celerion

Translating Science to Medicine

EBF Workshop 2022:

Points to Consider on Cut Points

28-29 April 2022

Disclaimer:

- The following case studies are based on true stories but all data has been anonymized
- Any likeness to actual data, living or dead is purely coincidental
- No animals were harmed in the making of this presentation
- And if the statistics in this presentation make you feel drowsy then they are probably working as intended.

Case Study 1: Low False Positive Rate

- Analyte: A Cytokine Biosimilar (Non-Pegylated)

- After screening analysis only 5 out of 900 samples were reactive and then all 5 were negative in the confirmatory assay…

- Great news! No immunogenicity issues!
- No Titers, no Nabs – Study Complete!

- BUT - this gave a false positive rate of ***0.6%,*** well below the target of 5% (or even 2% to 11%) and previous studies with this same Biosimilar had not shown this issue.

Case Study 1: Low False Positive Rate

- Possible impact of a Low False Positive rate?
  - Perhaps the assay was not sensitive enough?
  - Perhaps our cut point was too high and we could possibly be missing some potential positives?
  - Perhaps there was a difference between our negative control and the subject population?
  - Or all of the above…

Time to investigate!

Case Study 1: Low False Positive Rate

- Sensitivity issue? Our Low Positive Control was set to 40.0 ng/mL using a commercial antibody.
  - This level was statistically determined from 12 titration curves of the PC and showed good inhibition.
  - This is also below the target of 100 ng/mL.

- Cut point too high? The screening CF was 1.13 which is pretty reasonable.
  - This was also statistically determined in the validation from 50 individuals, 6 times each.

- Difference in Negative Control and Study Population?
  - Average NC response = 250 RLU
  - Average Pre-Dose response = 225 RLU

Case Study 1: Low False Positive Rate

- Our negative control was giving slightly higher responses than pre-dose samples.
- This could be because the populations were different:
  - The NC was made of a pool of individual serum lots from Australia
  - The subject population was from Zimbabwe
- Differences in populations could give different background responses, resulting in lower responses and a lower false positive rate.

- Solution? It was agreed with the sponsor to use an in-study Cut Point!

Case Study 1: Low False Positive Rate

- We already had a lot of data from pre-dose samples so it was a simple matter of running the data through our statistical models.
- Normally we would take 50 pre-dose results from different runs/days/analysts but since all pre-dose samples were analyzed in a single day using automation we decided to use 100 individual pre-dose results instead.

- The new screening Correction Factor was now found to be 0.975
  A cut point BELOW the negative control?

- Actually that's fine, we applied that new correction factor to all of our 900 sample results and found 110 newly reactive samples.

Case Study 1: Low False Positive Rate

- Each of those 110 newly reactive samples where then analyzed in the confirmatory format and once again all were found to be negative. (Yay?)
- But this now gave us a new false positive rate of 12%, now much higher than the 5% target and still outside of the 2% to 11% range…

- But all that actually means is that the assay was not very efficient, we were just running more confirmatory samples than we should be – no real impact on the study.

Case Study 1: Low False Positive Rate

- So, all samples were negative, but can we do any more to confirm that?
  - We took a close look at the confirmatory data and noted that all samples were giving inhibition responses well below the validated inhibition cut point of 35%, in fact the highest sample inhibition seen was only 25%.

- But can we dig deeper?
  - Using the sample inhibition data we generated a in-study iCP value of 33% - pretty close to the validated iCP, showing that even if the background rates between the populations were different, the ***inhibition rates were similar.***

- Deeper still?
  - As an additional test we looked only the inhibition data from reactive pre-dose samples (n=22), and there we calculated an iCP of 27%, still higher than the highest sample.

Case Study 1: Low False Positive Rate

- Outcome: All samples remained negative even when using a variety of in-study cut points.

- Further options: If necessary, we could always repeat the analysis using a negative control pool comprised of pre-dose samples, to ensure background values remain equal.

- Take home messages:
  - Populations matter! One healthy population may not be the same as another healthy population.
  - Have a procedure in place in case you do not meet false positive rate targets.
  - Keep your data flexible so you can re-evaluate if necessary.

Case Study 2: Failing LPC

- Analyte: A Pegylated Cytokine Biosimilar
- Several characterization runs were invalid because the LPC was failing to meet acceptance criteria:
  - Specifically, the LPC was showing poor inhibition when using the ***non***-pegylated competitor…
  - Hmm… 
- Just briefly, we wanted to know what the sample antibodies were binding too, was it the drug or the PEG, so we added them separately as competitors to see which inhibited the assay – pretty standard.

Case Study 2: Failing LPC

- Of course the answer was simple:
  - The assay was optimized to show inhibition with the Pegylated Drug.
  - The positive controls bind nicely to the PEG portion, but less so to the naked drug.

- We were still seeing some reasonable inhibition though, it was just routinely at or just below the validated iCP.

- Solution?
  - Re-evaluate the validation data using a lower confidence interval and calculate a lower characterization iCP

Case Study 2: Failing LPC

- The validation was performed using a 99% confidence interval (1% false positive rate) when assessing inhibition with the non-Pegylated competitor.
- But this criteria was tighter than it really needed to be. This was just a characterization step after positivity has been already been confirmed and we would also be comparing inhibition in parallel with PEG
- So we re-eveluated the validation data using a 90% confidence interval and generated a lower characterization iCP: dropping from 30% to 24% (Not actually a big drop!)
- The runs were now valid and results could be interpreted.

