Abstract

Purpose: To improve a validated immunogenicity assay for monitoring anti-DruK-Ab antibodies without interference from endogenous Drug-K target protein. Drug target protein could lead to false positive anti-drug antibody responses when using bridging immunogenicity methods. Methods: A therapeutic Drug-K is a monoclonal antibody that targets Drug-K protein. Endogenous Drug-K target protein levels could be significantly elevated in disease such as cancer. Presence of drug target protein could lead to false positive anti-Drug-K-Ab antibody responses when using a bridging method. In this study, a bridging electrochemiluminescence (ECL) method that incorporates biotin-labeled Drug-K, ruthenium-labeled Drug-K and Drug-K target soluble receptor in master mix was evaluated to detect anti-Drug-K antibodies. Results: The highest expected concentration of Drug-K target protein in disease samples is 100 ng/mL. The target protein soluble receptor at 1,000 ng/mL blocked target protein response up to 1,000 ng/mL level.

Methods

- Electrochemiluminescence (ECL) bridging methods were used for determination of ADA in study samples (Figure 1A and 1B).
- Updated method (Figure 1B) that incorporates biotin-labeled Drug-K, ruthenium-labeled Drug-K and Drug-K target receptor in master mix was evaluated to detect anti-Drug-K-Ab antibodies. Introduced Drug-K target receptor binds to endogenous Drug-K target protein which helps to diminish/eliminate false positive response in anti-drug antibodies detection. Results: (1) Certain types of cancer plasma samples were evaluated to determine Drug-K target receptor concentration needed to decrease high background possibly due to elevated Drug-K target protein levels. Selected Drug-K target receptor concentration was used in the assay master mix side by side with biotin-labeled Drug-K and ruthenium-labeled Drug-K. Results showed that selected 1,000 ng/mL receptor level could eliminate effect of target protein at concentrations up to 1,000 ng/mL, which is above detectable levels expected in cancer plasma samples. (2) The assay sensitivity was about 50 ng/mL on tested anti-DruK-Ab antibody positive control. Drug tolerance for 250 ng/mL, tested anti-DruK-Ab positive control was 100 µg/mL. Multiple types of cancer individual plasma samples were tested showing acceptable method selectivity in disease population. (3) The assay sensitivity, drug tolerance and overall performance were similar to those of the original assay (without Drug-K target receptor presence). Conclusion: A bridging ECL method for detection of anti-drug antibodies to overcome false positive responses from drug target protein was developed. Similar approach could be applicable to other therapeutic drugs. The method is being validated for analysis of plasma samples derived from various types of cancers.

Introduction

A therapeutic Drug-K is a monoclonal antibody that targets Drug-K protein. Endogenous Drug-K target protein levels could be significantly elevated in disease such as cancer. Presence of drug target protein could lead to false positive anti-DruK-Ab antibody responses when using a bridging method. Here we introduced Drug-K target soluble receptor that binds to endogenous Drug-K target protein and helps to diminish/eliminate false positive response in anti-drug antibodies detection.

Purpose

To improve a validated immunogenicity assay for monitoring anti-DruK-Ab antibodies without interference from endogenous Drug-K target protein.

Results

- Analysis of samples from disease subjects showed false positive responses using original method (Figure 1A) and cut point established based on normal subjects.
- Hypothesis: Drug-K target protein that is present in elevated level in disease samples could bridge with labeled drug to produce false positive response (Figure 2).
- The target protein interference could be diminished with addition of the target protein receptor. Table 1 shows Drug-K target protein tolerance by the receptor when present in same mixture and assayed by original method (Figure 1A).

<table>
<thead>
<tr>
<th>Drug-K Target Protein (ng/mL)</th>
<th>0</th>
<th>10</th>
<th>100</th>
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<tr>
<td>Drug-K Tolerance (RLU)</td>
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<td>362.0</td>
<td>281.75</td>
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</table>

Table 1. Titration of Drug-K Target Protein against Drug-K Target Protein Receptor in Normal Human Plasma

- All neat cancer samples showed response below cut point (< CP), while the spiked cancer samples displayed a positive with respect to plate cut point response (> CP).
- Table 2 shows RLU responses for neat individual samples from subjects with cancer (2 different types) in the original assay (Figure 1A) in the presence of varying concentrations of Drug-K target receptor protein.

Table 2. Effect of Drug-K Target Protein Receptor on Individual Cancer Sample ECL Responses

- Table 3 shows updated method (in the presence of Drug-K target receptor) method selectivity results for cancer samples that were neat and spiked at Low anti-DruK-Ab antibody positive control level (LPC).

Table 3. Updated Method (Figure 1B) Selectivity for Anti-DruK-Ab Antibody in Human Plasma Samples

- Table 4 shows comparison of original (Figure 1A) and updated (in the presence of Drug-K target receptor) parameters for Anti-DruK-Ab antibody in Human Plasma.

Table 4. Updated Method (Figure 1B) Drug Tolerance for Anti-DruK-Ab Antibody in Human Plasma

- Table 5 shows comparison of original (Figure 1A) and updated (in the presence of Drug-K target receptor) parameters for Anti-DruK-Ab antibody in Human Plasma.

Table 5. Comparison of Original (Figure 1A) and Updated Method (Figure 1B) Parameters for Anti-DruK-Ab Antibody in Human Plasma

- Conclusions

A bridging ECL method for detection of anti-drug antibodies to overcome false positive responses from drug target protein was developed. The method could be used for both normal and disease samples. Similar approach could be applicable to other therapeutic drugs. The method is being validated for analysis of plasma samples from various cancer types. Disease specific cut point in original method was considered, however, not evaluated. Cut point based on high RLU responses of samples from disease subjects could lead to false negative results.

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#M1041 Overcoming False Positive Response in Anti-Drug Antibody Method