VHH surface modification to lipid nanoparticles for targeted osteoarthritic synovitis therapy

Synovitis is a common feature in osteoarthritis, resulting in joint pain and swelling. Inflammatory processes involving different cell types, including macrophage-like and fibroblast-like synoviocytes, play a critical role in the development of the disease. Even though RNA-based therapeutics have been crucial in the Onpattro® and COVID-19 drug development, their nanocarriers often lack specificity. In this report, different variable domain of the heavy chain only antibodies (VHH) surface modification approaches to lipid nanoparticles (LNPs) are studied as a proof-of-concept for targeted osteoarthritic synovitis therapy. Comparisons are made between the two different conjugation approaches.

Conjugation of both VHHs with their respective reactive group containing compounds were studied. Next to the VHH, conjugations were also performed using a fluorescent dye as a model compound for the VHH. Fluorescence analysis and SDS-PAGE assays both demonstrate the conjugation potential for both reactive groups when attached to a specific lipid. SDS-PAGE results showed that one of the reactive groups consistently outperformed the other. In addition, increasing the spacer length between the lipid and the reactive group increases the conjugation efficiency.

Apart from the different lipids with reactive groups, the influence of a reducing reagent was studied. SDS-PAGE results and SPR analysis show that a higher concentration reducing agent led to a higher conjugation efficiency, whilst negatively influencing the binding affinity of the VHH to the target antigen.

A dot blot assay together with the SPR results displayed the conjugation potential of functionalized LNPs. An increased availability of VHH on the LNP surface seems to have a positive effect on the binding affinity of the VHH, balancing out the negative effects caused by the reducing agent. However, further optimalization of these experiments are required to draw better conclusions.

Future research should focus on the integration of a reactive peptide sequence into the VHH sequence as this appears to be the most promising approach due to its high coupling efficiency, as described in literature, and the controlled reactive group placement. Additionally, post-insertion methods might be considered to further optimize the conjugation efficiency.

Full report not provided due to confidentiality