

ARE TWO THERAPEUTIC ANTIBODIES BETTER THAN ONE? PRODUCTION AND IN VITRO TESTING OF HUMANIZED ANTI-ERBB2 F(AB)2-S AND THEIR PARENT ANTIBODIES

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AIMS: Trastuzumab, a humanized anti-ErbB2 antibody is a specific targeted therapy against ErbB2 positive tumors, with a history of both success and a high rate of therapy resistance. Another humanized antibody, pertuzumab inhibits ErbB2 heterodimerization. Combined application of the two antibodies may improve treatment outcomes by either enhancement at the molecular level (e.g. by hypercrosslinking-based enhanced internalization of ErbB2), 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. In the first phase, we have generated these fragments and compared them in vitro.

METHODS: F(ab)2 from trastuzumab and pertuzumab was 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 Ki 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.

RESULTS: Since protein A and protein G still showed binding of cleaved F(ab)2, chromatography optimized for exclusion size and ionic strength was used to produce proper F(ab)2 variants with intact antigen binding but no Fc region taggable with polyclonal anti-Fc. The produced F(ab)2 fragments effectively competed for ErbB2 binding sites with their parent antibodies on breast tumor cells as revealed by flow cytometry. The in vitro effect on proliferation of the in vitro trastuzumab sensitive BT-474, and the in vitro resistant JIMT-1 cell lines was not affected by removing the FC region. The only measurable difference was a slightly enhanced dimerization inhibition by the smaller pertuzumab F(ab)2 as opposed to its intact parent antibody. This effect appeared coherent with the decreased EC50 of the F(ab)2 compared to the parent pertuzumab.

Intact antibodies mediated ADCC-based killing by freshly isolated human peripheral blood mononuclear cells of both tested cell lines, while F(ab)2 fragments did not mediate ADCC.

Based on the EC50 values determined as single agents on BT-474 cells, isoboles for a range of combinations were also measured for both the whole antibodies and the F(ab)2 fragments. In spite of earlier observed potentiation effects with anti ErbB1 antibodies, in this model system combination were only additive, hinting that potentiation may only occur in vivo, in an ADCC dependent manner.

CONCLUSIONS: The set of trastuzumab and pertuzumab whole antibodies and their F(ab)2s are now ready for in vivo testing of their possible synergistic effects. Possibly enhancement by the combination of ADCC-only is currently being tested with the JIMT-1 xenograft model, which we have earlier shown to be killed by this mechanism exclusively. Mixed mode killing will be tested with BT-474 and N87 cell lines, sensitive in vivo to both interference of the antibody with signaling pathways and ADCC.