It’s All Relative

An alternative to the statistical cutpoint approach to pre-clinical immunogenicity assessment

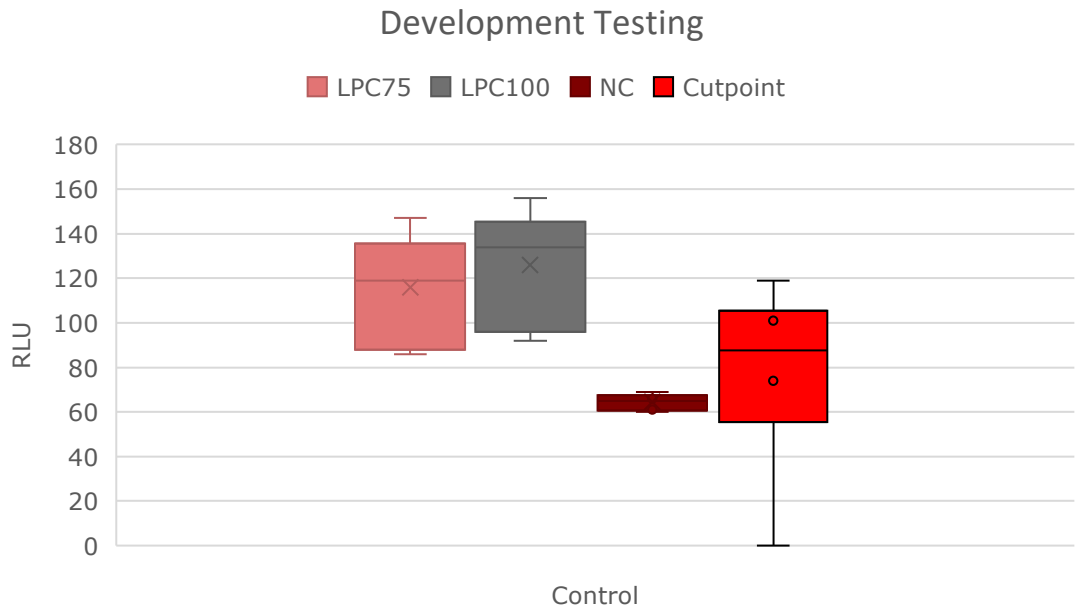
Introduction

- Initial 2 tier statistical approach
- Problems encountered
- Requirements for Pre-clinical Immunogenicity assessment
- Cut point sample alternative approach
- Recommendations for this approach
  - Development
  - Validation
  - Sample analysis

Initial 2 tier statistical approach

- Assay details:
  - Supporting a pre-clinical toxicology study for bi-specific antibody
  - Homogenous bridging assay using acid disassociation
  - Neutralise along with excess of labelled drug, (biotinylated drug capture, sulfo-tag drug detection)
  - Capture on streptavidin plates wash, then read (MSD)
- Screening assay development, floating cutpoint was very small (+10.13 RLU)
- After pre validation testing, it all passed:
  - LPC's
  - Selectivity
  - Drug tolerance etc all fine

Looking back- evidence of issues with cutpoint were already there



Problem Encountered in Validation

- Cutpoint assessment was run on full panel as described by Shankar (2008)
  - 15 individuals
  - 2 analysts
  - 3 plates each
  - Run over 2 days
- Statistical analysis:
  - Very little variability in data, Validation cut-point = Median of values!
  - Correction factor calculated as -0.13 RLU!

- So what to do?
  - Repeat cut point assessment?
  - Redevelop assay to increase variability?

- Time to consider our options…

Requirements for Preclinical ADA

- Pre-clinical ADA is not predictive of clinical response
- Data are used mainly to interpret PK/PD
- High sensitivity not required
  - 500- 1000 ng/mL

- The assay has a sensitivity of less than 75 ng/mL, much lower than requirements
- Why are we so desperate to see small levels of ADA when it is not required to do the interpretation?

Cut Point Samples (CPS)

- The chosen solution was cut-point samples (CPS)

- Basic principle:
  - Choose an appropriate cut point sensitivity
  - Run control samples spiked at this known concentration and compare unknowns to these

≥ Mean Response of CPS: Positive
< Mean Response of CPS: Negative
Sensitivity = Chosen conc. of CPS

How it Worked

- Validation plan was extensively amended
  - Removed confirmatory assessment
- CPS sample Prepared at 75 ng/mL
- LPC samples prepared at 100, 150 and 200 ng/mL
- Precision of responses was measured (intra and inter-assay), but no criteria set
  - <30% achieved
- Selectivity, Prozone, Drug tolerance and process stability were tested along with target interference,
  - All acceptable
- Sample analysis
  - No ADA detected in Control group, except one individual that was positive throughout profile (interference or Pre existing ADA)
  - Detected ADA in post dose animals correlated to PK profiles.

