DEVELOPMENT OF A FERRITIN NANOPARTICLE-BASED VACCINE PLATFORM AGAINST *Klebsiella pneumoniae*

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Klebsiella pneumoniae (*Kp*) is a Gram-negative pathogenic bacterium responsible for various diseases such as pneumonia, sepsis and urinary tract infections. Due to the emergence and spread of hypervirulent (hv) and hv multidrug-resistant strains, it is considered a public health threat. The World Health Organization has designated this pathogen as a critical priority for research and the development of new control strategies[1]. In recent years, the use of protein-based nanoparticles as vaccine platforms for antigen presentation has gained attention. Among them, ferritin nanoparticles (F-NPs) stand out due to their self-assembly capacity, thermal and pH stability. In this project, we propose the rational development of a glyconjugate-based nanovaccine specifically targeting hypervirulent *Kp*. To predict and identify potential *Kp* vaccine candidates, we developed a reverse vaccinology methodology, which allowed the *in-silico* identification of six vaccine candidates. Specific oligonucleotides were designed from the selected candidates. The vaccine candidates were cloned into the pJET 1.2/blunt vector and, using restriction enzyme sites, they were subcloned into the pET28a(+) expression vector. Finally, the expression and purification conditions were standardized, allowing the successful expression of the first vaccine candidate. On the other hand, the SpyTag/SpyCatcher system was used to design fusion proteins for the F-NPs platform [2]. Through protein engineering, three chimeric sequences were designed and optimized, these nanoparticles are composed as follows: signal peptide–SpyCatcher–linker–ferritin from *H. pylori* and *Aquarana catesbeiana*. These constructs were sent for synthesis to the company GenScript. Additionally, oligonucleotides were designed with recognition sites for the restriction enzymes, which enabled subcloning into the pET28a(+) expression vector. The F-NP are expected to be expressed in Expi293F cells, followed by functionalization with the vaccine candidates. Additionally, a strategy will be implemented to obtain K1 and K2 polysaccharides, which will be conjugated to the nanoparticles. This research project has established a methodology for predicting vaccine candidates, standardized the expression and purification conditions for vaccine candidates, and proposes an optimized, effective, and safe platform against *Kp* infection

References

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2. Hatlem D, Trunk T, Linke D, Leo JC. Catching a SPY: using the SpyCatcher-SpyTag and related systems for labeling and localizing bacterial proteins. International journal of molecular sciences. 2019;20(9):2129.