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Research paper

"Two-in-One" approach for bioassay selection for dual specificity antibodies

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ABSTRACT

Dual specific antibodies and bispecific antibodies that recognize two different antigen targets are currently being regarded as very effective therapeutics for complex human diseases. While effective, designing and developing a bioassay strategy for dual specific antibodies that is reflective of the mechanism of action (MoA) and also measures the dual activities of antibodies pose unique and exciting challenges. An *important* question asked while developing a bioassay for dual specific antibodies is, "*How many bioassays will be needed, one bioassay or two separate bioassays*?" Here we present an approach of using one bioassay for a dual specific antibody that targets two receptors in signaling pathways. The presented assay is able to measure the antibody effects on both target bindings, which would not be achievable using two separate assays. Furthermore, this assay can detect changes *in the binding of either target, which impact overall efficacy of the* antibody. Its improved sensitivity enables substituting two binding assays with this one bioassay for lot release and stability testing to measure any changes on either target binding, ensuring consistency between lots. This is a single-bioassay approach for a dual specific antibody that is MoA reflective of the intended therapeutic indication. The demonstrated assay development and bridging study strategy for this bioassay for a dual specific mAb1 could be applicable to the other dual specific, bispecific antibodies, and antibodies used for combination therapy.

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1. Introduction

The field of monoclonal antibody (mAb) therapeutics is growing fast. To date, more than 30 IgGs and their derivatives have been approved for various indications, and more are being developed (Beck et al., 2010). For many complex diseases such as cancer and infectious disease, which involve redundant or synergistic action of disease mediators such as crosstalk between their signaling networks, improved therapeutic efficacy may result from blocking multiple, different pathological factors and pathways (Gao et al., 2011; Huang et al., 2004; Ignar-Trowbridge et al., 1992). In recent years, interest in dual specific antibodies has grown because they are able to bind two target antigens and block signaling pathways and pathological factors more efficiently than conventional monoclonal antibodies (Garber, 2014; Huang et al., 2013; James et al., 2003; Kontermann, 2014; Mariuzza, 2006; Rouet and Christ, 2014; Schaefer et al., 2011) that target a single antigen. Compared with bispecific antibodies, whose structures have two different half mAb arms or antigen binding fragments (Fabs), the dual specific antibody is more like the traditional mAb having identical Fabs. As such, manufacturing, stability data, and PK properties could be leveraged from the known conventional mAb, presenting dual specific antibodies as attractive alternatives for monospecific antibodies with better efficacy (Fig. 1a).

A bioassay, commonly called potency assay, is required for the release and stability testing of biologic drug substances and products, and it is expected to be reflective of the mechanism of action (MoA) of the therapeutic antibody. Development of a suitable bioassay(s) is particularly challenging for dual specific antibodies, which bind two targets and have potential additive/synergistic effects on the two interactive downstream signaling pathways. How to develop a bioassay(s) that is not only reflective of the MoA but also measures the dual activities of the antibody is an outstanding question in the study of dual specific and bispecific antibodies. Here we present the strategy and development of a single "Two-in-One" bioassay for a dual specific antibody. The single bioassay was demonstrated to be MoA reflective and able to detect changes in recognition of either target. Furthermore, it was shown to be able to capture the additive effects, which would not be achieved using two separate assays measuring the antibodies effects on each target.

2. Materials and methods

2.1. Materials

Recombinant dual specificity antibody mAb1, mAb1 antibody mutants, mAb1 stressed samples, anti-HER3, anti-EGFR were generated at

