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Detection of low-affinity anti-drug antibodies and improved drug tolerance in immunogenicity testing by Octet® biolayer interferometry

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ABSTRACT

We assessed the utility of the FortéBio Octet® system for detection of anti-drug antibodies (ADAs) against an investigational therapeutic human IgG1 monoclonal antibody (mAb), CNTO X. To understand the relative merits of this technology, key performance requirements were compared with two popularly accepted ADA detection methods, a step-wise bridging ELISA and a Meso Scale Discovery (MSD) homogeneous (single step binding) bridging ECLIA. When used to detect 13 monoclonal ADAs of varying affinities and one polyclonal ADA, all three methods demonstrated their greatest apparent sensitivity to the polyclonal sample (1, 6, and 130 ng/mL, respectively for ECLIA, ELISA, and Octet). Sensitivity to monoclonal ADAs tended to vary in accordance with their affinities, however, the sensitivity of the Octet method varied much less between ADAs. As a result, the above ranking became reversed such that Octet was the most and ELISA least sensitive for detection of low-affinity ADAs. With regard to drug tolerance, the presence of CNTO X could lead to false-negative assay results, although each method was affected to a different degree, with the Octet method tolerating up to 10 times more drug than the ECLIA method, which in turn tolerated up to 10 times more than the ELISA. Finally, the ECLIA and Octet methods were applied to the bioanalysis of cynomolgus monkey sera from a pre-clinical multiple dose study of CNTO X. Octet indicated 3 positive animals developed ADA as early as day 15 of the dosing phase while drug was present at nearly 1 mg/mL. ECLIA detected only one of these, and only in a day 57 recovery sample after drug had cleared from circulation. We conclude that the Octet is a promising platform for detection of lower affinity ADAs and is particularly suitable for ADA detection when drug persists at levels that negatively impact bridging immunoassays.

1. Introduction

The administration of therapeutic biologic drugs can induce anti-drug antibody immune responses in study subjects. These immune responses can produce a range of effects from benign and asymptomatic to altered pharmacokinetics and/or pharmacodynamics and adverse clinical sequelae [1,2]. It is therefore important to use sensitive and reliable bioanalytical methods to monitor the ADA status of study subjects during investigational treatment with biologic drug products [3,4].

Numerous assay technologies have been used for the detection of ADAs, each of which is associated with relative merits and weaknesses [5]; however, electrochemiluminescent immunoassays (ECLIA) have become very popular, and a recent survey indicates that ELISA remains the most widely used technology for this purpose, even though 90% of laboratories are also considering other technologies for potential advantages in the detection of immunogenicity [6]. To date, all ADA assay technologies are prone to false-negative results due to interference caused by drug that remains in circulation after dosing [7–9]. To make assays more "drug-tolerant" it is common practice to pre-treat samples with acid to disassociate immune complexes; however, this may inactivate some ADAs without completely eliminating drug interference. Although many techniques are available to improve the sensitivity and drug tolerance of ELISA assays [10–15], it is often easier to reach the same goals using ECLIA [16,17]. ELISA- and ECLIA-based ADA assays are often designed as bridging methods in which ADA is captured by one molecule of drug then detected when concurrently bound to a second molecule of drug. Biolayer/biomolecular interaction (BLI) paired with surface plasmon resonance (SPR) detects binding events via changes in light reflectance as proteins accumulate on a sensor, and thereby circumvent the need for a detection label, thus eliminating one binding event required of a bridging format as well as eliminating the need to conjugate the drug to a label. They are ideal for use with simple buffer systems, but are less suitable for complex matrices, such as serum, which may present clots and varied background effects between individuals. Biacore® (GE

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Healthcare, Piscataway, NJ) and ProteOn® (Bio-Rad Laboratories, Hercules, CA) are SPR-based biosensor technologies successfully used to detect clinically relevant ADAs, particularly low-affinity antibodies [18,19], however, they have not been widely adopted due to the modest throughput compared to microtiter plate methods, specialized equipment, the high price of the equipment and consumables, the extensive training required to develop expertise, and the complexity of data interpretation. The Octet® System (FortéBio, Inc., Menlo Park, CA) is another biosensor technology that employs a related BLI system (for a comprehensive review of the principles of BLI, the reader is referred to a prior publication [20] or the manufacturer's website http://www.fortebio.com). With this system, analysis occurs on disposable fiber tips in a "dip and read" manner [21–23] using 96- or 384-well microtiter plates. For determination of binding kinetics there is evidence that the Octet generates kinetic binding constants comparable to other biosensor instruments [22,24,25]. Similar comparability assessments for non-kinetic uses have not been published; however, we predicted that Octet would maintain key benefits of related biosensor technologies, while permitting simplified data analysis without microfluidics that may become clogged by serum.

We compared Octet with two of the most widely employed methods, a step-wise bridging ELISA and a MSD homogeneous bridging ECLIA, for the detection of ADAs against CNTO X, an investigational therapeutic human IgG1 monoclonal antibody (mAb) that neutralizes a soluble inflammatory human cytokine. After optimizing each method, our comparisons focused on assay sensitivity and drug tolerance, characterizing performance against sera spiked with purified ADAs and/or CNTO X. Then the two most promising methods (ECLIA and Octet) were evaluated by analyzing cynomolgus monkey sera from a pre-clinical multiple dose study of CNTO X. Although the manufacturer suggests using the Octet to detect ADA responses, to the best of our knowledge this is the first demonstration of an Octet ADA assay being applied to a pre-clinical study.

