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Research paper

Identification and inhibition of drug target interference in immunogenicity assays

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ABSTRACT

A well-designed anti-drug antibody (ADA) immunoassay is critical for appropriately monitoring the immunogenicity profile of a therapeutic protein during its development. AMG 386 is a peptide-Fc fusion protein that inhibits angiogenesis by preventing the interaction of angiopoietins with the Tie2 receptor. In bridging immunoassays for ADA, interference by the drug target, present in the assay sample, can result in false positive antibody detection. We used a statistical design-of-experiments approach to identify angiopoietin interference in bridging immunoassays of anti-AMG 386 antibodies. We also demonstrated that a high-affinity monoclonal antibody, directed against an epitope on angiopoietin that competes with AMG 386 binding, could inhibit the angiopoietin interference while preserving the detection of ADA. This report describes the development and validation of methodologies for evaluating and addressing drug target interference in bioanalytical assays that involve interactions between drug, ADA, immune complexes, and drug target.

1. Introduction

Therapeutic proteins have the potential to elicit antibody responses in animals and humans (Mire-Sluis et al., 2004; Swanson, 2006; Koren et al., 2008). An immune response to therapeutic proteins has the potential to neutralize the therapeutic effect and can also influence the results obtained in studies of drug exposure and efficacy. In some cases, the anti-drug antibodies (ADA) can lead to adverse events, including acute hypersensitivity. The methods to detect specific ADA during preclinical and clinical development of therapeutic proteins have evolved from the use of direct binding to the bridging formats in the assay design. This evolution has led to a better understanding of the interference of high concentrations of drug. Examples of assays used to detect ADA include the BIAcore biosensor assay for detecting low-affinity and IgM antibodies, the enzyme-linked immunosorbent assay (ELISA), and the electrochemiluminescence (ECL) immunoassay for measuring high-affinity antibodies in the presence of high concentrations of circulating drug (Swanson, 2003; Swanson et al., 2004; Moxness et al., 2005; Patton et al., 2005; Thorpe and Swanson, 2005a,b; Lofgren et al., 2007; Sickert et al., 2008; Swanson and Chirmule, 2009). We have evaluated the potential of the drug target to interfere with these assays. Our results highlight the complexity of interactions that occur in immunogenicity assays and that include drug, ADA, and drug target, in free and complexed forms.

Ligand–receptor interactions between soluble factors, such as cytokines and growth factors in the downstream cell-signaling pathways, have been targeted successfully in the development of novel therapeutic proteins (Jones et al., 2008).

Abbreviations: ADA, anti-drug antibody; ELISA, enzyme-linked immunosorbent assay; ECL, electrochemiluminescence; peptibody, peptide-Fc fusion protein; Ang, angiopoietin; ACP, assay cut point; DOE, design-of-experiments; S/N, signal-to-noise ratio; NC serum, negative control serum.

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Angiogenesis, the formation of new blood vessels from pre-existing vessels, is essential for tumor growth and metastasis (Folkman, 1995; Fidler et al., 2001). There is evidence to suggest that the production of proangiogenic substances by cancer cells or the density of microvasculature in tumors may be a useful tool when determining the tumor prognosis in cancer patients (Leek, 2001; Mehta et al., 2001; Papamichael, 2001; Dvorak, 2002; Scappaticci, 2002; Reinmuth et al., 2003; Hurwitz et al., 2004). Human angiopoietins are secreted proteins that bind to the Tie2 receptor on vascular endothelial cells and hematopoietic cells and play key roles in the maturation and structural integrity of both blood and lymph vessels (Peters et al., 2004; Scharpfenecker et al., 2005; Shim et al., 2007; Augustin et al., 2009). Out of the 4 angiopoietins that have been identified to date, angiopoietin 1 (Ang1) and angiopoietin 2 (Ang2) are the best studied. Ang1 and Ang2 appear to have separate and sometimes antagonistic functions; Ang1 has been shown to stabilize blood vessels and Ang2 is involved in vascular remodeling (Augustin et al., 2009). Higher levels of Ang2 in some human tumors have been associated with more advanced disease and poorer prognosis (Bunone et al., 1999; Etoh et al., 2001; Leek, 2001; Mehta et al., 2001; Papamichael, 2001; Dvorak, 2002; Scappaticci, 2002; Reinmuth et al., 2003; Sfiligoi et al., 2003). AMG 386 is an investigational, angiopoietin antagonist peptide-Fc fusion protein (peptibody) that suppresses angiogenesis by selectively inhibiting the interaction of angiopoietins with the Tie2 receptor (Oliner et al., 2004).

