CD44-Targeted Antibody-Drug Conjugate with Cathepsin B-Cleavable Linker for Selective Delivery of Doxorubicin in Ovarian Cancer

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Ovarian cancer (OC) is a leading cause of cancer-related death among women, often diagnosed at the late stage, resulting in a low survival rate despite of advancement in cancer treatment. Major challenge associated with OC therapy is recurrence and resistance, largely driven by a small subpopulation of tumor-propagating cells called cancer stem cells (CSCs). Targeting CSC biomarkers like CD44, having crucial role in OC recurrence and resistance, is important for improving therapeutic outcomes. Antibody-drug conjugates (ADC) provide a promising approach by combining the specificity of antibodies with the potency of cytotoxic drugs. This targeted approach enhances cancer treatment efficacy minimizing off-target toxicity. In this study we developed a CD44-targeted antibody-drug conjugate (ADC) using doxorubicin (DOX), anti-CD44 monoclonal antibody, and a cathepsin B cleavable linker (MC-VC-PAB-PNP), to enhance drug delivery within the tumor microenvironment.

The drug-linker payload (MC-VC-PAB-DOX) was synthesized and characterized via UV-vis spectroscopy, HPLC, NMR, and LCMS. It was then further conjugated to the cysteine residue of anti-CD44 monoclonal antibody via thiol-maleimide conjugation chemistry. The drug antibody ratio (DAR) of the developed ADC was calculated using UV-Vis spectroscopy. Further characterizations were performed with HIC and SE-HPLC. *In vitro* evaluations were conducted on CD44-overexpressing SKOV3 and CD44-negative A2780 cells. *In vivo* biodistribution was carried out, using *in vivo* imaging system FOBI, to evaluate the distribution and targetability of the Cy5.5 labelled ADC in tumor and other vital organs in SKOV3 tumor bearing Balb/C nude xenograft mice. A preliminary antitumor study was caried out with three different doses of ADC (0.3 mg/kg, 1 mg/kg, and 3 mg/kg equivalent to Dox), administered on days 0, 4, 8, and 12 days once the tumor size reaches 150-200 mm^3. Tumor volume and body weight were measured every 2 days up to 24 days.

UV-vis spectroscopy, HPLC, ^{1}H-NMR, and LCMS confirmed the successful synthesis of the drug linker payload (MC-VC-PAB-DOX), with a molecular mass of 1042.65 Daltons. Final conjugates CD44-VC-DOX have drug-antibody ratio (DAR) of 3.97. HIC results showed an increase in retention time after payload conjugation, while SE-HPLC confirmed no aggregation of the ADC. CD44-VC-DOX demonstrated higher binding affinity to CD44-overexpressing SKOV3 cells, with significant internalization into lysosomes, indicating CD44-mediated uptake. Cytotoxicity studies revealed greater potency of CD44-VC-DOX, supported by live/dead assays, apoptosis, and cell cycle analysis. Biodistribution studies revealed a preferential accumulation of CD44-VC-DOX in SKOV3 tumor tissues over 72 h indicating enhanced delivery and targeting ability of the developed ADC to the CD44-overexpressing tumors. Preliminary antitumor studies demonstrated that the highest dose 3 mg/kg substantially reduced tumor growth, establishing it as the optimal dose for further studies.

In conclusion, CD44-VC-DOX effectively targets CD44-overexpressing OC cells. Its ability to target CD44 antigens is confirmed by enhanced binding, internalization, and cytotoxicity with selective tumor accumulation and antitumor efficacy in vivo. These findings highlight its potential as a possible treatment approach for CD44 overexpressing OC.