INTERNATIONAL MASTER IN SUSTAINABLE DRUG DISCOVERY

FORMULATION AND EVALUATION OF ADJUVANTED mRNA LIPID NANOPARTICLES FOR PROPHYLACTIC VACCINATION AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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SUMMARY

Tuberculosis remains a global health challenge, necessitating the development of new and improved vaccines. This thesis focused on optimizing mRNA vaccine formulations against TB by exploring lipid nanoparticles as delivery systems.

The first objective examined the potential of S-Ac-7-DOG as an alternative to the standard C12-200 lipid for LNP formulation. A 51 day stability study was conducted to analyse size stability and zeta potential stability by Dynamic Light, encapsulation efficiency by Ribogreen assay, and transfection efficiency through Flow cytometry. The overall stability of S-Ac-7-DOG LNPs was lower than C12-200 LNPs. However, when co-formulated with the adjuvant α-GC, S-Ac-7-DOG LNPs enhanced CD8+ effector and tissue-resident memory T cell responses in *in vivo* experiments suggesting potential for stimulating both adaptive and humoral immunity.

The second objective evaluated mRNA vaccines encoding the TB antigens ESAT-6 and Ag85B, with and without the adjuvant α-GC. The study involved determination of adaptive immune response through tetramer staining and intracellular cytokine staining. Results showed that these vaccines elicited robust immune responses, and α-GC further enhanced antigen-specific CD44+ T effector cells and Th1/Th2 cytokine production. However, due to lack of CD8 responses in mouse model, the adjuvant effect could not be seen. Importantly, vaccination induced a pronounced effect on tissue-resident memory T cells, indicating potential for long-lasting lung immunity. On top of that, the current lyophilization protocol impacted negatively the efficacy of adjuvanted LNPs, therefore further optimization is needed.

The third objective explored the use of alternative adjuvants MPLA and QS21. LNP production and characterisation was performed. MPLA improved LNP stability and mRNA translation/expression, while QS21 had a negative impact on these properties, indicating a need for further refinement when combining adjuvants.

In conclusion, this research provides valuable insights into the development of mRNA vaccines against TB. Further research should focus on optimizing LNP stability with S-Ac-7-DOG, exploring the long-term efficacy of α-GC-adjuvanted LNPs, refining the combination of adjuvants, and conducting preclinical studies to assess the protective efficacy of these vaccine candidates against TB infection.