



DESCRIPTION

Fab- α RFc-CL-DMDM is a Fab fragment of an anti-rat IgG Fc specific antibody conjugated to duocarmycin DM with a cleavable linker. The antibody portion is a Fab fragment of a polyclonal antibody which is specific to the Fc region of rat IgGs. Duocarmycin is a cytotoxic small molecule which causes cell death by binding to the minor groove of DNA and alkylating the adenine. The cleavable linker connecting duocarmycin to the antibody is stable in extracellular fluid, but can be cleaved by cathepsin upon entering cells.

APPLICATIONS

Antibody-drug conjugates (ADCs), which have become a new targeted therapy against cancer, consist of an antibody linked to a cytotoxic drug. The ADCs bind selectively to the target cancer cells via the monoclonal antibody portion. Internalization of the ADCs releases the drug to do its damage. Prior to testing the function of ADCs in cell-based assays, each monoclonal antibody is typically conjugated directly with a cytotoxic drug. This step is time consuming and expensive, requiring milligram quantities of purified antibody, separate conjugation of each antibody, and further isolation of the ADC from the unconjugated drug. Using secondary antibody-drug conjugates (2^oADC) in a cell-based cytotoxic assay is a quick and economical alternative to pre-screening monoclonal antibodies as ADC candidates against tumor cells. Instead of conjugating the monoclonal antibody with a cytotoxic drug, the naked monoclonal antibody is added directly to the cells in the presence of the 2^oADC. Internalization of the monoclonal antibody/2^oADC complex can achieve a similar effect of targeted drug release within the cells as the monoclonal antibody-drug conjugate. Furthermore, the 2^oADC can also be applied to screen protein ligands for receptor-mediated cell targeting.

Fab- α RFc-CL-DMDM is a 2^oADC for pre-screening antibodies with a rat IgG Fc moiety or recombinant rat IgG Fc fusion proteins to determine their cytotoxicity as duocarmycin bioconjugates. When applied in combination with tumor specific rat monoclonal antibodies, **Fab- α RFc-CL-DMDM** can help determine the cytotoxic potential for these antibodies against target cell lines. The mono-valence nature of Fab 2^oADC may have some advantage in certain applications than the full length IgG 2^oADC.

EXAMPLE DATA

It has been demonstrated that Herceptin-DM1 conjugates (T-DM1) displayed potent killing activity against Her2-overexpressing tumor cells but not normal Her2 expression or Her2 negative cells. Here cytotoxicity of a high affinity rat anti-human EGFR (α EGFR) mAb was tested in four breast cancer tumor cell lines expressing different amount of EGFR marker. MDAMB468 is an EGFR overexpressing cell line, followed by SKBR3 and HCC1954. MCF7 has very low level of EGFR expression. The unconjugated anti-rat IgG Fc antibody (α RFc) does not change the apparent effect of the α EGFR mAb against these cell lines. *In vitro* the growth of these cells were not significantly affected by the rat α EGFR mAb. However, in the presence of 1:6 ratio of rat α EGFR mAb/**Fab- α RFc-CL-DMDM**, the α EGFR mAb displays potent cytotoxicity against EGFR-overexpressing MDAMB468 cells, some killing of SKBR3, and no significant killing towards HCC1954 or MCF7 cells (Fig B). The 2^oADC **Fab- α RFc-CL-DMDM** alone has minimal toxicity towards these cells (Fig C).

Fig A. Cytotoxic Profile of Rat α EGFR mAb Alone

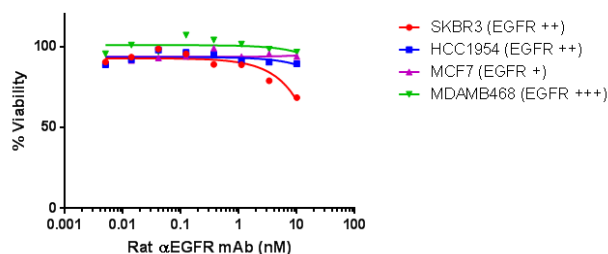


Fig B. Cytotoxic Profile of Rat α EGFR in the Presence of 1:6 Ratio of Fab α RFc-CL-DMDM

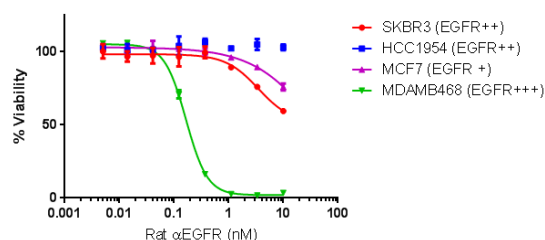
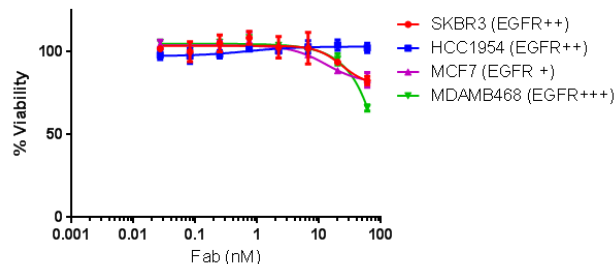


Fig C. Cytotoxic Profile of Fab α RFc-CL-DMDM Alone



STORAGE

Store in -20°C or -70°C manual defrosted freezer. Avoid repeated freeze-thaw cycle.