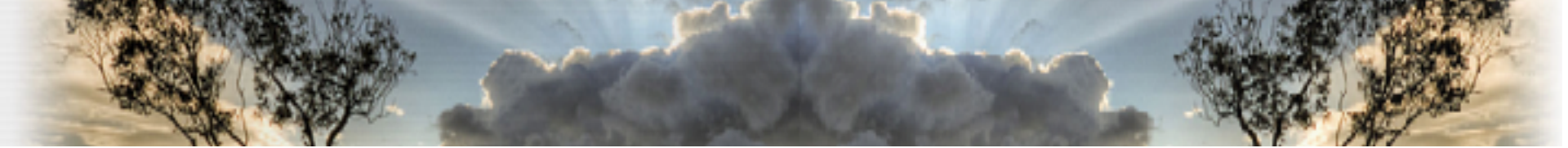


EBF

The Omicron Workshop

Points to Consider on Cut Points

Introduction to the session and ‘the problem statement’

Jo Goodman, on behalf of the EBF

28-29 April 2022 – in Cyberspace

Welcome to our first session …

13:00 14:00 Session 1: Introduction to the meeting and problem statement

Jo Goodman/Robert Nelson – on behalf of the EBF

Introduction presentation to introduce the items discussed at the meeting, includes but not limited to: Cut Points (CPs) – what and why; Difference from PK assays (CoU); In study vs. validation CP; Clinical relevance of CPs; regulatory challenges; the changing landscape on ADA and new modalities

Session includes Q&A for items that are not covered later in the workshop

Continuing the EBF discussions on immunogenicity

- Previous EBF discussions:
  - "Current analysis of immunogenicity – Best Practices and Regulatory Hurdles", September 27-28, 2016: https://e-b-f.eu/fw201609-slides/
  - FW Paper: https://pubmed.ncbi.nlm.nih.gov/29345496/
  - "Today's challenges and solutions in assessing immunogenicity in patients", September 19-20, 2018: https://e-b-f.eu/fw201809-slides/
  - "Training Day: managing the Practical Aspects of Immunogenicity", Cyberspace March 23-24, 2021: https://e-b-f.eu/fw202101-slides/
  - Recommendations and discussion points on immunogenicity, biomarkers, automation/technology and protein–MS from the 2021 European Bioanalysis Forum Focus Workshops: https://www.future-science.com/doi/10.4155/bio-2021-0200
  - A strategic approach to nonclinical immunogenicity assessment: a recommendation from the European Bioanalysis Forum: https://www.future-science.com/doi/full/10.4155/bio-2021-0028
  - Plus sessions in Barcelona

So why are we discussing cut points?

- Much discussion in the last decade on how to calculate a robust cut point that is **scientifically and clinically meaningful** (incidence of immunogenicity is not the full story)
- Technology and assays have improved which may cause operational challenges
- Use of surrogate controls
- Trials in multiple regions and potentially different disease states or populations
- Transfer of assays and cross validation
- Regulatory landscape has changed as experience has grown
- Immunogenicity assessment is becoming more complex as the modalities may be more challenging or novel

All therapeutic proteins have the potential to elicit an immune response; either wanted or unwanted

WANTED

- Typically related to vaccines
- Injecting antigen leads to an **immune response against the pathogen**
- Providing **protection from future exposure**

UNWANTED

- Some patients may mount an **undesirable response to a biotherapeutic** (seen as foreign)
- Anti-drug antibody (ADA)
- Breaking tolerance
- May **inactivate the effect of the drug and/or induce adverse events**

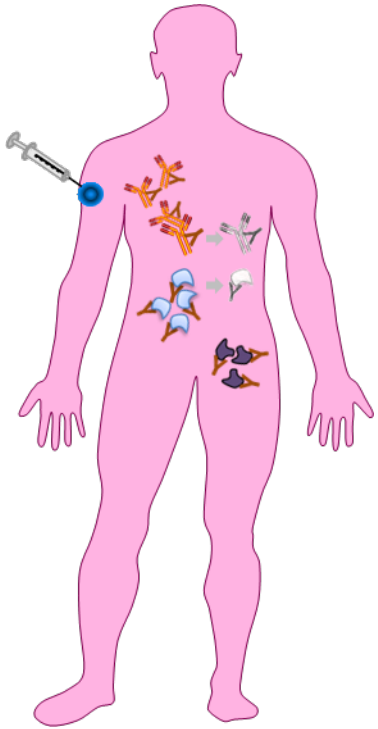
Potential clinical impacts of immunogenicity

