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Strategies for Overcoming Drug Target Interference in Anti-Pembrolizumab Antibody Detection

Amy Lavelle[1], Lindsay Silva[1], Marin Barden[1], Christopher Rabold[1], Loree Mewborn[1], Charles Kelsey[1]

[1]PPD® Laboratories bioanalytical lab, Richmond, Virginia

CONTACT INFORMATION: amy.lavelle@ppdi.com

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PURPOSE

Both EMA and FDA regulatory guidance for ligand binding assay (LBA) anti-drug antibody (ADA) method development recommend a sensitive and specific assay. Many factors may interfere with an assay's ability to accurately detect ADA, including endogenous and circulating drug, soluble drug target levels, or other serum factors. These factors may contribute to false positive or false negative results and potential over- or under-reporting of ADA results and titers in patient samples. Assay formats, sample pre-treatments, and assay reagents can be manipulated to help reduce target interference.

Pembrolizumab is commonly prescribed for the treatment of various cancer types. It is a humanized monoclonal IgG4 antibody directed against PD-1, which disrupts PD-1/PD-1L interactions and increases cytotoxic T-cell activity. Soluble PD-1 at concentrations of 10 ng/mL and higher generate false positive responses, indicating a disrupted ability to accurately measure pembrolizumab ADA.

OBJECTIVES

- Use laboratory model of target interference to test various mitigation strategies
- Identify mitigation strategy best suited for high throughput analysis of samples

RESULTS

- Acid dissociation, heat denaturing for 30 minutes at 56°C, and purification using melon gel were all unable to resolve the false positive response seen in the Pembrolizumab ADA assay (figure 1).
- Additionally, any combination of these three pre-treatments were insufficient at mitigating the target interference (figure 1).
- However, acid dissociation, followed by a competitive inhibitor (human PD-1 polyclonal antibody) added to the neutralization step, reduced the target interference without affecting assay sensitivity or true positive responses (figure 2).
- Acid dissociation followed by solid-phase extraction of rhPD-1 Fc with human PD-1 polyclonal coated magnetic beads reduced target interference by 10 ng/mL rhPD-1 Fc, but was unable to resolve the false positive response in the presence of 100 ng/mL rhPD-1 Fc (figure 3).

METHOD

We explored various methodologies to evaluate the mitigation of drug target interference in a bridging LBA pembrolizumab ADA assay. Target interference was simulated by pre-incubating negative, low-positive, and high-positive controls with increasing levels of rhPD-1 Fc.

Methodologies included:

- Acid dissociation,
- Melon gel extraction,
- Heat denaturation,
- Competitive antibody pre-treatment, and
- Solid phase extraction of soluble target.

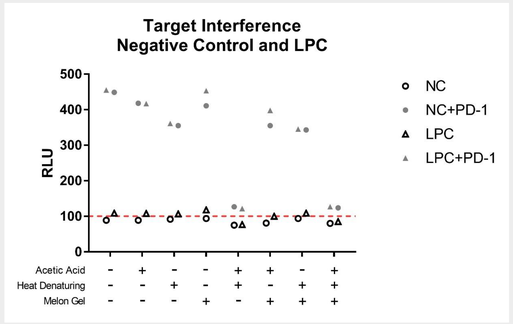


Figure 1. Assay performance with various pretreatments. In control condition, presence of PD-1 (100 µg/mL) resulted in false positive response in negative control (NC) sample, and elevated signal at LPC. Red line represents average plate cut point. n = 1 for each NC, PC at each treatment condition.

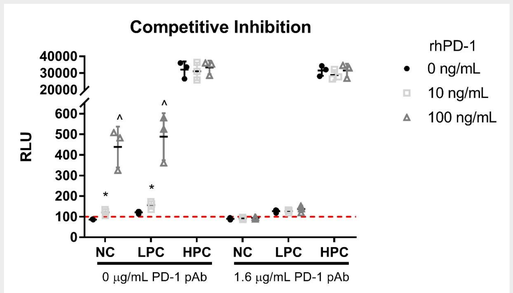


*Figure 2. Assay performance with competitive inhibition using 1.6 µg/mL PD-1 pAb. Red line represents average plate cut point. Each NC, PC run in duplicate at each condition in 3 independent assays. *indicates significantly greater than 0 ng/mL rhPD-1, p < 0.05. ^ indicates significantly greater than 0 ng/mL rhPD-1, p < 0.01.*

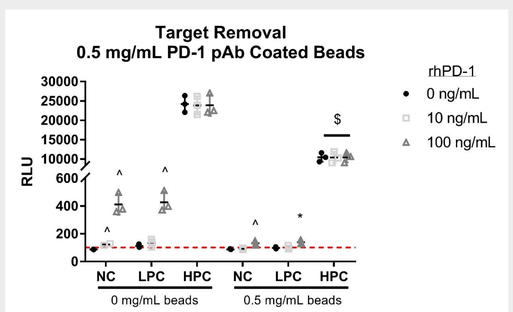
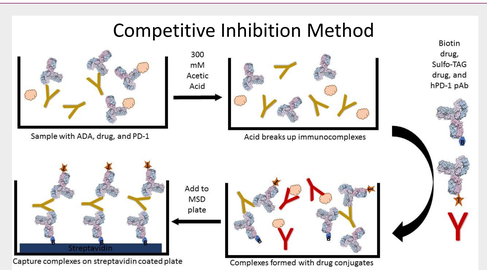


*Figure 3. Assay performance using 0.5 mg/mL SA Beads with 1 µg/mL Biotin-hPD-1 pAb. Red line represents average plate cut point. Each NC, PC run in duplicate at each condition in 3 independent assays. *indicates significantly greater than 0 ng/mL rhPD-1, p < 0.05. ^ indicates significantly greater than 0 ng/mL rhPD-1, p < 0.01. $ indicates significantly lower than 0 mg/mL beads, p < 0.01.*

CONCLUSIONS

It is important when optimizing an assay during method development to keep in mind the consequences of drug target interference. Disease state and normal/healthy circulating soluble target levels may affect whether an assay requires format changes for eliminating interference. Although circulating levels of PD-1 in healthy individuals do not appear to cause interference, interference is noted at 10.0 ng/mL rhPD-1, resulting in false positive responses. Therefore, certain disease states may have interfering levels that affect the assay's ability to accurately detect ADA.

Pembrolizumab is often used as a first line treatment or co-medication, therefore it is critical to be able to accurately detect anti-pembrolizumab ADA. Presence of ADA may exclude a patient from a future treatment or clinical trial, rendering false positive results of the utmost concern. To the best of our knowledge, these techniques have yet to be applied to addressing false positive responses in a pembrolizumab ADA bridging assay and can prove necessary in certain studies. Additionally, a high throughput, user friendly method for mitigating interference to produce accurate results with good precision and accuracy is necessary. Acid dissociation coupled with PD-1 antibody competitive inhibition produces accurate results that mitigate soluble PD-1 interference in a technically uncomplicated assay format.



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