Assessing the Impact of Ultra-Low Cut Points in Immunogenicity Assays

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Introduction

Meaningful cut points are essential to interpretation of the impact of immunogenicity on the safety and efficacy of biotherapeutics. With advances in immunogenicity assay techniques and technology, low screening assay cut points below 1.20 and ultra-low cut points (ULCP) below 1.10 are often observed.

The validity of these cut points and their ability to accurately assess immunogenicity risk in preclinical and clinical studies is often questioned.

Through six (6) case studies, we explore issues that could potentially arise from the application of an ultra-low cut point such as:

- Will the immunogenicity rate be abnormally high?
- Will the assay fail more often with a Low Positive Control (LPC) set close to the background?
- Are the cut points determined during validation applicable for sample analysis?

We also discuss areas for further examination in the case that a low or ultra-low cut point is calculated:

- The amount of data points that have been excluded as outliers
- Indications that an alternate statistical model may be more appropriate
- The distribution of normalized cut point sample responses

Methods

Cut points calculated from January 2022 – July 2024 using a statistical analysis tool were compiled to assess the frequency of low and ultra-low cut points. Cut points were calculated using the Tukey outlier approach followed by parametric or non-parametric methods as appropriate.

Case studies with ultra-low cut points were selected to provide a broad representation of drug development and include multiple species, assay types, and biotherapeutic modalities.

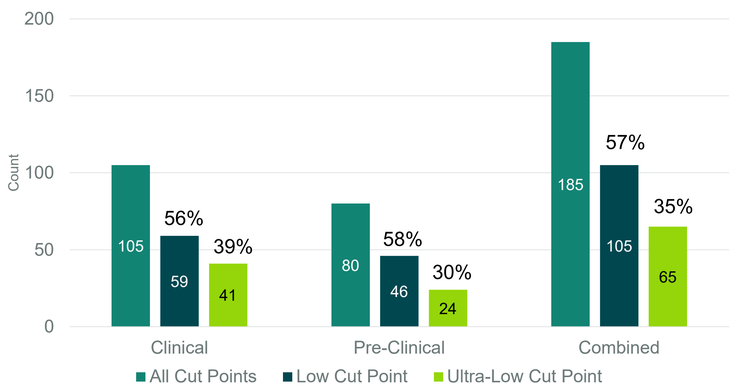
Results

Cut Point Analysis Summary

Cut points were collected from January 2022 – July 2024 for clinical and pre-clinical studies. The total number of cut points calculated were 185. The break down of pre-clinical and clinical cut points that are low or ultra-low are presented in **Figure 1**.

Surprisingly, more than 50% of all cut points (pre-clinical and clinical) were low screening cut points ≤ 1.20. Approximately one third of all cut points were ultra-low cut points ≤ 1.10.

Figure 1. Cut Point Analysis Breakdown



Discussion

Ultra-Low Cut Point Case Studies

Findings from six case studies where ULCPs were calculated during assay validation are detailed in **Table 1** and summarized below.

Case Study ICON -1

- Normal and disease state cut points were calculated in validation. The client prospectively requested an in-study cut point calculated for this Phase 3 program.
- Normal, disease state, and in-study cut points were all ultra-low and very similar.
- A 1% failure rate LPC was used and 5% of runs failed due to LPC acceptance. LPC was increased due to a shift in method performance.
- 71% of samples were ADA positive. In this case, the molecule is known to be immunogenic, so the high rate of observed immunogenicity is unlikely to be due to the ULCP.

Case Study ICON -2

- Per study False Positive Rate (FPR), an in-study cut point was not needed.
- <1% of runs failed due to LPC acceptance.
- Rate of immunogenicity was low.

Case Study ICON -3

- Per study FPR, an in-study cut point was not needed.
- <1% of runs failed due to LPC acceptance.
- As this is a Fc-fusion protein in a non-human species, the amount of immunogenicity observed is within expectation.

Case Study ICON -4

- Per study FPR, an in-study cut point was not needed.
- <1% of runs failed due to LPC acceptance.
- Rate of immunogenicity was low.

Case Study ICON -5

- Per study FPR, an in-study cut point was needed. In this case, 16.2% of data points were removed as outliers during validation cut point calculation, potentially contributing to the calculation of a ULCP.
- <1% of runs failed due to LPC acceptance using the validated ULCP.
- Rate of immunogenicity was low using the in-study cut point.

Case Study ICON -6

- Per study FPR, an in-study cut point was not needed.
- <1% of runs failed due to LPC acceptance.
- Rate of immunogenicity was low.

Impact of Outlier Removal on Cut Points

When ultra-low cut points are calculated, it is useful to consider the threshold for outlier removal. Excessive outlier removal is one way that ULCPs are generated.

The commonly applied Tukey outlier approach uses a multiplicative factor and the distribution of the dataset to identify outliers. With this approach, data below Q1-1.5*(Q3-Q1) or above Q3+1.5*(Q3-Q1) are removed. Q3-Q1 may also be referred to as the Inter-Quartile Range (IQR).

In instances where greater than 10-15% of data are removed using 1.5x IQR for identification of outliers, using 3x IQR for outlier identification may avoid an excessive reduction in variability that is not representative of the population.

Alternate Statistical Models

If ultra-low cut points are observed and there is concern that using the more traditional Tukey outlier approach has resulted in a dataset that is not reflective of the true variability of the population, then other statistical models can be evaluated such as mixed effect models. Mixed effect models do not exclude outliers, but rather model the variability from different factors to calculate a cut point.

In the authors' experience, instances where greater than 10-15% of data points are removed as outliers or where systematic patterns of outliers are observed (consistently tied to a particular day, operator, etc.) may be justification for use of an alternate statistical approach.