Safety

Injection site/infusion reactions
Anaphylaxis
Cytokine storm release
Hypersensitivity

Elevated Safety Concerns

Neutralisation of non-redundant essential endogenous protein(s) that can lead to loss of physiological function



Altered Efficacy

Binding and neutralising anti-drug antibodies (ADA) can impact PK, PD and efficacy
Clearing ADA
Sustaining ADA

Potential clinical impact is more important that incidence alone

- Risk of molecule can differ and is evaluated through **risk assessment**
  - Likelihood that a response will occur and the impact of the response
  - Modality type, patient, molecule characteristics, administration, consequence of the response

- Every product needs to be **evaluated for immunogenicity individually and an appropriate strategy adopted for each development programme**
  - Comparison of immunogenicity rates with other products is not appropriate

- **Incidence of immunogenicity alone is not necessarily clinically relevant or impactful** and requires characterisation

- A few patients with severe responses is more important that many patients displaying anti-drug antibodies (ADA) without apparent clinical impact

Immunogenicity is required for the development and approval of a biotherapeutic and is described in the drug label and submission documentation

Immunogenicity

Formation of anti-adalimumab antibodies is associated with increased clearance and reduced efficacy of adalimumab. There is no apparent correlation between the presence of anti-adalimumab antibodies and the occurrence of adverse events.

In patients with polyarticular juvenile idiopathic arthritis who were 4 to 17 years, anti-adalimumab antibodies were identified in 15.8% (27/171) of patients treated with adalimumab. In patients not given concomitant methotrexate, the incidence was 25.6% (22/86) compared to 5.9% (5/85) when adalimumab was used as add-on to methotrexate. In patients with polyarticular juvenile idiopathic arthritis who were 2 to < 4 years old or aged 4 and above weighing <15 kg, anti-adalimumab antibodies were identified in 7% (1/15) of patients, and the one patient was receiving concomitant methotrexate.

In patients with enthesitis-related arthritis, anti-adalimumab antibodies were identified in 10.9% (5/46) of patients treated with adalimumab. In patients not given concomitant methotrexate, the incidence was 13.6% (3/22), compared to 8.3% (2/24) when adalimumab was used as add-on to methotrexate.

Patients in rheumatoid arthritis studies I, II and III were tested at multiple time points for anti-adalimumab antibodies during the 6 to 12 month period. In the pivotal trials, anti-adalimumab antibodies were identified in 5.5% (58/1053) of patients treated with adalimumab, compared to 0.5% (2/370) on placebo. In patients not given concomitant methotrexate, the incidence was 12.4%, compared to 0.6% when adalimumab was used as add-on to methotrexate.

In patients with paediatric psoriasis, anti-adalimumab antibodies were identified in 5/38 subjects (13%) treated with 0.8 mg/kg adalimumab monotherapy.

In adult patients with psoriasis, anti-adalimumab antibodies were identified in 77/920 subjects (8.4%) treated with adalimumab monotherapy.

In adult plaque psoriasis patients on long term adalimumab monotherapy who participated in a withdrawal and retreatment study, the rate of antibodies to adalimumab after retreatment (11 of 482 subjects, 2.3%) was similar to the rate observed prior to withdrawal (11 of 590 subjects, 1.9%).

In patients with moderately to severely active paediatric Crohn's disease, the rate of anti-adalimumab antibody development in patients receiving adalimumab was 3.3%.

In patients with Crohn's disease, anti-adalimumab antibodies were identified in 7/269 subjects (2.6%).

