EBF Workshop: Points to Consider on Cut Points
29th April 2022

Integrated Summary of Immunogenicity (ISI):

ADA assay cut point strategy & method life-cycle history

Paul Chamberlain

Immunogenicity Integrated

© Immunogenicity Integrated 2022

Value proposition

Bioanalytical section of Integrated Summary of Immunogenicity (ISI) provides opportunity to communicate strategy and minimize questions

1. Help regulatory reviewer to navigate information distributed across multiple documents (MVRs, BARs) submitted in different sections of CTD dossier
2. Explain evolution of methodology & impact on assay performance
3. Summarize assay cut points applied for different clinical studies / clinical populations
4. Justify alternative statistical approaches

Regulatory basis:
EMA guideline, section 10 – Summary of the immunogenicity program
FDA 2019 guideline, section VIII - Documentation

© Immunogenicity Integrated 2022 |

ISI *vs.* MVR *vs.* BAR

Integrated Summary of Immunogenicity (ISI)

- CTD § 5.3.5.3
- Section 2 = Summary of immunogenicity assessment strategies / methods, with links to MVRs & BARs
    - Opportunity to present complete picture & link documents

Method Validation Reports (MVRs)

- CTD § 5.3.1.4
- Assay performance detail
- Cut-points for initial & subsequent method validations

Bioanalytical Reports (BARs)

- Usually appended to Clinical Study Report in CTD § 5.3
- Clinical sample analysis results
- Statistical report for clinical sample analysis, including in-study cut point determination

Immunogenicity Integrated

© Immunogenicity Integrated 2022

Use ISI to consolidate critical points for assessment

Method Validation Reports (MVRs)

- CTD § 5.3.1.4
- Assay performance detail
- Cut-points for initial & subsequent method validations

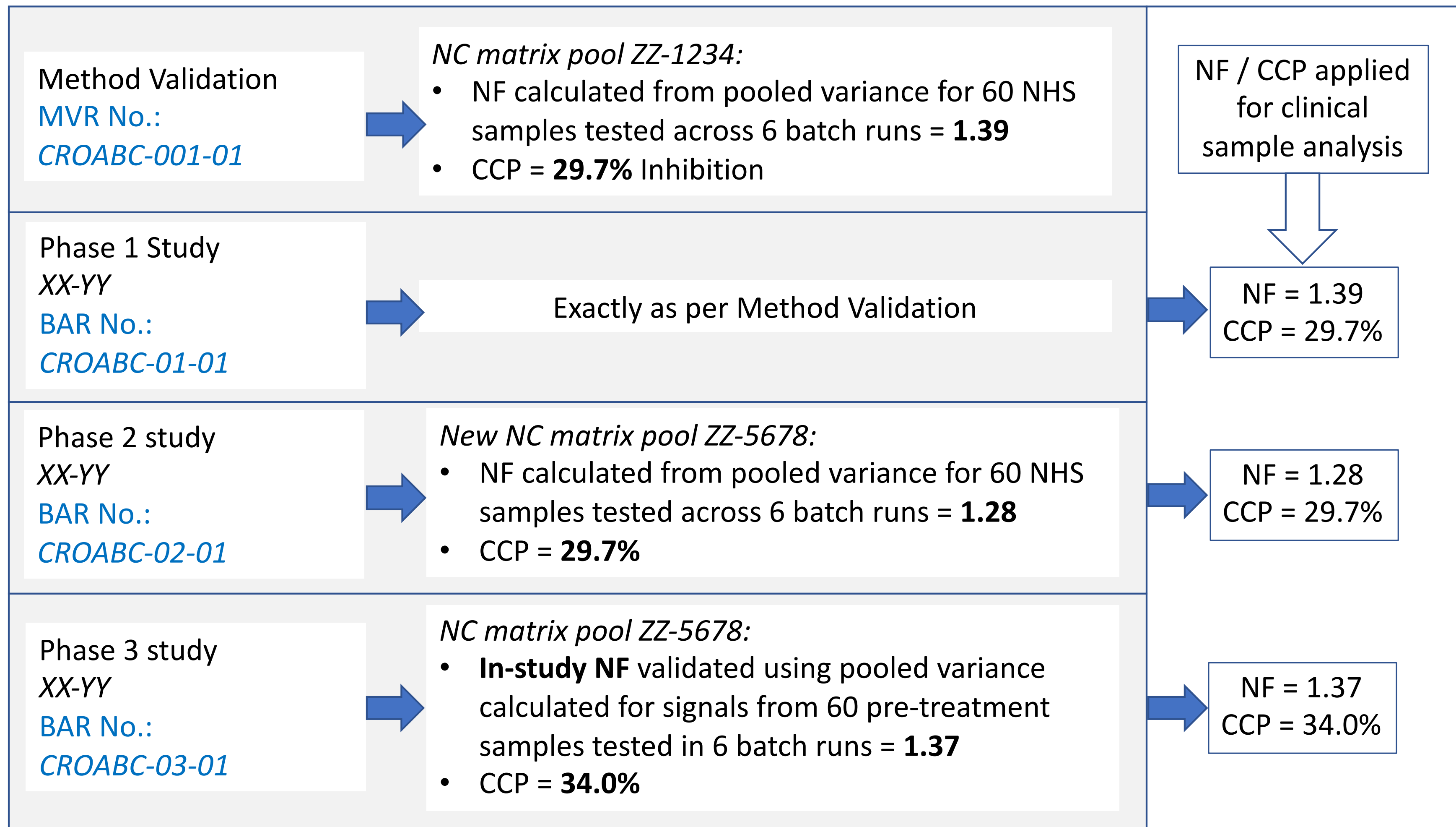
Bioanalytical Reports (BARs)

