A comprehensive platform for well-defined Antibody-Oligonucleotide conjugates

Daniele Addis1 , Soham Mandal1 , Mohammad Ghaem1 , Linda Neumann1, 1, Christopher Schmidt1 , Nadine Assmann1 , Jochen Deckert1 , Monika Krampert1 , Monique Slawik1 , Eileen Hereth1 1Axolabs GmbH, Kulmbach, Germany

Background: Antibody-oligonucleotide conjugates (AOCs) have emerged as a promising modality for the targeted delivery of nucleic acid therapeutics. However, their development remains challenging due to the need for controlled conjugation, defined oligonucleotide-to-antibody ratios (OAR), and comprehensive analytical characterization. Method: In this presentation, we describe a robust workflow for the synthesis, purification, and characterization of AOCs bearing small interfering RNA (siRNA). Conjugation was performed both by stochastic modification of native antibody and site-specific strategies on engineered antibodies, employing thiol-maleimide Michael addition or Strain-Promoted Alkyne-Azide Conjugation (SPAAC). Results: Site-specific conjugation yielded significant control on the resulting OAR, whereas random conjugation resulted in heterogeneous mixtures with variable OAR distributions. Chromatographic separation enabled the isolation of discrete OAR species. In addition, we established a comprehensive analytical toolbox to characterize the identity, purity, and stability of AOCs. Conclusion: The synthesized AOCs were further evaluated in functional assays to confirm retention of antibody-mediated target recognition and oligonucleotide activity. Binding performance was assessed using ELISA-based assays, demonstrating that conjugation did not abolish the interaction with the intended target. In addition, selected AOCs were tested in vitro for mRNA silencing activity, confirming that the siRNA payload remained biologically active after conjugation and was able to induce target mRNA knockdown in the relevant cells, in vitro through receptor mediated uptake.