

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

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INTRODUCTION

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. While these antibodies have been developed based on the in vitro direct cellular effect of their mouse parent antibodies, there is the possibility that their in vivo mechanism of action could be quite different, such as antibody dependent cellular cytotoxicity (ADCC) exerted by NK cells

AIM

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.

OBJECTIVES

1. To produce F(ab')₂ fragments from the trastuzumab and pertuzumab IgG antibodies
2. To separate the F(ab')₂-s from the IgG antibodies
3. To verify that we obtained the expected bivalent fragments that only differ in the lack of Fc chain from the parent IgG
4. To assess the effect of the therapeutic agents alone and in combination on in vitro tumor cell proliferation.
5. To assess the effect of pertuzumab and pertuzumab F(ab')₂ on ErbB2 heterodimerization
6. To verify the ability of the IgG and F(ab')₂ antibodies to evoke antibody-dependent cell-mediated cytotoxicity in vitro.

METHODS

Whole trastuzumab and pertuzumab antibodies were digested with pepsin-agarose. Separation of digested fragments was tested with protein A and G and 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 or their F(ab')₂s was tested in a real time adherence assay.

RESULTS

1. Production of F(ab')₂ from trastuzumab and pertuzumab was optimized with pepsin-agarose digestion
2. Since protein A and protein G showed binding of cleaved F(ab)₂, chromatography optimized for exclusion size and ionic strength was used to separate F(ab')₂ from IgG
3. Proper F(ab')₂ variants showed intact antigen binding but no Fc region taggable with polyclonal anti-Fc
4. The effect on proliferation of in vitro sensitive BT-474 and resistant JIMT-1 cell lines was not affected by the removal of the Fc region. Based on the EC₅₀ values determined as

single agents on BT-474 cells, isoboles for a range of combination were also measured for both the whole antibodies and the F(ab')₂ fragments. Enhanced dimerization inhibition by the smaller pertuzumab F(ab')₂ was identified as opposed to its intact parent antibody¹.

5. Intact antibodies mediated in vitro ADCC-based killing, while F(ab')₂ fragments did not mediate ADCC.

CONCLUSIONS

A set of antibodies and their functional F(ab')₂-s which do not mediate ADCC but exert similar direct cellular effects are now ready for testing on JIMT-1 and N87 xenografts in SCID mice which represent in vitro trastuzumab resistant and sensitive cell lines, respectively.

REFERENCES

1. Roszik J, Tóth G, Szöllősi J, Vereb G. Validating pharmacological disruption of protein-protein interactions by acceptor photobleaching FRET imaging. *Methods Mol Biol.* 2013; 986:165-78.