Optimizing formulations for mRNA delivery into the muscle

Jonas Füner1 , Simone Odau2 , Emanuele Cettul2 , José Dinis Faustino2 , Saveria Germani2 1preclinics discovery GmbH, Potsdam, Germany, 2preclinics GmbH, Potsdam, Germany

Background: Transfection of the skeletal muscle is of interest either to treat muscle diseases themselves or to utilize the muscle as an organ for recombinant protein therapy; in this context, the muscle can be an alternative to the liver. Advantage of the muscle are easy accessibility for injections and a large mass. Although muscle transfection is straightforward in principle, the various methods have advantages and disadvantages. We compared these methods and evaluated them using reporter genes and therapeutic applications. Method: We investigated various LNPs and LNP-free formulations with regard to their muscle-specific transfection efficiency, offtarget transfection, and inflammatory response. To this end, the various formulations were administered intramuscularly to Balb/C mice; subsequently, bioluminescence imaging was performed at 6h and 24h. After the last imaging serum was collected and organs were harvested. Cytokines in the serum were quantified using a multiplex assay, and the organs were analyzed via ddPCR to quantify both the vector-encoded mRNA and tissue cytokine mRNA. Results: While some LNPs exhibited enhanced muscle specificity, their administration consistently resulted in off-target transfection and potential immunogenicity. Conversely, our LNP-free formulations significantly reduced immunogenicity and completely prevented lymphatic translocation, achieving high muscle specificity at the expense of lower transfection rates. Conclusion: There is a need for the development of muscle treatment with mRNA. It is important to increase muscle specificity while simultaneously reducing immunogenicity; LNP-free formulations could be a solution for this, however, LNPs have so far achieved higher absolute transfection