Synthesis and Biological Evaluation of DNA Decoys Bearing Modified Internucleosidic Linkages

Marie Joyeux[a,b] **Megane Debiais**[b] **Wael Jdey**[b] **and Michael Smietana**[a]

[a]*IBMM, Université de Montpellier, CNRS, ENSCM, Montpellier*
[b]*Valerio Therapeutics*

E-mail: m.joyeux@valeriotx.com

Gene therapy is based on strategies controlling the expression of disease-associated genes. Beyond antisense oligonucleotides and interfering RNAs, double-stranded DNA containing a transcription factor consensus sequence, commonly called DNA decoys, represents an alternative strategy to modulate the expression of a target gene, either through inhibition or activation.[1] Nonetheless, as with other oligonucleotides, their vulnerability to nuclease degradation remains a major limitation and significantly constrains their therapeutic applicability. Phosphorothioate (PS)-type internucleoside modifications are widely used to enhance stability, but their limited specificity and associated toxicity pose major limitations.[2] In this context, an alternative modification using mesylphosphoramidate (MsPA) linkages was explored. Initially developed for antisense oligonucleotides,[3] these linkages are applied here for the first time to the DNA decoy approach. Different MsPA linkages were incorporated into a model DNA decoy to assess their impact on stability and specificity compared to conventional PS modifications. In addition, *ex vivo* toxicity studies were carried out to evaluate the biocompatibility of these modifications. The results clearly demonstrate the strong potential of these modifications to enhance the performance of DNA decoys, paving the way for future therapeutic applications. These findings will be presented in detail.

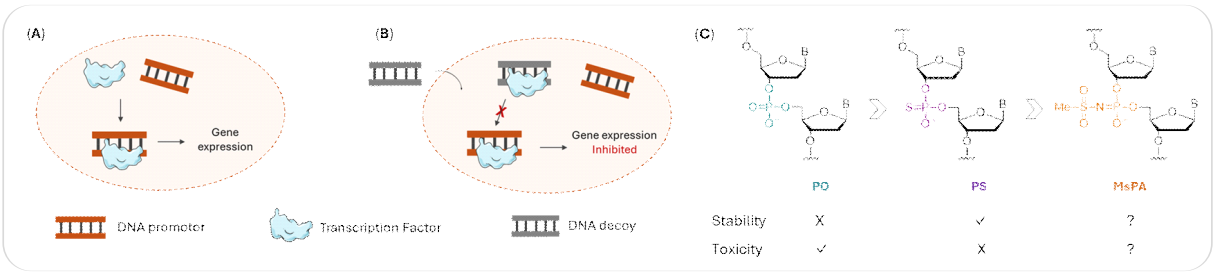

Figure 1 : Schematic overview of transcriptional activation and its inhibition by chemically modified decoy oligonucleotides. (A) Transcriptional activation through specific binding of a transcription factor to the promoter of the target gene. (B) Competitive inhibition mediated by a decoy DNA that traps the transcription factor, thereby blocking its binding to the promoter. (C) Introduction of internucleosidic MsPA modifications into decoy oligonucleotides and assessment of their biological impact on stability and cytotoxicity.

References

[1] M. J. Mann, V. J. Dzau, *J. Clin. Invest*. **2000**, 106, 1071-1075.
[2] S. P. Henry, M. V. Templin, N. Gillett, J. Rojko, A. A. Levin, *Toxicol. Pathol.* **1999**, 27, 95-100.
[3] S. K. Miroshnichenko, O. A. Patutina, E. A. Burakova, B. P. Chelobanov, A. A. Fokina, V. V. Vlassov, S. Altman, M. A. Zenkova, D. A. Stetsenko, *Proc. Natl. Acad. Sci.* **2019**, 116, 1229-1234.