- Usually appended to Clinical Study Report in CTD § 5.3
- Clinical sample analysis results
- Statistical report for clinical sample analysis, including in-study cut point determination

It can be very challenging for naïve reviewer to understand to relationship of different method versions to particular clinical study sample analyses:

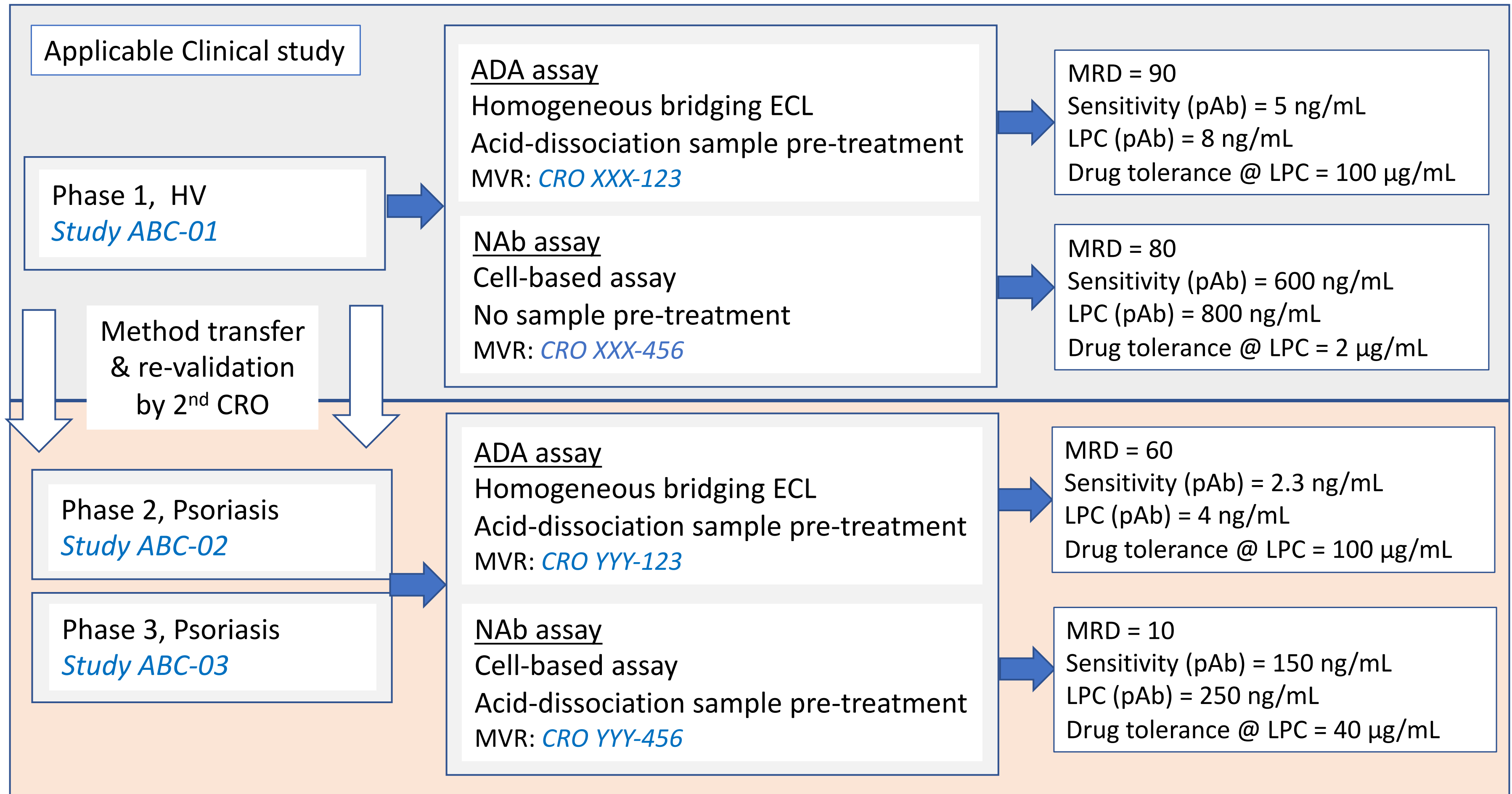
➢ Use Bioanalytical section of ISI to explain what was done and why