Immunogenicity Information in Human Prescription Therapeutic Protein and Select Drug Product Labeling — Content and Format

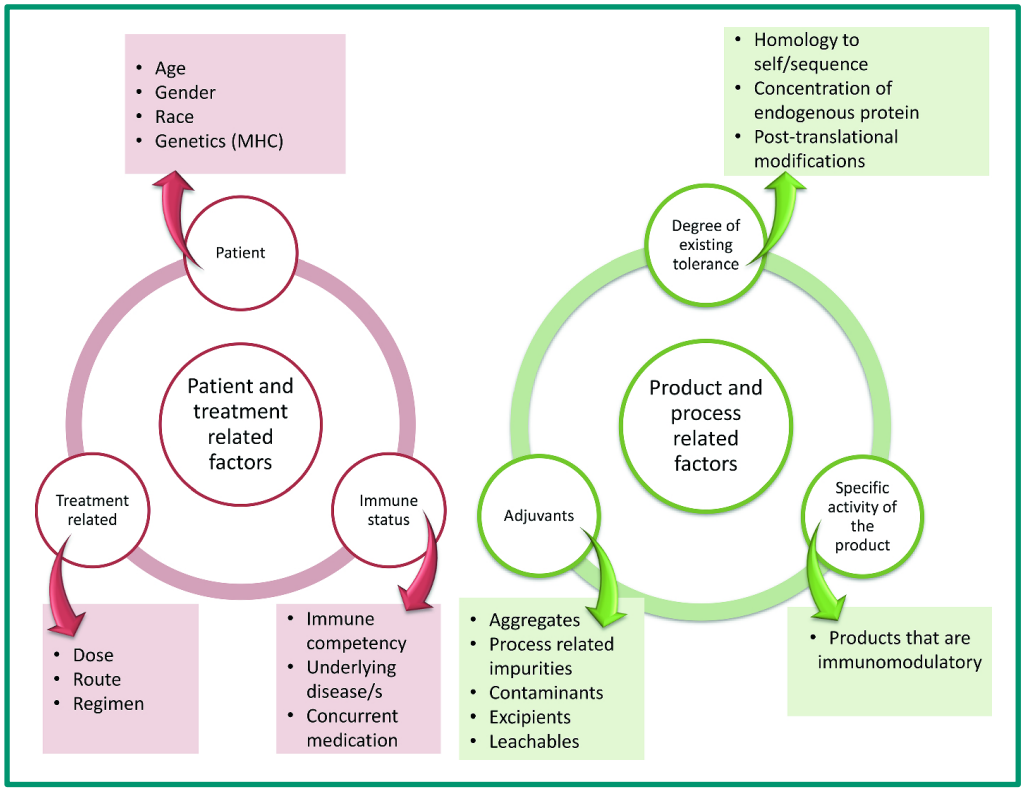
Guidance for Industry

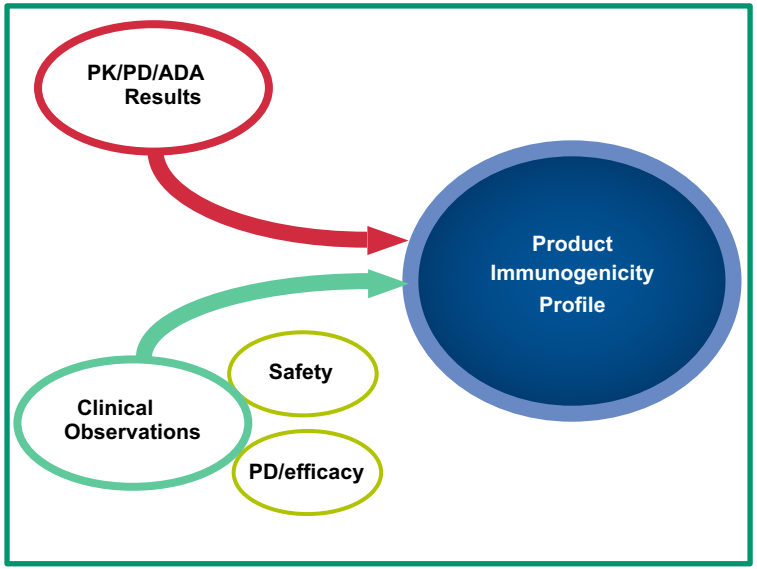
DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

https://www.ema.europa.eu/en/documents/product-information/humira-epar-product-information_en.pdf

