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Enhancing efficiency and quality of statistical estimation of immunogenicity assay cut points through standardization and automation

Cheng Su [a,*], Lei Zhou [a], Zheng Hu [b], Winnie Weng [a], Jayanthi Subramani [c], Vineet Tadkod [b], Kortney Hamilton [d], Ami Bautista [b], Yu Wu [a], Narendra Chirmule [d], Zhandong Don Zhong [b,**]

[a] Global Biostatistical Science, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[b] Pharmacokinetics & Drug Metabolism, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[c] Information Systems, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA
[d] Clinical Immunology Medical Sciences, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA.

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ABSTRACT

Biotherapeutics can elicit immune responses, which can alter the exposure, safety, and efficacy of the therapeutics. A well-designed and robust bioanalytical method is critical for the detection and characterization of relevant anti-drug antibody (ADA) and the success of an immunogenicity study. As a fundamental criterion in immunogenicity testing, assay cut points need to be statistically established with a risk-based approach to reduce subjectivity. This manuscript describes the development of a validated, web-based, multi-tier customized assay statistical tool (CAST) for assessing cut points of ADA assays. The tool provides an intuitive web interface that allows users to import experimental data generated from a standardized experimental design, select the assay factors, run the standardized analysis algorithms, and generate tables, figures, and listings (TFL). It allows bioanalytical scientists to perform complex statistical analysis at a click of the button to produce reliable assay parameters in support of immunogenicity studies.

1. Introduction

Biotherapeutics can elicit immune responses from animals and humans, inducing anti-drug antibodies (ADA). The presence of ADA can have a significant impact on the pharmacokinetic (PK) profiles, safety, and efficacy of the candidate molecule. Such antibody responses can sometimes result in adverse clinical sequelae ranging from hypersensitivity to neutralization of the biological activity of an endogenous protein. A well-designed, robust immunogenicity assay is the foundation for appropriately evaluating the immunological properties of a biotherapeutic. Much effort has been given to the advancement of bioanalytical methods for the detection and characterization of ADAs. Several regulatory guidelines and industry recommendations have been published concerning the development and validation of ADA assays, sample testing, and data reporting and interpretation (US Food and Drug Administration, 2009, 2013; Gupta et al., 2007; Mire-Sluis et al., 2004; Committee for Medicinal Product for Human Use, 2007; Shankar et al., 2014). Vigorous statistical analyses have been woven into the immunogenicity assessment process throughout the development cycle of a biotherapeutic, starting from assay optimization by utilizing the design of experiments principle (Chen et al., 2012; Hammond et al., 2008; Zhong et al., 2010); evaluation of method robustness and ruggedness during validation and transfer of the assay between test laboratories (Tatarewicz et al., 2009); monitoring the assay performance during in-study sample testing (Barger et al., 2010); and evaluation of the impact of ADA on the PK profile, pharmacodynamic (PD) effect, and the safety profile of the molecule of interest (Chen et al., 2013; Chirmule et al., 2012; Kelley et al., 2013; Sailstad et al., 2014; Thway et al., 2013).

A tiered approach is utilized for ADA testing (Shankar et al., 2008). Initially, a sample is tested for the potential presence of ADA in a screening assay, by applying a screening assay cut point (ACP) that is predetermined through appropriate statistical analysis of drug-naïve samples from the population of interest. The screening assay is expected to detect some false positive samples to maximize the probability of identifying all potential positive samples and reduce the probability of false

Abbreviations: ADA, anti-drug antibody; CAST, customized assay statistical tool; ACP, screening assay cut point; DCP, depletion cut point; ECL, electrochemiluminescence; S/N, signal to noise ratio; NC, negative control; NAb, neutralizing antibody; Log, logarithm; ANOVA, analysis of variance; SW, Shapiro–Wilk.

* Correspondence to: C. Su, 1120 Veterans Boulevard, Mailstop ASF3 2011, South San Francisco, CA 94080, USA.
** Correspondence to: Z.D. Zhong, One Amgen Center Drive, Mailstop 30E-3-B, Thousand Oaks, CA 91320, USA.
E-mail addresses: csu@amgen.com (C. Su), zhoul@amgen.com (L. Zhou), zhengh@amgen.com (Z. Hu), nweng.email@gmail.com (W. Weng), jayanthi@amgen.com (J. Subramani), vtadkod@amgen.com (V. Tadkod), kortneyh1101@gmail.com (K. Hamilton), amic@amgen.com (A. Bautista), yuw@amgen.com (Y. Wu), chirmule@gmail.com (N. Chirmule), zhongz@amgen.com (Z.D. Zhong).

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negative results. Following the detection of positive reactivity of a sample during the screening step, a competitive inhibition test is performed by treating a sample with the drug. The resulting reduction of assay response greater than the signal depletion cut point (DCP) would confirm that the positive reactivity is indeed specific to the drug. Additional experiments may be necessary to confirm the specific binding to be that of antibody origin, to determine the antibody levels, to identify the ADA binding epitopes, to measure the affinity of the ADA, and to characterize the ADA isotypes and subclasses. Most importantly, the ability of the ADAs to neutralize the biological activity of the biotherapeutic is further evaluated in a functional biological assay (Gupta et al., 2007).

