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Collaborative study using common samples to evaluate the performance of anti-drug antibody assays constructed by different companies

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ABSTRACT

This study was undertaken to evaluate the performance of anti-drug antibody (ADA) assays constructed by each participating company using common samples including ADA, drug and human serum. The ADA assays constructed by each company showed good sensitivity and precision for evaluation of ADA. Cut points for screening and confirmatory assays and assay selectivity were determined by various calculation methods. In evaluations of blind ADA samples, nearly similar results were obtained by the study companies in determinations of whether samples were positive or negative except at the lowest sample concentration (5 ng/mL). In measurement of drug tolerance, for almost samples containing ADA and drugs, more positive results were obtained in assays using acid dissociation compared to those without acid dissociation. Overall, the performance of ADA assays constructed by the 10 companies participating in this study was acceptable in terms of sensitivity and reproducibility for detection and evaluation of immunogenicity in both patients and healthy subjects. On the other hand, based on results for samples containing ADA and drugs, validity of results for ADA assays conducted without acid dissociation was less meaningful and more difficult to evaluate. Thus, acid dissociation was confirmed to be useful for improving drug tolerance.

1. Introduction

Immunogenicity is the propensity of a therapeutic protein product to provoke immune responses that result in induction of anti-drug antibody (ADA), cytokine, or other molecules that are

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associated with various potential clinical consequences such as reduction or loss of efficacy, as well as altered pharmacokinetics and hypersensitivity, and is a major concern in the development and post-marketing use of biotechnology-derived products [1]. Therefore, immunogenicity should be evaluated by assays for which the validity has been fully and adequately evaluated. There are several types of ADA assays, including enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence (ECL), radioimmunoprecipitation and surface plasmon resonance [2].

Several white papers and other resources are available to help ensure that ADA assays produce meaningful results to enhance patient safety and product efficacy [3–11]. ADA generation is usually evaluated by tiered approaches, including screening, confirmation and characterization [2]. The samples are analyzed by screening assays to detect antibodies that bind to a given drug and then analyzed by confirmatory assay(s) to determine whether the binding to the drug is truly specific. If an ADA-positive sample is found in screening and confirmatory assays, further characterization of the ADA is conducted to determine the ADA neutralizing activities and isotypes.

However, in practice, objective evaluations of the validity of a given ADA assay can be difficult for pharmaceutical industrial companies and contract research organizations (CRO) because there are few opportunities for mutual comparisons of individual ADA assay performance. According to a survey by the American Association of Pharmaceutical Scientists, various methods are used to detect and evaluate ADA assays between companies [12]. Thus, the use of different methods that can have different detection and evaluation approaches may make the interpretation of ADA efficacy and safety challenging.

With this background, we initiated for the first time a joint government-private sector effort in Japan to construct and evaluate ADA assays of common samples using the methods defined and designed by each company and CRO. The ADA assays that were confirmed to be acceptable are expected to yield more reliable detection of ADA and evaluation of the influence of immunogenicity on the efficacy and safety of products in clinical trials and post-marketing surveillance by the companies and CROs, and to provide efficacious and safe products to the health care market.

2. Materials and methods

2.1. Materials

Individual human serum samples and pooled human serum were purchased from Biopredic International (Saint Gregoire, France). Belimumab was purchased from OZ International JAPAN (Tokyo, Japan).

2.2. Preparation of antibodies against belimumab

Belimumab is a fully human IgG1λ recombinant monoclonal antibody directed against BLys [13] that is used to treat systematic lupus erythematosus and as an immunogen for ADA preparation. ADA preparation was outsourced to Medical & Biological Laboratory Co., Ltd. as described below. All animal experiments were performed according to protocols approved by the Animal Care and Examination Committee of Medical & Biological Laboratory Co., Ltd. To generate an ADA positive control, three rabbits weighing on average 3 kg were immunized by an initial administration of a 50% emulsion of 0.1 mg belimumab in Freund's complete adjuvant followed by four weekly booster injections of a 50% emulsion of 0.1 mg belimumab in Freund's incomplete adjuvant. Whole blood was collected from the rabbits three weeks after the final booster injection. ADA was purified by belimumab affinity chromatography followed by human IgG affinity chromatography.

2.3. ADA assay format

2.3.1. ECL

Companies A-J referenced below indicate anonymous names of companies participating in the study. ECL was used by all companies, except Company I, to measure ADA. Measurement of ADA was mainly performed according to the manufacturer's instructions [14], except for that used by Companies E and F (see below). Although some companies made minor modifications to the protocol, these modified protocols did not differ substantially from the original. Streptavidin Gold plates were used by all companies using ECL. Supplementary Table 1 lists the concentrations of SULFO-TAG labeled and biotinylated belimumab solutions, rather than the final concentration in the assay mixture. Reagents and test solutions used for the assay were independently prepared at each company unless otherwise noted.

Company E used acid dissociation in all assays wherein 50 μL sample and 24 μL 1 M acetic acid were added to a polypropylene plate and incubated for 5 min. The mixture was then incubated for 1 h with reaction buffer consisting of biotinylated belimumab solution, SULFO-TAG labeled belimumab solution and 1 M Tris-HCl (pH 9.5) at a vol:vol ratio of 25. A 50 μL aliquot of the incubation mixture was used for further experiments. Other experimental conditions were the same as those described in the manufacturer's instructions [14]. Company F also used acid dissociation in some experiments by mixing 25 μL sample with 12 μL 1 M acetic acid for 5 min instead of 40–45 min, which is the recommended time indicated in the manufacturer's instructions [14]. Other experimental conditions were similar to those described in the manufacturer's instructions [14].

2.3.2. ELISA

ELISA was used for measurement of ADA by Company J. The basic protocols were carried out as previously described [15,16]. Biotinylated and digoxigenylated belimumab solutions were prepared according to standard protocols [17]. Biotinylated belimumab (100 μL of 100 ng/mL) in reaction buffer consisting of PBS containing bovine serum albumin (5 mg/mL), Block Ace (1% v/v, DS Pharma Biomedical (Osaka, Japan)) and Tween 20 (0.05% (v/v)) was incubated in a streptavidin-coated Micro Titer Plate for 90 min. The wells were then washed 4 times with T-PBS, PBS (pH 8.0) containing 0.05% Tween 20. Sample (100 μL) was added to the well and then incubated for 3 h at 37 °C before washing 4 times with T-PBS. A 100 μL aliquot of anti-DIG-HRP obtained from Roche Diagnostics GmbH (Mannheim, Germany) was diluted 2000-fold with reaction buffer, added to each well and incubated for 30 min at 37 °C. The wells were then washed 4 times with T-PBS at 37 °C before 100 μL of TMB solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD USA) was added to each well and incubated for 10 min at room temperature. The enzyme reaction was terminated by adding 100 μL sulfuric acid (1 M) to each plate. After slightly mixing the solutions, absorbance values were measured at 450 nm and at 620 nm for reference. The absorbance at the reference wavelength was calculated by subtracting the absorbance at 620 nm from the absorbance at 450 nm.

2.4. Validation items

2.4.1. Minimum required dilution

The minimum required dilution (MRD) was mainly determined based on the recovery of the response ratio of ADA samples at several concentrations (Supplementary Table 2), except for