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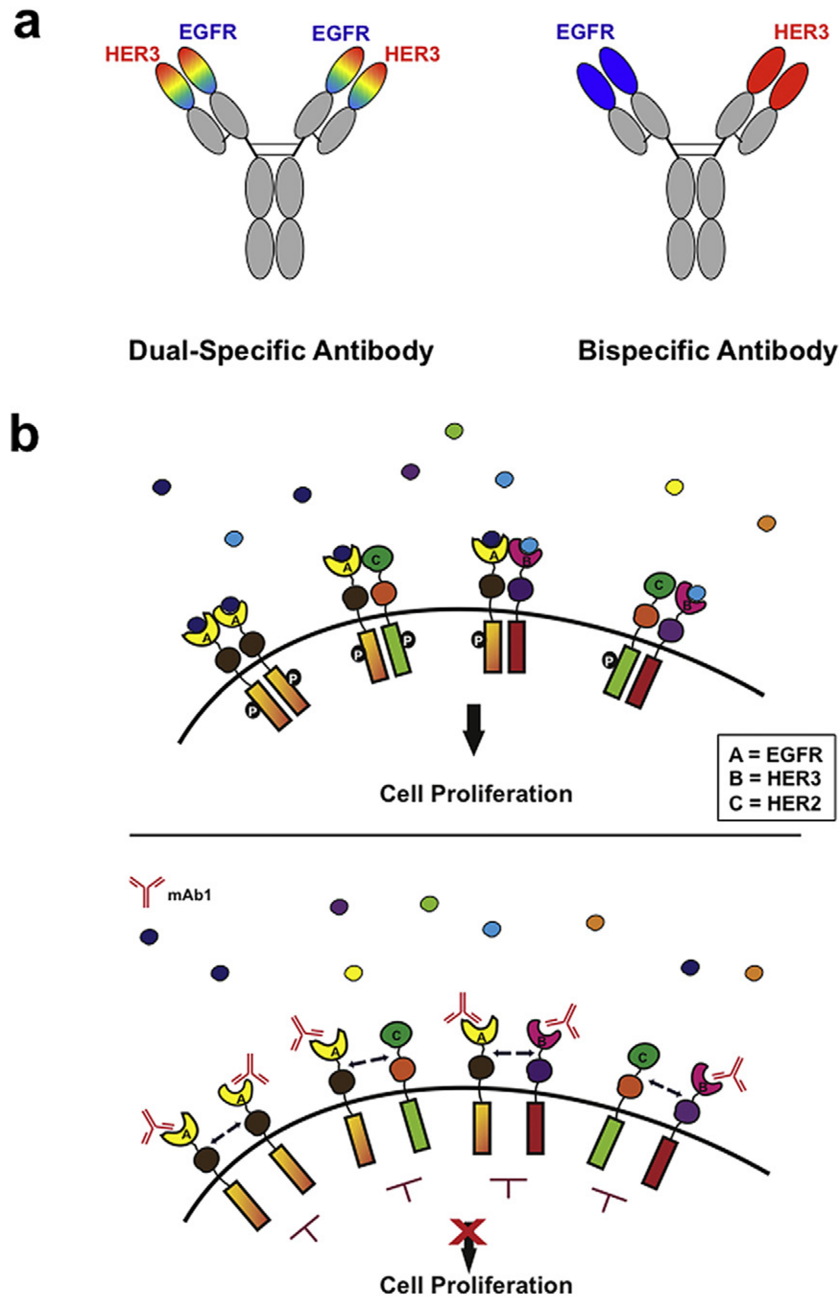

Fig. 1. Dual specific antibody mAb1. (a) The dual specific antibody has two identical arms that recognize two antigens EGFR and HER3. A bispecific antibody has two different arms, each recognizing a different antigen EGFR or HER3. (b) Mechanism of action of mAb1 blocking signaling pathways and the effect on cell proliferation.

Genentech (a member of the Roche group, South San Francisco, CA). HER3 and EGFR protein reagents (EGFR extracellular domain [ECD], HER3 ECD) used for the binding assays were also generated at Genentech. Cell line FaDu was originally obtained from ATCC.

2.2. Methods

2.2.1. HER and EGFR binding assay

Briefly, 96-well Maxisorp immunoplates were coated with HER3 or EGFR overnight at 4 °C. The following day, the plates were blocked with 200 μL/well blocking buffer (0.5% bovine serum albumin [BSA] in phosphate buffered saline [PBS]) followed by the addition of 100 μL of mAb1 samples diluted in assay diluent (PBS, 0.5% BSA and 0.05% Polysorbate-20) in duplicate wells. The plates were incubated at room temperature with agitation for 1–2 h, washed with wash buffer (PBS, 0.05% Polysorbate P20) three times, followed by the addition of 100 μL horseradish peroxidase (HRP) conjugated anti-human IgG and incubated for 1–2 h. Finally, the plates were washed and the HRP substrate 3,3′,5,5′-tetramethylbenzidine was added to the wells. Color development was stopped by the addition of 0.6 N sulfuric acid, and absorbance was measured at 450 nm, with reference at 650 nm, using a plate reader. Results, expressed in optical density, were plotted against the antibody concentrations using a four-parameter curve fitting program.

2.2.2. Anti-cell proliferation assay (cell-based assay)

The anti-cell proliferation assay measures the ability of mAb1 to inhibit the proliferation of FaDu cells, which express endogenous HER3 and EGFR. In this assay, cells were plated at 1.0×10^5 cells/mL in a volume of 50 μL in normal growth media with 1% fetal bovine serum (FBS). mAb1 standard, control, and samples were diluted and added to the plate. Because the cells used for the assay secrete ligands for receptors (autocrine), no additional ligand was added for the assay. Following a

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