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Correlation of screening and confirmatory results in tiered immunogenicity testing by solution-phase bridging assays

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ABSTRACT

Biotherapeutic proteins induce undesired immune responses that can affect drug efficacy and safety. For this reason, immunogenicity assessment is an integral part of drug development and is mandated by the regulatory authorities. Immunogenicity is typically evaluated by a tiered approach consisting of a screening assay followed by a competitive inhibition with unlabeled drug serving as confirmatory assay and additional characterization of the immune response. The confirmatory assay is intended to reduce the number of false positive responses generated in the screening tier and ensure that all samples are correctly classified as positive or negative. The positive–negative sample decisions are based on screening and confirmatory assay cut points that are statistically derived through evaluation of drug-naive samples. In this paper, we describe the analysis of cut point data for the presence of statistical correlation between the screening and confirmatory results. Data were obtained from validations of solution-phase bridging assays for detection of anti-drug antibodies against monoclonal antibody therapeutics. All data sets showed moderate to strong positive correlation, indicating that the screening and confirmatory assays were not independent and were likely to generate similar information. We present theoretical evidence that correlated results may be a general feature of the tiered approach when the same test platform is used for both screening and confirmatory assays. The competitive inhibition test, therefore, may be of limited value beyond reduction of the overall false positive rate. Our results indicate that similar sample results could be obtained by using just the screening assay with the false positive rate set to 1%.

1. Introduction

Therapeutic biotechnology products such as human monoclonal antibodies are known to cause undesired anti-drug antibody (ADA) responses in patients, negatively affecting drug efficacy and safety [1–3]. The clinical manifestations of drug immunogenicity range from no effect to loss of drug efficacy and harmful effects on patient health [4–6]. Due to potential risks to patients treated with biotherapeutics, regulatory authorities issued several guidance documents concerning the development and validation of immunogenicity assays for the detection of ADA in clinical samples [4,5,7]. Moreover, several "white papers" have been dedicated to the development and validation of immunogenicity assays [8–11]. Given the large number of samples that must be analyzed in clinical trials, a multi-tiered approach is recommended for testing patient samples [5,12]. As illustrated in Scheme 1, all clinical ADA samples are subjected to a high throughput and relatively simple screening assay to evaluate the potential presence of ADA. Samples giving signal at or above the screening cut point are classified as potentially positive and are tested in the confirmatory tier, where specificity of the immune response to the drug is evaluated. Samples with responses at or above the confirmatory cut point are declared confirmed positive and may be characterized further for titer, presence of neutralizing antibodies, and for immunoglobulin isotypes. The aim of the multi-tiered approach is to identify the positive samples and eliminate negative samples from unnecessary testing in costly and time-consuming assays. The screening and confirmatory cut point values serve as decision thresholds whether to classify a sample as negative and terminate its analysis or to classify it as positive and continue to the next tier. Screening and confirmatory cut point

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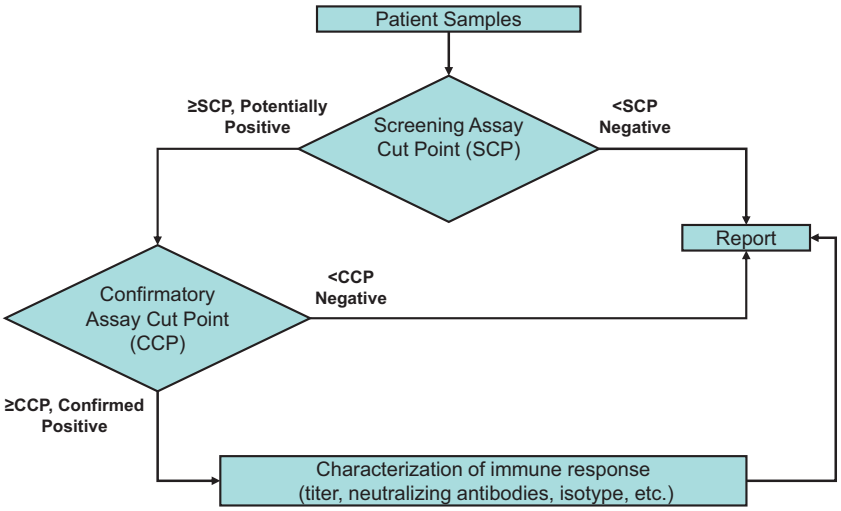

Scheme 1. Typical multi-tiered immunogenicity testing approach employed in clinical trials.

values are determined during validation of analytical methods. The screening cut point is established using drug-naive samples from the prospective study population. To ensure the ability of the assay to detect low positive samples, the screening cut point value is set at the upper limit of approximately 95th percentile of responses which yields a false positive rate of 5% [5,10–12].