Interplay of multiple factors can impact immunogenicity and requires an integrated approach





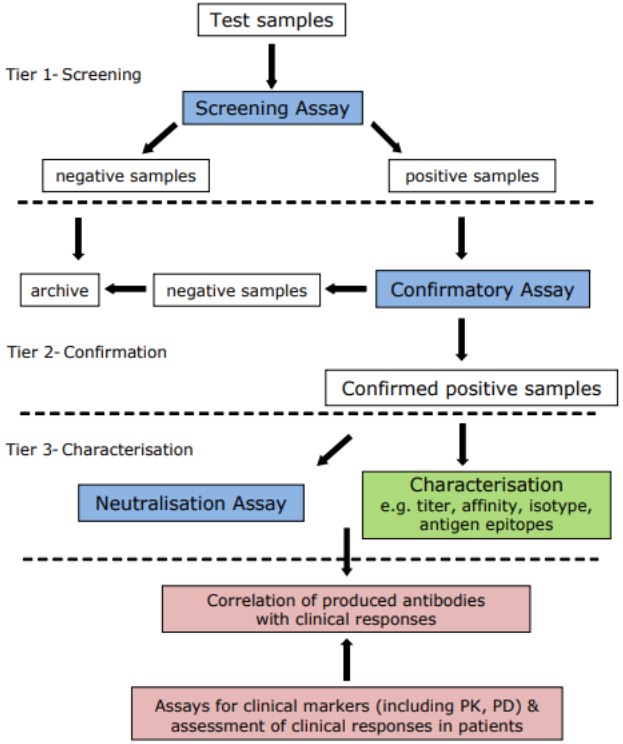
Rao and Verthelyi (2019)

Immunogenicity assays are not PK assays

	PK Assay	Immunogenicity Assay
Assay type	Quantitative	Qualitative/Quasi-quantitative
Assay control	Drug itself, usually well-characterized QCs with nominal values	Surrogate antibody Positive controls
Result determination	Calculated based on the standard curve	No standard curve Positive or negative based on cut points Use of titer for magnitude and duration of response
Assay sensitivity	Lowest concentration with acceptable accuracy and precision	Level of positive control antibody that gives signal equal to or above the cut point
Accuracy and precision	Both are assessed	Only precision is used
Common challenges for immunogenicity assays	N/A	Drug interference Target interference Pre-existing antibody responses Appropriate cut points

Tiered approach for clinical immunogenicity assessment

18 May 2017 EMEA/CHMP/BMWP/14327/2006 Rev 1 Committee for Medicinal Products for Human Use (CHMP)



Initial Positive/Negative assessment

Confirm the response is against drug/drug domains

Characterisation of the immune response

Interpretation and correlation

Context of Use (COU) can differ

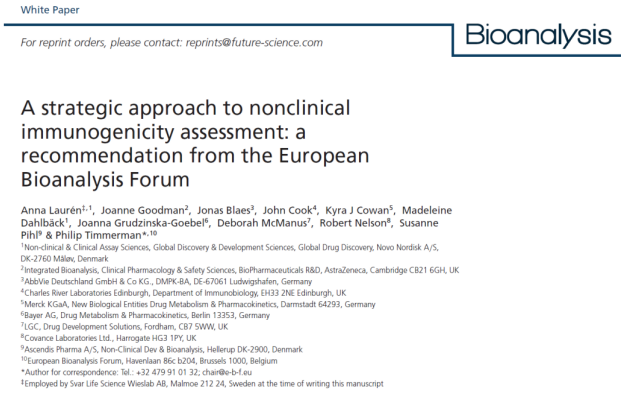
- Just like biomarker assays have a different COU based on the **purpose of the assay and the decisions being made with the data**, ADA assays also have a COU

- Similar challenges exist as for the Biomarker COU discussion:
  - Understanding the ability and limitation of the assay(s)
  - Use of the data and decisions being made
  - Scientific value
  - Stakeholder management
  - Stage of development (nonclinical, clinical, Ph1 *Vs*. Ph3)
  - Tier of immunogenicity assessment

- Just because assays are compliant with current regulatory guidance, it may not be good science and guarantee a successful submission
  - New modalities

