Background Antibodies targeting aberrantly glycosylated proteins are ineffective in treating cancer. Antibody-drug conjugates have emerged as effective alternatives, facilitating tumor-specific drug delivery. Previous studies have assessed the aberrantly glycosylated tandem repeat region of MUC1 glycoprotein as three site-specific glycosylated neoantigen peptide motifs (PDTR, GSTA, and GVTS) for binding with a monoclonal antibody. Neoantigen epitopes are created by the mechanism of glycosylation deficiency caused by defective function of COSMC (C1GALT1C1), the chaperone protein required for O-glycan elongation. This study aimed to develop an antibody-drug conjugate for cancer treatment based on monoclonal antibodies against the aforementioned three neoantigen peptide motifs.

Methods Internalization of monoclonal antibodies was assessed via immunofluorescence staining and colocalization with lysosomal markers in live cells. Antibodies were also labelled with \(^{89}\text{Zr}\) for measuring their internalization ex vivo and in vivo in tumor-bearing mice. Antibody positivity in tumor and peritumoral tissue samples was assessed via immunohistochemistry. The efficacy of anti-MUC1 ADCs was evaluated using various cancer cell lines and a mouse tumor xenograft model. Humanized antibodies were designed for GSTA neoantigen-specific 16A according to its co-crystal structure with glycopeptide epitope (PDB ID: 7V7K).

Results An anti-MUC1 ADC was synthesized by conjugating GSTA neoantigen-specific 16A with monomethyl auristatin E (MMAE), which displayed potent antitumoral efficacy with an IC\(_{50}\) ranging 0.2–49.4 nM toward various cancer cells. In vivo, 16A-MMAE inhibited tumor growth in a dose-dependent manner in a mouse xenograft model established using the NCI-H838 NSCLC cell line, at a minimum effective dose of 1 mg/kg. At 3 mg/kg, 16A-MMAE did not cause significant toxicity in a transgenic mouse expressing human MUC1. In vivo, 16A antibody labelled with \(^{89}\text{Zr}\) showed clear preference of internalization by COSMC knockout cells, as compared to parent cell lines with wild type COSMC genotype.

Conclusions The high antitumoral efficacy of 16A-MMAE suggests that aberrant glycosylated MUC1 neoantigen is a potential target for the development of ADCs for treating various cancers. Personalized therapy may be achieved through such glycosite-specific ADCs. Ongoing studies are being focused on multiple types of cancer with high positivity for 16A epitope expression and several drug payloads including topoisomerase inhibitor.

Acknowledgements Supported by National Key Research and Development Plan grants 2021YEE0200500 and 2017YFA0505901, Fundamental Research Funds for the Central Universities 22120200163, National Natural Science Foundation of China grant 31870972, and Shanghai Science and Technology Commission grant 15002360172.

REFERENCES
