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Advantages of Linear DNA over Circular DNA plasmids

Conventional, circular DNA plasmids have been widely used either directly or indirectly for the development of cell and gene therapy drug products.

In this white paper, we discuss how novel, linear DNA technologies, such as Mediphage's linear, double-stranded, and covalently closed ministring (msDNA) can significantly reduce the risk of insertional mutagenesis.

Insertional mutagenesis or ***genotoxicity*** is defined as the unintended insertion of viral or non-viral sequences into the host genome that can disrupt the normal regulation of cell development and proliferation, and lead to transactivation of nearby proto-oncogenes or the inactivation of tumor suppressor genes which can result in oncogenesis[1]. In 2007, the U.S. Department of Health and Human Services and Food and Drug Administration's Center for Biologics Evaluation and Research published an industry guidance document on the "Considerations for Plasmid DNA vaccines for Infectious disease indications" highlighting plasmid DNA persistence and integration concerns, including risks of tumorigenesis if unwanted ***insertions reduce the activity of a tumor suppressor or increase the activity of an oncogene***[2]. Other highlighted concerns are the generation of ***chromosomal instability*** through the induction of chromosomal breaks or rearrangements due to DNA insertion.

DNA vectors with ***circular topology*** have an increased risk of insertional mutagenesis due to 1) crossover events and 2) non-homologous end joining (NHEJ) via open ends generated through nicking or breakage events[3].

On the contrary, msDNA reduces the risk of insertional mutagenesis due to its unreactive covalently closed ends. If illegitimate integrations take place, a "safety" switch that prompts cell death is activated.

More information about msDNA and the "safety" switch can be found in Nafiseh et al. 2014.

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[1]Schlimgen, Ryan PhD; Howard, John MD; Wooley, Dawn PhD; Thompson, Maureen RN; Baden, Lindsey R. MD; Yang, Otto O. MD; Christiani, David C. MD, MPH; Mostoslavsky, Gustavo MD, PhD; Diamond, David V. MD; Duane, Elizabeth Gilman MS, RBP, CBSP; Byers, Karen MS, RBP, CBSP; Winters, Thomas MD; Gelfand, Jeffrey A. MD; Fujimoto, Gary MD; Hudson, T. Warner MD; Vyas, Jatin M. MD, PhD. Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. Journal of Occupational and Environmental Medicine 58(12):p 1159-1166, December 2016. | DOI: 10.1097/JOM.0000000000000879

[2]Center for Biologics Evaluation and Research. Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. FDA Guidance for Industry, November 2007. 2005D-0047.

[3]Nafissi, Nafiseh PhD; Alqawlaq, Samih PhD; Lee, Eric A. MSc; Foldvari, Mariana PhD; Spagnuolo, Paul A. PhD; Slavcev, Roderick A. PhD. DNA ministrings: highly safe and effective gene delivery vectors. Molecular Therapy Nucleic Acids 3(6):e165, May 2014. | DOI: 10.1038/mtna.2014.16. PMID: 24892724; PMCID: PMC4078758.