Discussion (cont'd)

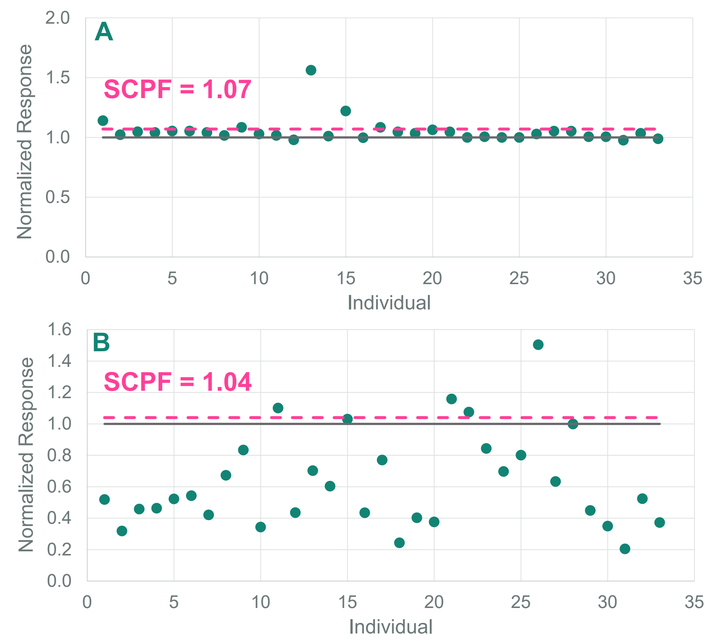
Negative Control and Distribution of Individual Data as an Indication of Cut Point Appropriateness

The screening cut point should reflect the variability of the individual population responses and their relationship to the negative control (NC).

Figure 2A describes a case in which the normalized individual responses exhibit low variability and are centered around 1.0. In such a case, an ultra-low cut point is meaningful for defining a positive or negative response.

In **Figure 2B**, the normalized individual responses are more variable and most are < 1.0. In this case, an ultra-low cut point is less meaningful and does not appropriately represent the observed variability. Selection of a NC that is more representative of the individual population would result in a more meaningful cut point.

Figure 2. Comparison of Normalized Individual Responses



Conclusions

Low screening assay cut points below 1.20 and ULCP below 1.10 are increasingly common, accounting for over 50% of all cut points surveyed.

Examination of six case studies indicates that concerns regarding high immunogenicity rates, increased run failures due to the LPC, and the applicability of ULCPs to study samples are not always warranted:

- 5/6 cases showed acceptable rates of immunogenicity with application of a ULCP (e.g. high immunogenicity attributable to low cut points was not observed).
- 5/6 cases had no run failures attributable to the LPC. Of the four cases with 1% FR screening LPCs, only one necessitated adjustment.
- 5/6 cases did not require an in-study cut point. A prospective in-study cut point was calculated for ICON -1, but it was very similar to the normal and disease cut points calculated during validation.

The authors suggest evaluation of the cut point data to determine if a low or ultra-low cut point is appropriate prior to application of an alternative statistical model.

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References

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Table 1. Case Studies with Ultra-Low Cut Points

Method Information						Cut Point and Control Information								
Case Study ID	Drug Modality	Assay Type	Species	Matrix Type	Platform	Validated Screening Cut Point Factor (SCPF)	Validated Confirmatory Cut Point (CCP)	% outliers removed	Low Positive Control (LPC) 1% Failure Rate	Adjustment of 1% Failure Rate LPC Needed	Study False Positive Rate (FPR)	In-study Cut Point needed	Sample Immuno-genicity Rate	% Run Failure due to LPC
ICON -1	mAb	Bridging w/ Acid Dissociation	Human	Plasma (K2EDTA)	ECLIA	1.05 (normal), 1.08 (disease)	12.2% (normal), 12.7% (disease)	11.3% (normal) 30.0% (disease)	Yes	Yes - screening and confirmatory LPCs were increased due to a shift in method performance	10.0% [a]	In-study SCPF of 1.07 and CCP of 9.8%	71.1%	16/319 runs (5%)
ICON -2	humanized mAb	Bridging w/ Acid Dissociation	Human	Serum	ECLIA	1.09	10.5%	8.78%	No [b]	NA	11.4%	Not needed, based on evaluation of pre-dose samples	5.41%	0/28 runs (0%)
ICON -3	Fc fusion protein	Bridging w/ Acid Dissociation	Non-human primate	Serum	ECLIA	1.09	8.92%	3.33%	No	NA	2.63%	Not needed, based on evaluation of pre-dose samples	37.9%	0/40 runs (0%)
ICON -4	mAb	Bridging w/ Acid Dissociation	Human	Serum	ECLIA	1.04	16.9%	6.67%	Yes	No - screening LPC adjustment not needed Yes - confirmatory LPC was increased	7.71%	Not needed, based on evaluation of pre-dose samples	2.58%	0/137 runs (0%)
ICON -5	humanized mAb	Bridging w/ Acid Dissociation	Human	Serum	ECLIA	1.07	20.1%	16.2%	Yes	No	23.0%	Needed, based on evaluation of pre-dose samples. In-study SCPF of 1.43	1.15%	0/19 runs (0%)
ICON -6	mAb	SPEAD	Human	Serum	ECLIA	1.08	26.0%	10.0%	Yes	No	7.50%	Not needed, based on evaluation of pre-dose samples	1.34%	0/8 runs (0%)

[a] FPR calculated using in-study cut points
[b] 1% Failure Rate LPC included on runs, but not applied for run acceptance

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