A5. Preclinical development
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Development of TB recombinant mycobacterial vaccine without antimicrobial resistance marker

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Background: Even though Bacillus Calmette–Guérin (BCG) vaccine protective capacity decreases in adulthood, this vaccine is the only form of prophylaxis currently licnensed against tuberculosis. mc2-CMX is a live attenuated recombinant vaccine composed of Mycobacterium smegmatis (mc2) expressing CMX, a fusion protein of three M. tuberculosis antigens expressed during TB infection, whose ability to protect against tuberculosis and its immune responses has been very well characterized in preclinical studies. To apply for human clinical trials, it is necessary to comply with the Geneva agreement that restricts the use of live vaccines with antimicrobial selective marker. Aim: To develop the mc2-CMX without antimicrobial resistance marker.

Methods: Integrative plasmids containing the gene sequence of the CMX protein and the apramycin resistance (apraR) gene flanked by diff regions (DifA) were constructed in silico and used to transform mc² by electroporation. Apramycin-resistant transformants were grown without the presence of the antimicrobial to loose resistance.

Results: Transformants that became sensitive to apramycin were analyzed by polymerase chain reaction (PCR) and next-generation sequencing (NGS) of chromosomal DNA and the presence of the CMX gene and absence of apraR was confirmed. The CMX expression by the live attenuated vaccine was confirmed by western blot. The new mc2-CMX vaccine is stable.

Conclusion: mc2-CMX was developed in agreement with the Geneve agreement. The next steps of the study are to evaluate if the protection and immune response achieved with the former recombinant vaccine developed with plasmids without genome integration and containing antimicrobials selection mark was maintained.

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Conflicts of Interest

None