Preclinical and clinical immunogenicity have a different COU

- Nonclinical studies
  - EBF publication on a strategic approach
  - For a GLP study, aim to understand safety and exposure to allow progression into man
  - ICH S6(R1)
  - Administration of a human protein to animals will be recognised as ‘foreign’ and some animals may mount a response which may impact exposure
  - Companies may not assess ADA unless there is a safety event potentially due to immunogenicity against the biotherapeutic
  - If ADA is assessed, then a single tier is typical, e.g. screening assay with a cut point at a lower % false positive rate
- Clinical studies
  - Strategy may differ due to product risk
  - Consequence of adverse events or impact on efficacy
  - Cut points may need to change due to COU


White Paper

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

A strategic approach to nonclinical immunogenicity assessment: a recommendation from the European Bioanalysis Forum

Anna Laurén[‡,1], Joanne Goodman[2], Jonas Blaes[3], John Cook[4], Kyra J Cowan[5], Madeleine Dahlbäck[1], Joanna Grudzinska-Goebel[6], Deborah McManus[7], Robert Nelson[8], Susanne Pihl[9] & Philip Timmerman*[,10]

[1]Non-clinical & Clinical Assay Sciences, Global Discovery & Development Sciences, Global Drug Discovery, Novo Nordisk A/S, DK-2760 Måløv, Denmark

[2]Integrated Bioanalysis, Clinical Pharmacology & Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge CB21 6GH, UK

[3]AbbVie Deutschland GmbH & Co KG., DMPK-BA, DE-67061 Ludwigshafen, Germany

[4]Charles River Laboratories Edinburgh, Department of Immunobiology, EH33 2NE Edinburgh, UK

[5]Merck KGaA, New Biological Entities Drug Metabolism & Pharmacokinetics, Darmstadt 64293, Germany

[6]Bayer AG, Drug Metabolism & Pharmacokinetics, Berlin 13353, Germany

[7]LGC, Drug Development Solutions, Fordham, CB7 5WW, UK

[8]Covance Laboratories Ltd., Harrogate HG3 1PY, UK

[9]Ascendis Pharma A/S, Non-Clinical Dev & Bioanalysis, Hellerup DK-2900, Denmark

[10]European Bioanalysis Forum, Havenlaan 86c b204, Brussels 1000, Belgium

*Author for correspondence: Tel.: +32 479 91 01 32; chair@e-b-f.eu

‡Employed by Svar Life Science Wieslab AB, Malmoe 212 24, Sweden at the time of writing this manuscript

Immunogenicity assays use cut points rather than calibration curves

- The cut point of the screening assay is **the level of response of the immunogenicity screening assay at or above which a sample is defined to be positive and below which it is defined to be negative**
- Confirmatory cut point where specificity of the immune response is determined by spiking drug (or separate domains if applicable) to determine inhibitory response
- For the titration assay, the screening cut point may be used or a specific titration cut point
- Cut points for neutralising antibody (nAb) assays

- Set pre-study (during validation) using drug naïve samples
- Statistically calculated to set a pre-determined rate of false positives (FPR) and remove the risk of false negatives

Cut points statistically set with drug naïve samples

- Over the last decade, statistical methods have been described in the literature (Shankar, Devanarayan, Shen etc.)
  - **Fixed** – uses a fixed normalisation factor based on validation data and the same value is used in the in-study phase
  - **Floating** – normalisation factor from the signal of the mean or median NC signal from the same plate during in-study
  - **Dynamic** – does not use variation estimates from validation - not recommended as it usually highlights an assay issue

Description	EMA	FDA	NMPA
Cut Point and False Positive Rate (FPR)	Statistical approach where justified although real data is acceptable (e.g. double background) **Screening:** preferably 5% FPR **Confirmatory**: not specified	**Screening**: 5% FPR: 90% one-sided lower confidence interval for the 95th percentile of the negative control population (Shen *et al*. 2015). **Confirmatory:** 1% FPR: 80% to 90% one-sided lower confidence interval for the 99th percentile.	**Screening**: 5% FPR **Confirmatory:** not specified

