
Cell culture studies (microscopy and Flow cytometry) revealed that L NLS is less stable after 48 hours of treatment. Stability studies provided strong evidence stating D NLS is more stable in serum. Binding studies confirmed the absence of nonspecific interactions with RNA and DNA. Targeted delivery to the tumor environment is achieved. We established that PNA L-NLS and PNA D-NLS have shown nuclear uptake and are effective in downregulating the downstream targets. Still, PNA D-NLS is more stable in human serum, which is confirmed to have a similar activity to PNA L-NLS

Conclusion
We have identified a stable nuclear delivery system that can hinder the Oligonucleotides from the enzymatic degradation and transporting ASO’s to invade the Genomic DNA by enhanced nuclear delivery. Results presented in this study establish a new platform for targeting genomic DNA

Keywords: NLS, Stability, genomic DNA, c-Myc PNA