

mRNA-based strategy for the prophylaxis of Plasmodium falciparum malaria

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Plasmodium falciparum reticulocyte-binding protein homologue 5 (RH5) is a leading blood-stage malaria vaccine antigen. Clinical trials of RH5-targeting vaccines have identified anti-RH5 monoclonal antibodies (mAbs) that inhibit erythrocyte invasion by the parasite, preventing the pathogenesis of malaria. R5.016 is among the most potent of these neutralising mAbs, preventing the essential interaction of RH5 with its erythrocyte receptor, basigin. As recombinant mAbs are currently unfeasible for at-scale prophylaxis of malaria due to prohibitive manufacturing costs, we aim to use *in vitro*-transcribed (IVT) mRNA technology to synthesise R5.016 *in vivo*. Using an mRNA platform featuring a novel combination of untranslated region sequences, we confirmed expression of R5.016 in HEK-293T cells. As high R5.016 titres will be required to provide protection, we next aimed to increase the translational capacity of the mRNA construct by exploring the use of different cap analogs, modified nucleosides and poly(A) tail designs. After synthesizing a library of mRNA constructs, we transfected HEK-293T cells and measured the R5.016 concentrations in the supernatants by standardized ELISA. The combination of using a Cap1 analog instead of Cap0, engineering the sequence of the poly(A) tail, and optimizing the 3' terminus of the poly(A) tail resulted in a ~30-fold increase to the R5.016 concentration produced from the mRNA construct *in vitro*. Replacement of uridine with N1-methylpseudouridine dramatically decreased the yield of R5.016 produced by each construct design. These results provide proof of concept for using mRNA technology to express antimalarial mAbs and supports progression to preclinical studies in mice. Using our mRNA platform to express the RH5 antigen, we plan to benchmark the quality and quantity of the anti-RH5 IgG response produced from a conventional mRNA vaccine approach with this novel antimalarial mAb-encoding mRNA strategy.