Generation of Antibody Oligonucleotide Conjugates Using a Novel Conjugation Chemistry

Wang, Brady (School: Horace Greeley High School)

Oligonucleotides, especially siRNAs, have been identified as a point of interest due to their capability for precision targeting of "undruggable" targets. siRNAs (Small Interfering RNA) bind to and guide the RNA Induced Silencing Complex (RISC) in the cytoplasm, leading to degradation of specific targeted mRNAs. Despite advancements in oligonucleotide chemistry, delivery to extrahepatic tissues remains a challenge. Current FDA-approved delivery systems such as lipid nanoparticles (LNPs) and trivalent GalNAc, primarily target the liver. Antibodies, however, are emerging as a promising modality for targeted extrahepatic delivery of oligonucleotides. Antibody oligonucleotide conjugates (AOCs) leverage antibodies' receptor-based uptake for precise delivery of siRNA to targeted regions of the body. However, current chemistries for AOC generation are either inefficient or non-site specific. My research introduces a novel site-specific conjugation technology for generating AOCs. This method combines an enzymatic transglutaminase reaction with an Inverse Electron Demand Diels-Alder (IEDDA) reaction. Specifically, a tetrazine linker is added to the antibody via transglutaminase, followed by purification through size exclusion chromatography (SEC). The siRNA is then conjugated to the antibody-linker using IEDDA and purified with SEC, achieving an oligonucleotide to antibody ratio (OAR) of ~1, confirmed by UV-Vis and then hydrophobic interaction chromatography. Aggregation was minimal (<5%), and endotoxin levels were low (<0.5 EU/mg). This novel conjugation chemistry successfully generates AOCs, which will be tested in vivo for knockdown efficacy. Future applications include applying this chemistry to generate other modalities such as antibody-tethered LNPs (Ab-LNPs).

Awards Won: