charles river

Leaner Approaches to Clinical Analysis

Does the Confirmatory Assay Always Add Value?

John Cook

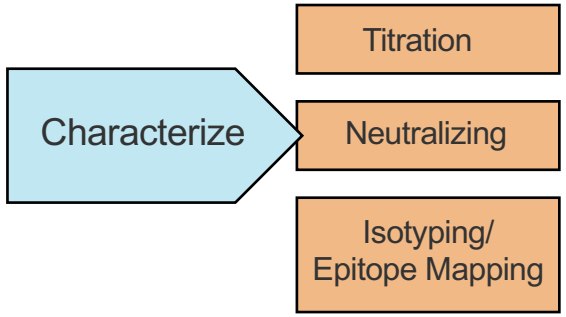
11/29/23

Clinical Immunogenicity Workflow

Current tiered analysis approach

- Typical workflow for the analysis of Clinical ADA samples

- Depending on clinical stage and therapeutic, characterization can be extensive



charles river

Clinical Immunogenicity Workflow

“How long and how much?”

Possible Analysis Required	Result	Cost
Screening	Negative	🕒 | €
Screening & Confirmatory	Negative	🕒🕒 | € €
Screening, Confirmatory & Titer	Positive (1:X)	🕒🕒🕒 | € € €

+ Any further characterization required…

- Current strategy has implications on both time and cost
  - ➢ Difficult to accurately predict analysis required
  - ➢ Cost and time is variable depending on analysis required
  - ➢ Batch analysis of immunogenicity samples can be the rate limiting step for database lock

Leaner Approaches

“Is there a faster, more cost effective option?”

- Is the same strategy of screening, confirming and titration always required?
- Could a leaner approach offer equivalent value?

Option 1:

Eliminate (or combine) the screening assay

Possible Analysis Required	Result	Cost
~~Screening~~	~~Negative~~	~~€~~
Screening & Confirmatory	Negative	€ €
Screening, Confirmatory & Titer	Positive (1:X)	€ € €

+

- Potential to save time and cost in case of high ADA incidence
  - Single analytical occasion to provide qualitative sample result
  - Fewer analytical batches overall?

–

- More costly approach in cases where ADA incidence is low?
  - Fewer samples per batch = Greater number of batches
  - Greater reagent expense?

Leaner Approaches

“Is there a faster, more cost effective option?”

Option 2:

Eliminate the titer assay

Possible Analysis Required	Result		Cost
Screening	Negative	🕒	€
Screening & Confirmatory	Negative	🕒🕒	€ €
~~Screening, Confirmatory & Titer~~	~~Positive (1:X)~~	~~🕒🕒🕒~~	~~€ € €~~

\+

- Offers greatest benefit in regards to cost saving
  - Greatest impact on overall batch numbers
  - Most unpredictable assay tier

–

- Signal-to-noise not always appropriate
  - Signal saturation for colorimetric approaches

Leaner Approaches

“Is there a faster, more cost effective option?”

Option 3:

Eliminate the confirmatory assay

Possible Analysis Required	Result	Cost
Screening	Negative	🕒 | €
~~Screening & Confirmatory~~	~~Negative~~	~~🕒🕒 | € €~~
Screening, ~~Confirmatory~~ & Titer	Positive (1:X)	🕒🕒🕒 | € € €

- Does the confirmatory assay offer unique value?:
  - Is the confirmatory assay only eliminating “marginally positive” samples?
  - Would a more stringent cut point (e.g. 1% or 0.1% FPR) serve the same purpose?

Evaluation of Clinical Studies

Study	Method	Sample No.	Samples Screened Positive (SCP)	Samples Confirmed Positive (CCP)	Signal:TCP
1	A	192	8	0	-
2	A	160	13	0	-
3	B	57	18	0	-
4	B	96	8	0	-
5	B	93	7	4	1.21 - 1.59
6	C	144	24	17	0.86 - 54.1
7	D	287	18	3	0.93 - 1.60
8	E	218	23	14	0.75 - 20.2

- Results from 8 Phase I clinical studies
- Mix of MAb and Bi-Specific Ab test items
- Tiered strategy adopted for analysis
  - Range of titres normalised

Would these results have been different if a confirmatory assay had not been included?

- Results reprocessed assessing the screening data against a titer cut point with a 0.1% FPR

Reprocessed Results

Study	Method	Sample No.	Samples Confirmed Positive (CCP)	Samples Screened Positive (TCP)	Discrepancies		
					Total Discrepancies	Confirmed Negative / Positive Against TCP	Confirmed Positive / Negative Against TCP
1	A	192	0	0	0	0	0
2	A	160	0	1	1	1	0
3	B	57	0	4	4	4	0
4	B	96	0	4	4	4	0
5	B	93	4	5	1	1	0
6	C	144	17	15	7	2	5
7	D	287	3	2	5	2	3
8	E	218	14	12	2	0	2

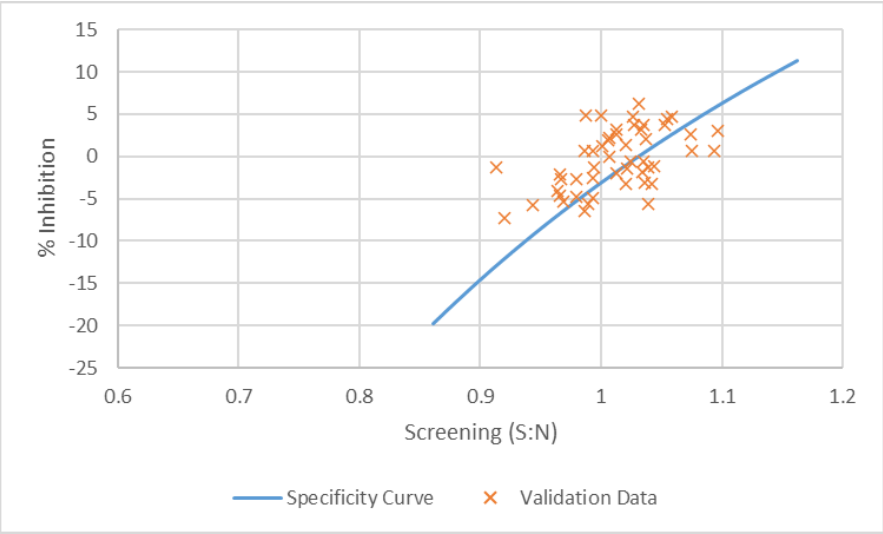
- 100% correlation, cut point with lower FPR would have achieved sample result as confirmatory assay (Green)
- Cut point with lower FPR results in higher % incidence rate reported; confirmatory assay eliminates samples identified as positive due to non-specific binding. No impact of patient safety? (Yellow).
- Low responders identified as positive in confirmatory assay are identified as negative against a cut point with a lower FPR. Were these associated with clinical signs? (Orange).
- Mix of both scenarios, minimal impact to % ADA incidence but different sample population reported as positive. How does this tie in with clinical signs? (Red).

What (if any) impact would these discrepancies have on the studies?

Could we have predicted where the greatest discrepancies would be?

Assessing the Impact – Method A

Study	Method	Discrepanices		
		Total Discrepancies	Confirmed Negative / Positive Against TCP	Signal:TCP
1	A	0	0	-
2	A	1	1	1.00



Method A	
SCP	1.086
TCP	1.173
Analytical Outliers	4
Biological Outliers	0
CCP	12.7%
Analytical Outliers	2
Biological Outliers	0
Selectivity	10/10 Individuals met acceptance

Impact of Removing Confirmatory Tier:

- Study 1 – 100% correlation; no impact
- Study 2 – 1 sample >TCP at MRD; no impact?

Review of Method:

- Low variation in CP population (SCP & CCP)
- Low outliers
- No issues identified in selectivity assessment

charles river

Assessing the Impact – Method B

Study	Method	Confirmed Positive Samples	Signal:TCP (Confirmed Positive)	Discrepanices		
				Total Discrepancies	Confirmed Negative / Positive Against TCP	Signal:TCP
3	B	0	-	4	4	1.01 - 1.06
4	B	0	-	4	4	19.8 - 25.4*
5	B	4	1.21 - 1.59	1	1	1.84

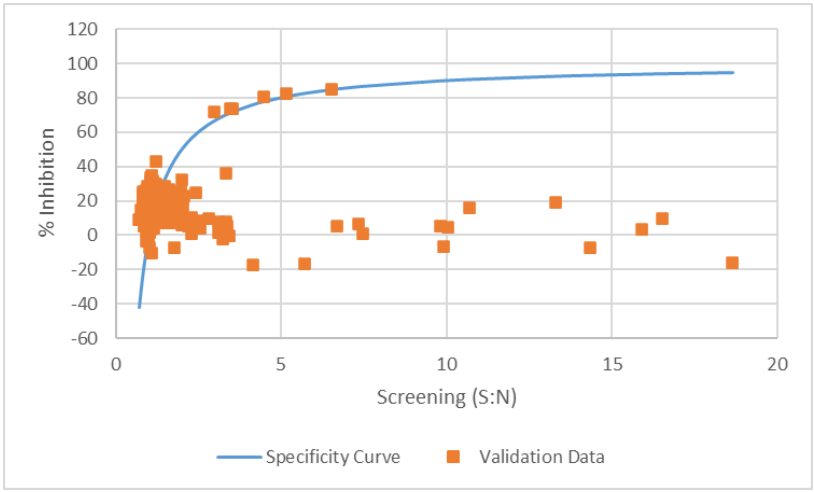
Impact of Removing Confirmatory Tier:

- Study 3 – 4 samples >TCP at MRD; no impact?
- Study 4 – 4 samples; 1 patient; Pre-existing response, no evidence of treatment boosted response; no impact.
- Study 5 – 1 additional sample > TCP at MRD; no impact?

	Method B
SCP	1.258
TCP	2.011
Analytical Outliers	33
Biological Outliers	7
CCP	31.6%
Analytical Outliers	8
Biological Outliers	3
Selectivity	9/10 Individuals meet acceptance criteria (unspiked)

Review of Method:

- High variation (SCP & CCP)
- High number of outliers
- One individual observed to be ≥ CP during selectivity assessment



charles river

Assessing the Impact – Method C

Study	Method	Confirmed Positive Samples	Signal:TCP (Confirmed Positive)	Discrepancies			
				Total Discrepancies	Confirmed Negative / Positive Against TCP	Signal:TCP	Confirmed Positive / Negative Against TCP
6	C	17	0.86 - 54.1	7	2	1.01 - 1.07	5

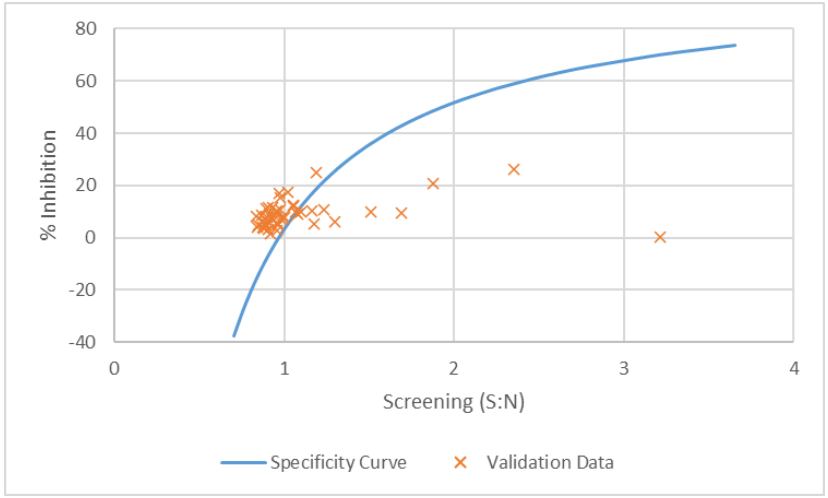
Impact of Removing Confirmatory Tier:

- 2 additional samples reported as Positive (≥ TCP)
  - Low responders
  - No impact of safety
- 5 samples now reported as negative (<TCP)
  - All samples ≥ SCP; < TCP
  - Were these samples associated with clinical signs?

	Method C
SCP	1.197
TCP	1.431
Analytical Outliers	9
Biological Outliers	5
CCP	20.9%
Analytical Outliers	13
Biological Outliers	3
Selectivity	9/10 Individuals met acceptance (Unspiked) 9/10 Individuals met acceptance (Spiked; 1 samples < SCP

Review of Method:

- Low number of outliers
- No issues observed during method validation



charles river

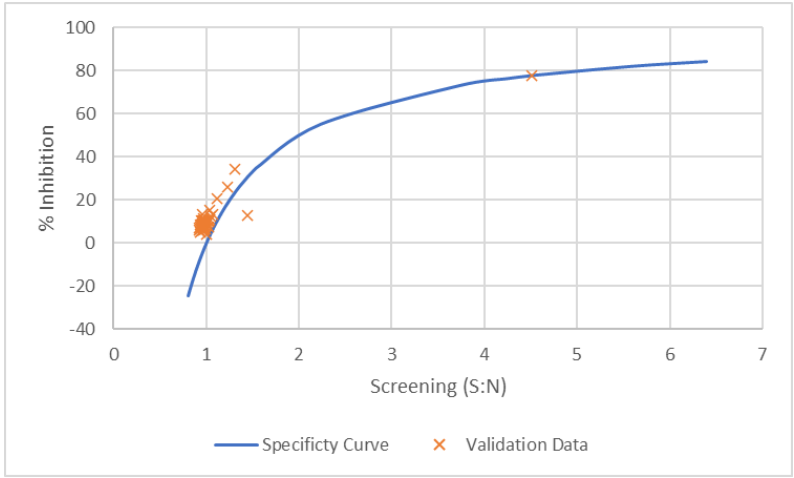
Assessing the Impact – Method D

Study	Method	Confirmed Positive Samples	Signal:TCP (Confirmed Positive)	Discrepancies			
				Total Discrepancies	Confirmed Negative / Positive Against TCP	Signal:TCP	Confirmed Positive / Negative Against TCP
7	D	3	0.93 – 1.60	5	2	2.27 – 3.90	3

Impact of Removing Confirmatory Tier:

- 2 samples reported as Positive (≥ TCP)
  - High responses
  - Non-specific binding
- 3 samples now reported as negative (<TCP)
  - All samples ≥ SCP/CCP; < TCP
  - Were these samples associated with clinical signs?
- ADA incidence for study remains comparable (~1%), however different individuals identified

	Method D
SCP	1.104
TCP	1.212
Analytical Outliers	9
Biological Outliers	5
CCP	26.3%
Analytical Outliers	3
Biological Outliers	5
Selectivity	9/10 Individuals met acceptance criteria (unspiked)



Review of Method:

- Mid-high variation observed in assessment of CCP
- Higher CCP better at eliminating NSB?

charles river

Assessing the Impact – Method E

Study	Method	Confirmed Positive Samples	Signal:TCP (Confirmed Positive)	Discrepancies	
				Total Discrepancies	Confirmed Positive / Negative Against TCP
8	E	14	0.78 – 20.2	2	2

Impact of Removing Confirmatory Tier:

- 2 Samples now reported as negative
  - ≥ SCP/CCP; < TCP
  - When assessed in titer assay both samples < TCP at MRD

	Signal:TCP
Sample 1	0.78
Sample 2	0.84

- Were these samples associated with clinical signs?