Development Recommendations

Based on experience and scientific developments since this study we recommend the following

- Establish reagent conditions and timings as you would normally
- Use normalisation of response divided by NC response to reduce run to run variability
- Check that arbitrary cut point will not allow false positives
  - Run 15 individual samples and check that mean +3.09 SD is below CPS
- Establish Low PC level
  - Run multiple CPS against dilution curve of positive control material
  - LPC concentration should be just above the interpolated conc. of mean response +3.09 SD of the CPS samples.
- Advised to check drug tolerance and selectivity at this point
- Look at impact of singlate vs duplicate wells
  - Singlate is preferable

Validation Recommendations

- Validation can be simple
  - 3-4 plates
- Precision and Accuracy
  - Duplicate wells; 2 NC, 4 CPS, 2 LPC samples per plate bracketing other validation tests
  - Singlate wells; 3 NC, 6 CPS, 3 LPC samples per plate, place at start middle and end
  - Include HPC when quasi-quantitative (ratio) results will be reported for samples

Acceptance Criteria (Ratio to NC)
1 (NC) < CPS < LPC < HPC

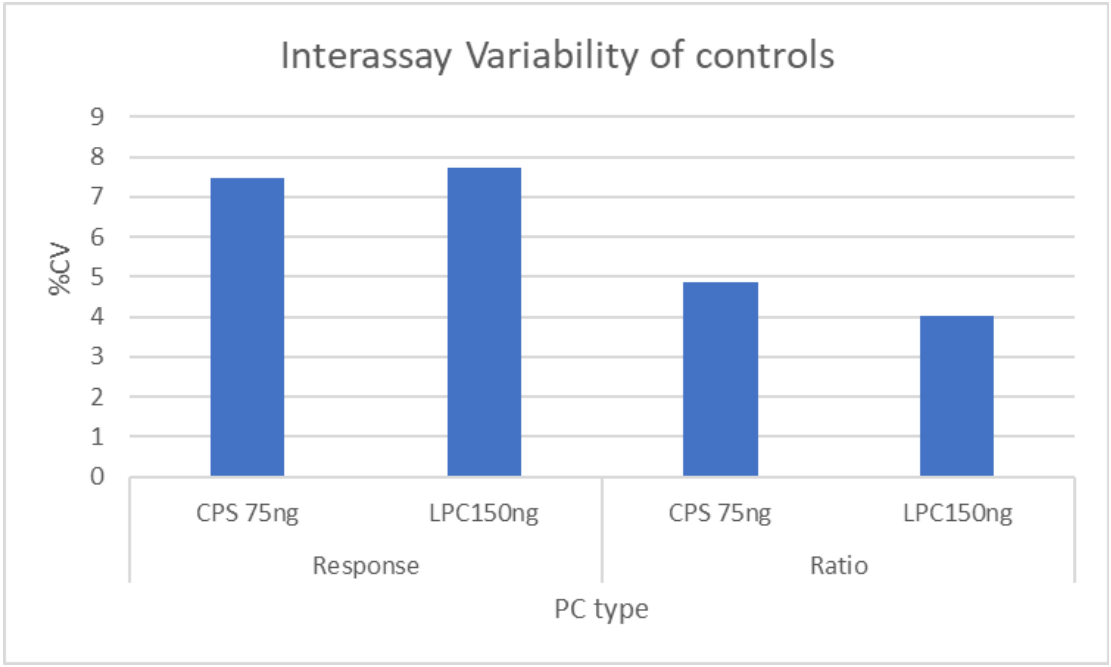
- Drug Tolerance: Serially Dilute Drug in LPC from approx. 4x expected concentration in samples
- Selectivity: Spike 6 individuals with LPC & run alongside unspiked
  - NC spike control (for troubleshooting)
- Prozone
- F/T and RT stability of LPC

Sample Analysis recommendations

- Run samples alongside 2 NC samples,4 CPS samples and 2 LPC samples.
  - 3 NC, 6 CPS and 3 LPC if using singlate analysis
- Analyse all samples in profile (most studies will only have a few plates worth of samples)
- If project teams require a quasi-quantitative result, then the ratio of sample to NC should be used.
  - An HPC should be used to demonstrate the assay is working across the active range.

Acceptance Criteria (Ratio to NC)
1 (NC) < CPS < LPC < HPC

- We do not recommend applying a range criteria of response ratio to the PC samples because of the limited number of assays.
  - Inter-assay variation is already reduced using Ratio to NC



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