Mapping to different clinical studies & BARs



Immunogenicity Integrated

ADA / NAb assay evolution *vs.* applicable clinical studies



Immunogenicity Integrated

© Immunogenicity Integrated 2022 |

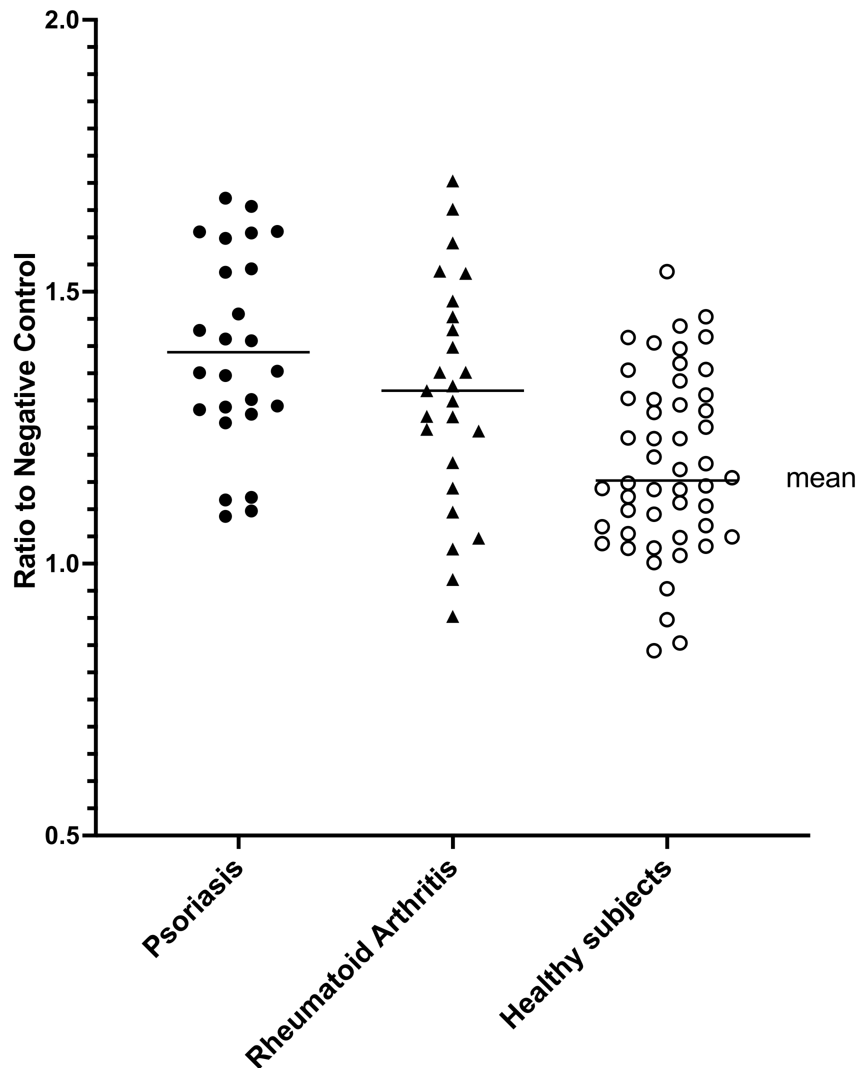
Explaining changes & impact

Table X: Changes Between 1st- and 2nd-generation ADA Assays

Aspect	1st-generation method	2nd-generation method
Applicable clinical study(ies)	*ABC-001*	*ABC-002* & *ABC-003*
CRO	*XXX*	*YYY*
SOP No.	*XXX-1234*	*YYY-1234*
MVR No.	*XXX-5678*	*YYY-5678*
MSD Detector	QuickPlex	Sector Imager 600
Biotin-Drug concentration in Master-Mix	1 µg/mL	2 µg/mL
Sulfo-TAG-Drug concentration in Master-Mix	1 µg/mL	2 µg/mL
pAb Positive Control	In house, Lot # 1234	In house Lot # 5678
mAb Positive Control	Supplier A, lot no. 2345	Supplier A, lot no. 6789
Negative Control	NHS Pool 2020-01-101	NHS Pool 2020-01-102
MRD (final in-plate dilution)	90	60
Sensitivity (pAb)	5 ng/mL	2.3 ng/mL
Low Positive Control (pAb)	8 ng/mL	4 ng/mL
Drug Tolerance at LPC	100 µg/mL	100 µg/mL
Selectivity	Validated for normal human serum only	Additional validation for psoriasis matrix