The purpose of the confirmatory assay is to assess whether the potential reactivity identified in the previous tier is due to specific binding to the drug. Specificity and saturability of the drug–ADA interaction is typically demonstrated by competitive inhibition of the signal upon treatment with unlabeled drug. Due to its relative simplicity, the competitive drug inhibition has been broadly adopted by the biotechnology industry as the confirmatory assay [13]. However, determining a level of inhibition that indicates specificity of the drug–ADA interactions (i.e. the confirmatory cut point) presents a special challenge [14,15]. Ideally, the confirmatory cut point should be obtained by testing samples from a true positive population but such samples cannot be easily obtained since immunogenicity assays have to be established and validated before the start of clinical trials or assignment of treatment groups. An ADA-positive population may be mimicked by spiking negative samples with a surrogate positive control consisting of anti-drug antibodies derived from animals. However, this approach is problematic since it is unclear to what extent anti-drug antibodies obtained by a deliberate immunization of animals are able to mimic a true positive signal in a clinical study [14]. In absence of true positives, the confirmatory cut point is typically determined by testing a drug-naive population in presence and absence of spiked excess of the unlabeled drug and evaluating the level of signal inhibition. This method relies on the assumption that a true positive sample analyzed in the confirmatory assay should produce a signal inhibition that is larger than the baseline cutoff established in the naive population. A value that corresponds to the upper limit of 99th or 99.9th percentile of all responses in the drug-naive population is typically recommended as the confirmatory cut point [11,16].

While extensive discussions have focused on determination of the screening assay cut points [11,17–19], there is a dearth of literature concerning the experimental design, statistical analysis and cut point determination of confirmatory assays. Recently, Wakshull and Coleman [15] discussed this topic and introduced a concept of orthogonality between the screening and confirmatory assays results. Their argument states that screening and confirmatory results for a drug-naive population should be orthogonal (i.e. non-correlated) which would ensure that the two assays generate independent and non-overlapping information about each tested sample. A failure to demonstrate orthogonality between the screening and confirmatory cut point data should prompt re-evaluation of the analytical method. In this paper, both through empirical data analysis and theoretical argument, we demonstrate that non-orthogonality is an inherent feature of screening and confirmatory assays relying on competitive inhibition with unlabeled drug. As the confirmatory assay is expected to distinguish a false positive from a true positive response, in this paper we investigated statistical requirements needed to make this classification most effective.

2. Methods

2.1. Reagents

Experimental data were obtained during validation studies of immunoassays for detection of ADA against three different fully humanized monoclonal antibody drug candidates. Positive controls were generated by MedImmune and consisted of goat polyclonal antibodies directed against the idiotype of each monoclonal antibody drug. Individual human serum samples and pooled human sera were obtained from Bioreclamation (Hicksville, NY). Multi-array standard streptavidin-coated plates (MA6000), Blocker A, Read Buffer T, and ruthenium (II) sulfotrisbipyridine N-hydroxysuccinimide ester were obtained from Meso Scale Discovery (Gaithersburg, MD). EZ-Link biotin sulfo-N-hydroxysuccinimide ester was purchased from Thermo Scientific (Rockford, IL).

2.2. Bioanalytical methods

Immunogenicity assays employed an electrochemiluminescence-based (ECL) bridging assay format on Meso Scale Discovery (MSD) platform. Aliquots of each antibody drug type were labeled with biotin and ruthenium complex using procedures recommended by the manufacturer. Serum samples were diluted 10-fold (validation Study 1) or 30-fold (validation Studies 2 and 3) with assay buffer (phosphate buffered saline/10% MSD Blocker A/0.1% Tween 20, pH 7.3) containing an equimolar mixture of the two labeled forms of the drug (ruthenylated and biotinylated) at 1 μg/mL each. Samples were incubated overnight for 17–22 h with gentle agitation at room temperature (RT) in the dark. Streptavidin (SA)-coated MSD plates were blocked with MSD Blocker A (150 μL/well) for approximately 2 h at RT and washed four times with washing buffer (phosphate buffered saline/0.1% Tween 20, pH