Cut point is a fundamental criterion in immunogenicity studies. Regulatory guidelines and industry white papers recommend a risk-based approach to establishing cut points statistically to reduce subjectivity (US Food and Drug Administration, 2009; Mire-Sluis et al., 2004; Shankar et al., 2008; Buttel et al., 2011). Statistical principles used to estimate cut points are consistent across immunogenicity applications. Proper statistical approach requires careful consideration in experimental design, data distribution, and analysis methods. Furthermore, as the cut points of an ADA assay are greatly dependent upon the biological status of the study population, it is important for the bioanalytical scientists to continuously monitor the cut points as well as other assay parameters for the relevant study populations during in-study sample analysis. In order to enhance the efficiency and quality of statistical analysis in support of immunogenicity studies, we have developed a comprehensive web-based customized assay statistical tool (CAST) to automate and standardize data analysis for determining cut points as well as other assay parameters. The CAST tool provides an intuitive web interface for a user to import experimental data generated from standardized experimental designs or study baseline samples, select assay factors, run the standardized analysis algorithms, and generate the tables, figures, and listings (TFL). The tool has been validated with a risk-based approach following the relevant regulatory guidance. It allows users with limited statistical training and experience to perform complex statistical analyses with a click of the buttons and generate the results within a few minutes for the intended use, which is a valuable functionality for monitoring ADA assay parameters during in-study sample analysis. In this manuscript, we report on the development of this CAST system for cut point estimation. We will describe in detail experimental and statistical considerations for the standardized designs of cut point experiments and development of the analysis algorithms.

2. Methods

2.1. Software platform

CAST is a web-based, multi-tier statistical analysis system that is built on Ext JS, Java, XML, SAS, and Oracle technologies to support data analysis for immunogenicity applications (Fig. 1). Ext JS is used to create intuitive and flexible user interfaces. The Java application platform is used to process user input, create and submit SAS jobs to the SAS engine. Data preprocessing, analyses, algorithms, and TFL creation are implemented using SAS V9.3. The user can access CAST data analysis through a web browser.

2.2. Experimental designs

Standardized experimental designs are devised for estimating cut points for ADA detection. The electrochemiluminescence (ECL) bridging assay is used as a model system to test CAST. As examples, two designs having 4 assay factors, also known as assay variables, are shown in Table 1. In the experimental designs, a set of donor samples are randomized into different donor groups, also known as sequences (eg, S1, S2, S3, and S4), which are stratified based on gender and disease population and allocated to different ECL detection plates. The donor groups are generated and used across all assay runs in a cut point experiment. Specifically, an assay run consists of testing the entire donor set across all assay factors of an experimental design. The assay factors are designated as VAR1, VAR2, etc., with the actual names being assigned through the user interface to provide flexibility in choosing an experimental design. An additional variable is also provided through the user interface for evaluating potential differences between two populations, eg, two disease populations, through equivalence evaluation.

Up to 4 assay factors can be incorporated into an experiment, such as date, analyst, instrument, and ECL detection plate lot. Both full and reduced versions are available for each experimental design. In the full design version, a complete set of donors are tested across all factors in each assay run. In the reduced version, only subsets of the donors are tested for each assay condition, with the complete set of donors tested in each assay run. The tool offers over 20 unique experimental designs, encompassing a number of assay factors, full and reduced versions of each design, and equivalency assessment. A flexible experimental design is also available to accommodate calculation of population-specific cut point during in-study sample analysis.

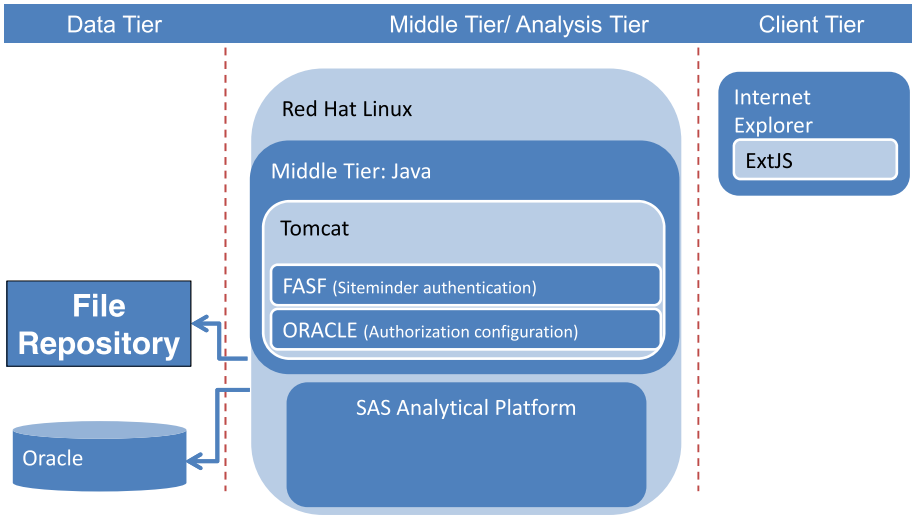

Fig. 1. CAST technology.