Tailoring approaches to mitigate the presence of pre-existing antibodies; considerations for different assays throughout the immunogenicity cascade related to an ADC drug product

Martin Roberge[1], André Forté[1], Justine Bélanger[1], Frédéric Bergeron[1], Mathilde Yu[1,2]

[1] Cerba Research, [2] Presentor

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Abstract

Novelty

This poster presents distinct tailored approaches to prepare matrices for anti-drug antibody (ADA) and neutralizing antibody (NAb) assay cut-point determinations for an antibody-drug conjugate (ADC) with high incidence and high levels of pre-existing ADAs.

Methods

The immunogenicity cascade included a multi-tiered ADA bridging assay (screening, titer, domain characterization (antibody and toxin)), as well as a cell-based NAb screening assay.

Excess drug was added to naïve matrix samples to determine the cut-points for every tier of the ADA immunogenicity cascade, including screening and domain characterization tiers (antibody and toxin). Addition of excess drug could not be used for the NAb assay cut-point determination. Instead, naïve matrix samples were pre-selected based on ADA and NAb assay results.

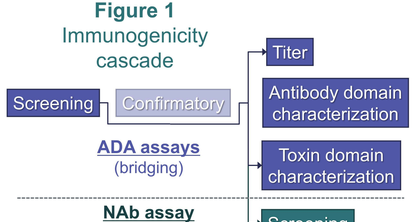
Results/Conclusions

Strategies used to determine the ADA and NAb assay cut-points generated sensitive assays which were used during subsequent sample analyses, bringing validity to the clinical immunogenicity results and their interpretation to properly assess the immune response in ADC-treated patients.

Immunogenicity Cascade

Since most people have been exposed to the bacterial toxin domain of the ADC, a very high incidence of specific pre-existing antibodies against the toxin was observed during method development. Therefore, the confirmatory tier of the cascade was not used, and domain characterization tiers were included to determine the ADA specificity. Samples positive for ADA were also further characterized in a titer tier as well as for their neutralizing activity (Figure 1).

Figure 1
Immunogenicity cascade



Challenges

Throughout the immunogenicity cascade, cut-points required for the different assays need to be established using negative samples.

For this ADC drug product, there were major challenges:

- There is a high incidence and high levels of pre-existing ADAs in naïve matrix samples
  - Protein A/G columns and protein G HP multitrap alternatives to remove pre-existing antibodies were tested but were not effective enough
- Finding a strategy to generate negative samples given that the ADA bridging assay and the NAb cell-based assay have completely different formats; the likeliness of a single strategy being effective for both assay formats was low.

ADA Assay Methods and Results

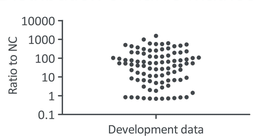
The electrochemiluminescent (ECL) bridging immunoassay to detect ADAs includes an acid dissociation step followed by a complex formation step in the presence of biotin- and sulfo-TAG-labeled ADCs. The complex is then captured onto a streptavidin-coated 96-well plate for detection.

The distribution of signals in the ADA assay was extremely variable in randomly selected naïve matrices due to pre-existing antibodies (Figure 2); determining a cut-point with such data was not feasible.

The strategy used to generate surrogate negative samples for cut-point determination was to add excess amounts of unlabeled drug to the tested naïve matrix samples; addition of excess amounts of toxin was also effective at creating negative samples, but the toxin was less readily available. The unlabeled drug binds to the pre-existing antibodies, preventing bridging of the labeled drugs, rendering the matrices "ADA-negative" for the purpose of cut-point determinations (Figure 3). The same strategy was used for determination of the cut-points for the characterization assay tiers.

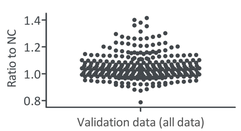
Sequestering of pre-existing anti-drug antibodies with excess drug was effective as ratio to negative control (NC) which varied between ~1 and ~1500 fell to between ~0.85 to ~1.30. Subsequent outlier removal was performed and led to cut-points that could generate a sensitive assay (Figure 4).

Figure 2
Distribution of naïve matrices



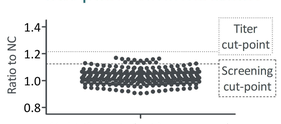
Add excess unlabeled drug

Figure 3
Distribution after ADA sequestering



Remove outliers

Figure 4
Cut-point determination



Notes

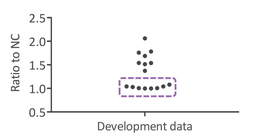
NC: Negative control; Each symbol = result from 1 testing

NAb Assay Methods and Results

The cell-based assay to detect NAbs uses a cell line expressing the receptor targeted by the antibody portion of the ADC. Pre-treated samples (heat inactivation + solid-phase extraction with acid dissociation (SPEAD)) are added to cells in 96-well plates. Cells are incubated for 3 days after which the cell viability is measured using a commercial cell viability reagent. When NAbs are present, they bind ADCs, thereby reducing their cytotoxic effect on cells.

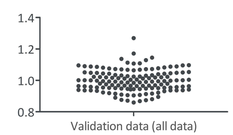
The distribution of NAb measurements in randomly selected naïve matrices shows that the pre-existing antibodies are neutralizing in many samples. The "excess drug addition" strategy to generate "NAb-negative" matrices could not be used given that the cells are sensitive to the cytotoxic effect of the drug. Based on cumulated data from ADA and NAb assay developments (Figure 5) as well as significant amounts of additional naïve matrix testing in the ADA assay (Figure 6), matrices with no or relatively low levels of pre-existing antibodies in the ADA assay (ratio to NC < 3) and NAb assay (ratio to NC of ~ 1) were selected for the NAb assay cut-point determination (Figure 7 and Figure 8).

Distribution of naïve matrices
Figure 5 (NAb assay)



Pre-select "NAb-negative" samples

Figure 7
Distribution of selected naïve matrices



Remove outliers

Figure 6 (ADA assay)

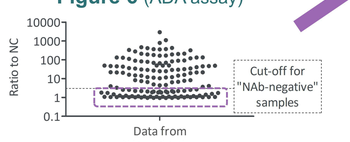
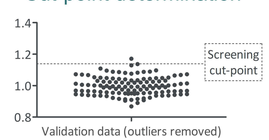


Figure 8
Cut-point determination



Note: Ratios of 1 and 2 in the NAb assay correspond to inhibitions of ~0% and ~100%, respectively

Final ADA and NAb assay characteristics

Assay	Screening	Titer	Charact. (toxin)	Charact. (antibody)	Sensitivity
	Cut-point				
ADA	1.125 ratio to NC (5% FP rate)	1.216 ratio to NC (0.1% FP rate)	18.9% inhibition (1% FP rate)	17.8% inhibition (1% FP rate)	~25 ng/mL
NAb	1.139 ratio to NC (1% FP rate)	-	-	-	~150 ng/mL

FP = false-positive

Conclusions

- Because of the toxin domain of this drug product, most of naïve samples had pre-existing antibodies. Strategies to generate ADA-negative samples required for cut-point determinations:
  - Needed to consider the incidence and levels of pre-existing antibodies
  - Needed to be tailored to the different assay formats; addition of excess drug product to remove pre-existing antibody signal in the bridging ADA assay could not be used for the cell-based NAb assay as the excess drug product would result in cell killing
- The testing cascade can sometimes be adapted; in this case, the ADA assay confirmatory tier was removed. In hindsight, removing the ADA assay screening tier could have also been considered as the titer and characterization tiers provided all required information.