UNIVERSITY OF NAPLES FEDERICO II

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XXXV CYCLE

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Engineering the HEK293 packaging cell line to improve simian Adenoviral vaccine production.

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1. Abstract

The rise of the coronavirus disease 2019 pandemic has highlighted the requirement for fast, flexible, economical vaccine development and production. Consequently, the detection and adoption of new strategies that lower costs and make vaccines affordable is essential purpose.

Among the most popular vaccine delivery platforms, replication-defective adenoviral vectors offer several advantages in their development such as easy and fast manipulation, high antigen expression level, potent and durable immune response, efficient manufacturing, and scalable production. Nevertheless, the generation and the amplification process of Adenoviral vector-based vaccines remain highly time-consuming and labor-intense, fostering research into alternative or improved ways to intensify the efficiency of the production process. This study employed several approaches to expedite and improve the non-human Adenoviral vector-based vaccine production process in the HEK293 packaging cell line.

The production process starts from the transfection of the Adeno vector plasmid, previously genetically manipulated, in the packaging cell line (rescue) that allows the generation of the viral particles. Chimpanzee Adenoviruses (chAd) precursor terminal protein (ch.pTP) overexpression in HEK293 cells provides the acceleration, improvement, and higher quality viral particles of non-human Adenovector vaccine derived from Chimpanzee (chAd) or Gorilla (GRAd).

Once obtained the Adenoviral particles from the vector rescue, the next step is scalable amplification to obtain high-dose vaccine production. ZNF622 protein silencing in HEK293 cells increases viral and infectious particle titers and confers greater thermostability over time of non-human Adenovector vaccine derived from Gorilla (GRAd).

In conclusion, the engineered HEK293 packaging cell line enables the acceleration and the optimization of simian-derived Adenoviral vaccine production as well as the improvement of viral preparation quality for large-scale vaccine production.