Review of Method:

- CCP at low level?

Method E	
SCP	1.272
TCP	1.694
Analytical Outliers	7
Biological Outliers	6
CCP	15.6%
Analytical Outliers	4
Biological Outliers	1
Selectivity	8/10 Individuals ≥ SCP when spiked at LPC

Comparing ADA Incidence

Study	Method	Sample No.	3-Teired Approach (Original Approach)		0.1% Screening / No confirmatory Approach	
			No. Samples Reported as Positive	% ADA Incidence	No. Samples Reported as Positive	% ADA Incidence
1	A	192	0	0.0	0	0.0
2	A	160	0	0.0	1	0.6
3	B	57	0	0.0	4	7.0
4	B	96	0	0.0	4	4.1
5	B	93	4	4.3	5	5.4
6	C	144	17	11.8	15	10.4
7	D	287	3	1.0	5	1.7
8	E	218	14	6.4	12	5.5

- Minimal difference in overall % ADA incidence for all studies reviewed
- Tendency for reported ADA incidence to increase
  - 5/8 increase
  - 1/8 no change
  - 2/8 decrease
- Caution – Studies reviewed all have relatively low sample numbers
  - Larger number of studies required to assess further
  - Opportunity to assess Phase III studies of *ca.* 20,000+ samples

Evaluating Benefits

Resource Revisited

Study	3-Teired Approach (Original Approach)				0.1% Screening / No confirmatory Approach				
	Screen	Confirm	Titer	**Total**	Screen	Titer	**Total**	No. of Plates Saved	**% Decrease**
1	7	1	0	**8**	7	0	**7**	1	**12.5**
2	6	1	0	**7**	6	1	**7**	0	**0.0**
3	2	2	0	**4**	2	1	**3**	1	**25.0**
4	4	1	0	**5**	4	1	**5**	0	**0.0**
5	4	1	1	**6**	4	1	**5**	1	**16.7**
6	5	2	2	**9**	5	2	**7**	2	**22.2**
7	10	2	1	**14**	10	1	**11**	2	**14.3**
8	8	2	2	**13**	8	2	**10**	3	**23.1**
								Mean	**14.2**

- Approximate requirements for analysis with each strategy
  - Assumes batch analysis (no interim analysis)
  - Assumes titer of interest identified in first run
- Saving in regards to no. of plates modest
  - Small study size
  - Comparable savings in Phase III studies would be substantial

Possible Analysis Required	Result	Cost
Screening	Negative	🕒 | €
Screening & Confirmatory	Negative	🕒🕒 | € €
Screening, Confirmatory & Titer	Positive (1:X)	🕒🕒🕒 | € € €

Summary, Conclusions & Questions

One size does not fit all..

- For studies reviewed, marginal difference in overall ADA incidence reported
- Tendency to see greater number of “positive” samples; greater impact from non-specific binding
- Difficult to predict value of confirmatory based only on validation data
- Where difficulties in specificity are encountered, confirmatory assay can be of value

- Could results from early clinical studies be used to justify leaner approach for Phase III?
- If confirmatory assays determined to add value can a different tier be eliminated?
- Do differences observed correlate with adverse events or clinical findings?

- Additional benefit if combined with other strategies such as S:N rather than titer analysis:

Study	3-Teired Approach (Original Approach)	0.1% Screening / S:N	% Decrease
1	8	7	12.5
2	7	6	14.3
3	4	2	50.0
4	5	4	20.0
5	6	4	33.3
6	9	5	44.4
7	14	10	28.6
8	13	8	38.5
		Mean	30.2

Thanks & Acknowledgements

- EBF Organising Committee

<u>CRL:</u>

- Sebastien Boridy
- Katie Sime