

O-VI.8 Gábor Tóth, László Simon, János Szöllősi, György Vereb

Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

ARE TWO THERAPEUTIC ANTIBODIES BETTER THAN ONE? COMBINATION OF TRASTUZUMAB AND PERTUZUMAB HAS SYNERGISTIC EFFECT MAINLY THROUGH ADCC

Trastuzumab, a humanized anti-ErbB2 antibody is a specific targeted therapy against ErbB2+ tumor. Another humanized antibody, pertuzumab inhibits ErbB2 heterodimerization. According to animal experiments, combined application of the two antibodies may improve treatment outcomes by either enhancement at the molecular level or offering sterically complementary docking sites for NK cells implementing ADCC. Distinction between these routes can be ascertained by also testing the F(ab)2 fragments of these antibodies.

We have generated these fragments and compared them in vitro. F(ab)2-s from IgG-s were produced with pepsin-agarose and separated with gel filtration. Affinity and lack of Fc fragment on F(ab)2 was tested with immunofluorescence in flow cytometry. In vitro EC50 was assessed with an MTT based assay. ADCC in the presence of the whole antibodies and absence thereof with F(ab)2 was tested in a real time adherence assay. The effect of the antibodies and their combination on the growth of subcutaneous xenograft breast cancer tumors in SCID mice were tested.

Chromatography was used to produce proper F(ab)2 variants with intact antigen binding but no Fc region taggable with polyclonal anti-Fc. The effect on proliferation of in vitro sensitive and resistant cell lines BT-474 and JIMT-1 was not affected by removing Fc region. Slightly enhanced dimerization inhibition by the smaller pertuzumab F(ab)2 as opposed to its intact parent antibody was measured. Based on the isobol curves of the in vitro proliferation inhibition, the combination of both the two whole and F(ab)2 antibodies seemed to be additive. Intact antibodies mediated ADCC-based killing of both tested cell lines, while F(ab)2 fragments did not. The F(ab)2 fragments did not decrease the growth of the JIMT-1 tumors in vivo. The whole antibodies introduced alone were same effective, though, and the combination of them showed strong synergism.

The results so far confirm that despite the lack of in vitro synergism, combination of trastuzumab and pertuzumab enhances ADCC in a synergistic way and this could serve as base for clinical combination therapies even in case of tumors that show resistance in vitro.