Analysis of Drug Attachment Site: Implications for ADC Potency and Stability

Abstract

Analysis of Drug Conjugates (ADC) have emerged as new therapeutics for the treatment of various cancers in targeted settings. These therapeutic molecules are constructed by conjugating a cytotoxic agent to an antibody. Before conjugation, the linker arm is designed to be the shorter to allow intra-cellular internalization; allowing for the emergence and propagation of the species into type. Single-strand linkage have been shown to enhance linker stability through stabilization of the antibody chemistry, such as modifications such as Cys to Asp, which can cause opening of the ring which influences in vivo potency.

Results

For this study, a commercial IgG1 mAb was joined to SMCC-Biotin (C3) to serve as the conjugate model. The peptide and cytotoxic agent that can affect efficacy.

Conclusions

Micro-environment of attachment site can cause chemical changes to linker. This was demonstrated with the multi-cysteine containing peptide where C3 attachment site was hydrolyzed and others were not. Sequence analysis showed neighboring cysteines and Arginines could participate in supporting solvent bound network.

Mass Spectrometry can detect these sites. Detection can be accomplished with High Resolution High Accuracy Mass spectrometers such as the LTQ-Orbitrap.

Open Maleimide Forms are less susceptible to Maleimide Exchange. Albumin and Glutathione can remove linkages by reverse Michael Reaction in vitro and in vivo.