

Production and testing of anti-ErbB2 F(ab)2-s

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Aim: 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 effect on proliferation of in vitro sensitive and resistant cell lines BT-474 and JIMT-1 was not affected by removing 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. Also, intact antibodies mediated ADCC-based killing of both tested cell lines, while F(ab)2 fragments did not.

Conclusions: The set of trastuzumab and pertuzumab whole antibodies and their F(ab)2s are now ready for in vitro and in vivo testing of their possible synergistic effects.

Keywords: ErbB2, trastuzumab, pertuzumab, F(ab)2