2. Materials and methods

2.1. Pre-clinical cynomolgus monkey study

A non-GLP toxicity study of CNTO X was previously conducted by our company to evaluate the tolerability of the biologic and to establish a toxicokinetic profile of this mAb when administered subcutaneously (SC, 20 or 100 mg/kg) or intravenously (IV, 100 mg/kg) to cynomolgus monkeys weekly for 4 weeks. Twelve (3 per CNTO X treatment group and 3 treated with control vehicle) female cynomolgus monkeys (Mauritius-origin, Covance Research Products, Alice, TX) were used in this study. The animals were dosed on days 1, 8, 15, and 22, and measurements of CNTO X serum concentrations and detection of ADAs were performed on samples collected at several time points during the dosing phase of the study as well as a sample taken on day 57 (35 days following the last injection). CNTO X concentration determinations were performed using an ECLIA (Meso Scale Discovery, Inc.) method that employed biotin- and ruthenium-conjugated anti-idiotypic antibodies to capture and detect CNTO X. A standard curve was employed (lower limit of quantification, 0.02 μg/mL) to quantify CNTO X in the samples. ADA detection was performed using both the bridging ECLIA (Section 2.6) and the Octet method (Section 2.7) using the Octet-RED device. Individual subjects' serum concentration–time profiles were generated for comparison against ADA results.

2.2. Generation of anti-CNTO X antibodies

In accordance with applicable regulations concerning the ethical use of laboratory animals, ten Balb/c mice (12–14 weeks old) were immunized with CNTO X, a human anti-cytokine monoclonal antibody. Lymphocytes were isolated from the immunized mice and were subsequently fused to FO myeloma cells. Solid phase ELISA was used to screen hybridoma supernatants for CNTO X binding antibodies. The IgG fraction of the hybridoma culture supernatant was purified by protein G affinity chromatography. Thirteen mAbs reactive to the variable region of CNTO X were identified and further characterized.

To generate a polyclonal antibody reagent, two cynomolgus monkeys were hyperimmunized by an initial administration of a 50% emulsion of 1 mg CNTO X per kg body weight in Hunter's TiterMax (CytRx Corp., Los Angeles, CA) followed every third week by booster injections of a 50% mixture of Imject Alum (Pierce, Rockford, IL) and 0.1 mg CNTO X per kg body weight. Blood was collected from the animals after multiple rounds of boosting. Polyclonal antiserum from the animal with the greater anti-CNTO X antibody titer was purified by protein G followed by CNTO X affinity chromatography.

2.3. CNTO X conjugation

For the ELISA and Octet applications, CNTO X was labeled with biotin using EZ-Link Sulfo-NHS-LC biotinylation kits (Pierce, Rockford, IL) according to the manufacturer's instructions. The biotin:protein ratio for ELISA was 9:1 and for Octet was 3:1.

For the ECLIA application, CNTO X was conjugated with a ruthenium bipyridine-sulfo-NHS (BvTAG) according to the manufacturer's (Meso Scale Discovery, Gaithersburg, MD) instructions. The 8:1 conjugate:protein labeling ratio was optimized to provide the greatest signal-to-noise ratio between an ADA-containing sample and a negative matrix sample in the ECLIA.

2.4. Antibody affinity determination

The binding affinities of 13 ADAs (mouse monoclonal anti-CNTO X antibodies) were determined using a Biacore® 3000 optical biosensor equipped with a research-grade CM5 sensor chip (GE Healthcare, Piscataway, NJ). Amine coupling reagents, N-ethyl-N′-dimethylamino-propylcarbodiimide (EDC), N-hydroxy-succinimide (NHS) and sodium ethanolamine–HCl, pH 8.5, were obtained from GE Healthcare. Standard coupling protocols were used to tether a goat anti-mouse Fc_γ polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) onto the biosensor surfaces [20,26]. The experiments were performed at 25 °C in PBS containing 3 mM EDTA and 0.005% P20. Anti-drug antibodies were diluted to approximately 1 μg/mL and captured on each of three immobilized anti-mouse Fc surface. Between 20 and 50 response units (RU) of each ADA were captured by the goat anti-mouse Fc_γ-specific antibody. After ADA capture, randomly ordered concentrations of CNTO X, spanning 4–500 nM, were injected in running buffer at 60 μL/min for 3 min. Dissociation was monitored for 15 min and each measurement was repeated in triplicate. Between measurements, the biosensor surfaces were regenerated with two 6-s pulses of 50 mM phosphoric acid. To increase data confidence, multiple buffer cycles were included in each of the triplicate assays [27]. Each data set was fitted globally to a 1:1 interaction model (BIA evaluation 3.1, GE Healthcare, Piscataway, NJ) to determine the kinetic parameters k_a and k_d. Apparent affinities were then calculated as a ratio (k_d/k_a) of these rate constants.

2.5. Bridging ADA ELISA

This method was developed and optimized using the hyperimmunized monkey polyclonal ADA obtained previously. Microtiter plate wells were coated with 1 μg/mL CNTO X in 0.1 M carbonate