In this manuscript, we report on the development and validation of a sensitive bridging ECL immunoassay. We have used a statistical design-of-experiments (DOE) approach to demonstrate that the presence of angiopoietin can interfere with the detection of anti-AMG 386 antibodies in the bridging ECL immunoassay. Further, we have developed a novel method to address drug target interference in bridging immunoassays.

2. Materials and methods

2.1. Equipment

The following instruments and reagents were used: Meso Scale Discovery (MSD) Sector® Imager 6000 instruments, MSD Read Buffer T, and MSD Avidin High Bind plates (MSD, Gaithersburg, MD); Freedom EVO liquid handling workstations (Tecan, Mannedorf, Switzerland), and ELX-405 Plate Washers (BioTek Instruments, Winooski, VT).

2.2. Antibodies and assay reagents

AMG 386, affinity-purified anti-AMG 386 rabbit polyclonal antibody, and human anti-angiopoietin monoclonal antibodies (MAb1, MAb2, and MAb3) were produced and prepared by Amgen Inc. (Thousand Oaks, CA). Biotinylation and ruthenium conjugation of AMG 386 were carried out using MSD SULFO-TAG™ NHS-Ester (MSD) according to the manufacturer's instructions. Ang1 and Ang2 were purchased from R&D Systems (Minneapolis, MN). Pooled normal human serum was obtained from Bioreclamation Inc. (Oceanside, CA).

2.3. Serum samples

Serum samples were collected from a total of 70 patients with solid tumors enrolled in two phase 1b studies. Patients were treated either with AMG 386 plus various chemotherapy regimens (22 patients) (Mita et al., 2007a,b) or with AMG 386 in combination with various VEGF inhibitors (48 patients) (Hong et al., 2008). Five milliliters of blood was drawn pre-infusion of AMG 386 at week 1 (predose baseline), week 2, week 4, and every 4 weeks thereafter during drug treatment, and serum was prepared. A total of 557 serum samples were collected at various time points and stored at −20 °C or colder before analysis for anti-AMG 386 antibodies.

2.4. Acid-dissociation, bridging ECL immunoassay

An acid-dissociation, bridging ECL immunoassay (henceforth referred to as the bridging ECL assay) was used to measure anti-AMG 386 antibodies (Fig. 1). Samples were diluted to 20% in 300 mM acetic acid in a nonbinding polypropylene 96-well plate (acid treatment plate) to enable antibody–drug complex dissociation before analysis. The acid treatment plate was incubated for 1 to 2 h with shaking at an ambient temperature.

Fifty microliters of the acidified sample from each well of the acid treatment plate was then transferred to the designated wells of another nonbinding polypropylene plate (incubation plate) containing an assay reagent mixture of 14 µL of a Tris base solution (1 M, pH 9.5), an equal-mole mixture of 0.125 µg/mL of biotinylated AMG 386 and 0.125 µg/mL of ruthenium-conjugated AMG 386, and 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS). The incubation plate was incubated for >18 h in the dark at an ambient temperature.

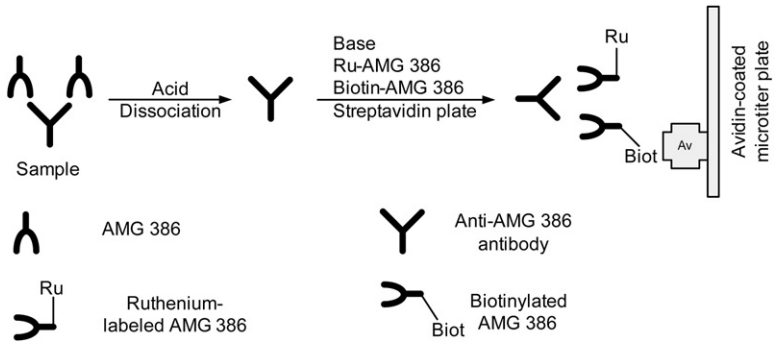

Fig. 1. Acid-dissociation, bridging ECL immunoassay for detection of anti-AMG 386 antibody.