Case Study 2: Failing LPC

- Impact: Changing the confidence interval changes the expected false positive rate of the ***characterization assay*** from 1% to 10%

- But what is the REAL impact?
  - We already know the samples are positive, they've already gone through the confirmatory step.
  - We also have parallel data showing that for all samples that were characterized as positive for Drug antibodies they were also negative PEG antibodies, and vice-versa – so all data remained internally consistent.
  - So a higher false positive rate in a characterization step had little to no impact.
  - Worse case scenario? We might have gotten some samples that could have been showing up as positive for both competitors, but we had multiple samples per subject, and this did not happen.

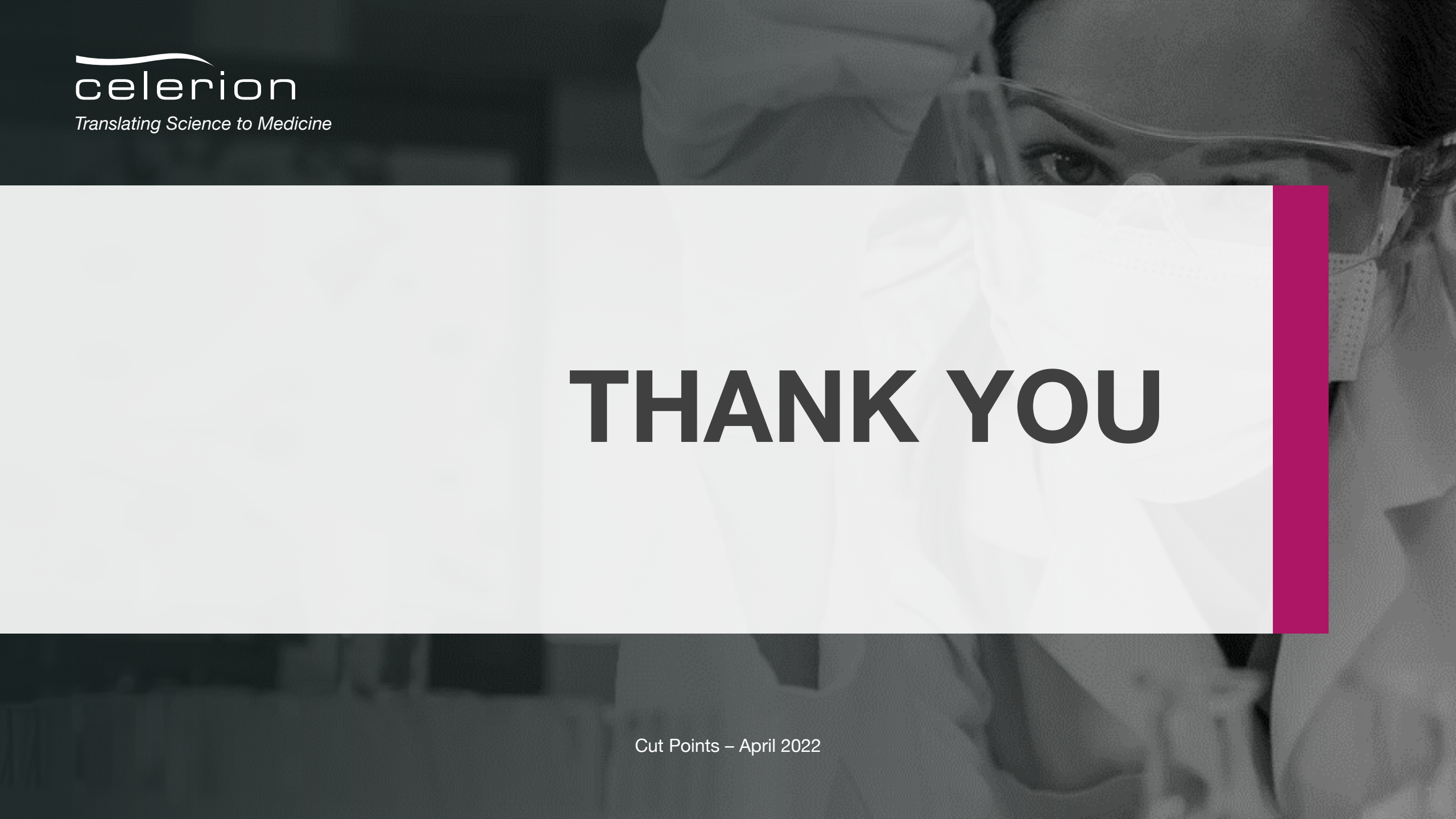
- Take home messages:
  - Assess impacts of failures logically – what is the true impact?
  - Keep your data flexible so you can re-evaluate if necessary.

Acknowledgements:

- Special thanks to Anonymous Sponsor Number 1 and Anonymous Sponsor Number 2!
  - They were actually both very open to sharing these stories but we decided to keep names and details out of it just for simplicity.

- Thanks to Marleen and her team at Celerion for generating and evaluating all this data.

- Thanks to Philip and the EBF community for this opportunity and all of the great advice over the years!



THANK YOU