Immunogenicity Integrated

Graphical presentation of data from different populations



	Psoriasis	Rheumatoid Arthritis	Healthy subjects
Test for normal distribution			
Shapiro-Wilk test			
W	0.9406	0.9857	0.9815
P value	0.1531	0.9703	0.6179
Passed normality test (alpha=0.05)?	Yes	Yes	Yes
P value summary	ns	ns	ns

One-way ANOVA	
F	12.04
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.1989

Bartlett's test	
Bartlett's statistic (corrected)	2.153
P value	0.3407
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

- Different Means
- Similar Variances
- Adjust NF for mean SCP/NC ratio

See Devanarayan V. and Tovey M.G. 2011. In Detection and quantification of antibodies to biopharmaceuticals. Ed. Tovey M.G. Wiley. New Jersey. Pages 289-308

False Positive Error Rate (FPER)

- Calculation of the FPER for screening assay provides an empirical reliability check versus expected false positive rate;
  - see Tan CY et al; AAPS Journal, 2020, 22, 19
- Calculate FPER for pre- and post-treatment samples
- Higher than expected FPER may reflect overly conservative screening cut point for the actual clinical sample population
  - *Unrepresentative negative control?*
  - *Is assay associated with non-specific binding?*
  - *Aggregated labeled antigen reagents?*
  - *Excessive "outlier" exclusion?*
  - *Different data distribution in clinical population?*
  - *Influence of target binding?*
- May provide justification for application of an in-study cut point rather than cut points established during method validation

© Immunogenicity Integrated 2022 |

False Positive Error rate (FPER) for screening assay

FPER = [(A – B) / Total sample number – B] x 100

A = T1 Positives = Total number of samples that screen positive

B = T2 Positive = Total number of samples that confirm positive

Table X: FPER for different clinical studies

Statistic	Treatment group			Overall
	Study *ABC-01*	Study *ABC-02*	Study *ABC-03*	
Total sample number screened	480	1856	3876	6212
Total number of samples that screen positive	96	734	2370	3200
Total number of samples that confirm positive	72	672	2293	3037
FPER	5.9	5.5	5.1	5.4

Immunogenicity Integrated

Justifying alternative statistical approaches

- Criteria for outlier exclusion may be inappropriate for actual study population, resulting in excessive exclusion of “biological outliers”
- Regulatory agencies accept alternative approaches for outlier exclusion criteria, if adequately justified by the data

Example:
Zhang J et al. J Immunol Methods, 2020, 484-485
A new method for identification of outliers in immunogenicity assay cut point data

- Problem = Elimination of outliers by the box plot method with 1.5 IQR whiskers is very efficient in stripping the data from most of biological variability and leading to cut points that are very close to the minimum values
- Proposed alternative method accounts for relative magnitude of biological versus analytical variability within the sample population
  - Combines identification of possible biological outliers using Tukey's box-plot outlier ranges using 1.5 or 3 IQR, but excludes only those values beyond a pre-defined biological variability / analytical variability ratio

Immunogenicity Integrated

Calculation of Normalization Factor

- FDA reviewers have recommended (in BLA review comments) that the Normalization Factor should be calculated to reflect the variance for the pooled data set generated across 6 batch analyses, rather than the average of the NF values calculated separately for each of the 6 batch analyses.
  - Justification = averaging of the NF values for each batch run results in an underestimation of the pooled variance across the 6 batch analyses.

Calculation of NF from average of each batch run						
Run ID	VA-1	VA-2	VA-3	VA-4	VA-5	VA-6
NF (screening) per run	1.19	1.22	1.59	1.31	1.28	1.16
Mean NF (screening)	1.29					

NF calculated by pooling individual values for all = 1.41

Immunogenicity Integrated

Main points

- Regulatory assessors have limited time to review the multiple documents supporting the clinical immunogenicity evaluation
- Regulators are interested in understanding:
  - Rationale for choice of bioanalytical methodology
  - Nature of changes made to methods during clinical development & impact on method performance, particularly on drug tolerance
  - Empirical data (FPER) to support reliability of screening assay cut point for relevant clinical populations
  - Changes in critical reagents, including positive and negative control reagents
- ISI provides opportunity to consolidate communication of the essentials!

© Immunogenicity Integrated 2022