Cut points statistically set with drug naïve samples

- Statistically calculated to set a pre-determined rate of false positives and remove the risk of false negatives
- Balanced design to reduce confounding factors
- Data may be transformed to achieve a normal distribution (e.g. log transformation)
- Appropriate outlier removal is needed
  - May inflate or deflate False Positive Rate (FPR)
  - The number of screened positives but not confirmed positive

Analyst	Assay Run	Validation Samples			
		Assay Plate	S1-S16	S17-S32	S33-S48
A1	R1	P1	X		
		P2		X	
		P3			X
	R2	P1		X	
		P2			X
		P3	X		
	R3	P1			X
		P2	X		
		P3		X	
A2	R4	P1	X		
		P2		X	
		P3			X
	R5	P1		X	
		P2			X
		P3	X		
	R6	P1			X
		P2	X		
		P3		X	

So coming back to why are we discussing cut points …

- How to calculate a robust cut point that is scientifically and clinically meaningful
- Outlier removal approaches
- Technology has improved
  - Can result in low cut points
  - Operationally may analyse a large number of positives without clinical consequence
  - Often the confirmatory tier may barely differentiate from screening tier (Kubiak 2012)
  - Approach for outlier removal
- Pre-existing antibodies can impact cut point setting
  - Strategies to overcome these
  - Vaccination with COVID-19 vaccines that use lipid nanoparticles
- Cut points may need to be re-assessed
  - Critical reagent batches
  - Transfer of assays to CROs or in new regions (cross validation)
  - Cut point may not be applicable for the disease state/population
- Regulatory expectations may differ or change

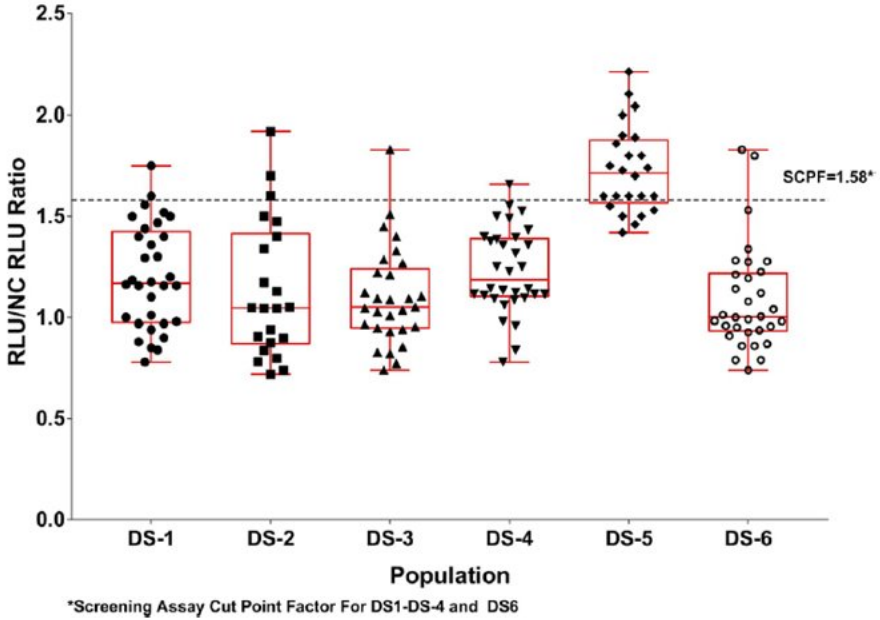
Pre-study cut point may not be reflective of the clinical population

- Do the cut points set in pre-study validation match the clinical samples/population(s)?
- Often derived from commercially sourced matrix (healthy and/or disease state)
- Separate pre-study evaluation may be needed
  - At least 20 subjects evaluated in 2 or more assay runs from the clinical population
  - Means and variances tested
  - However, the population may not be available commercially or you may not have a big enough population
  - What constitutes a different population?
  - The populations tested may give differing results

Pre-study versus In-study cut points

- In-study cut point may be needed if the positive rate among the pre-dose samples are outside the expected FPR
  - Ideally this is 2-11%
  - Needs a reasonably sized population for reliable assessment
- More regulatory concern if the FPR is <2% rather than >11%
  - A higher FPR will generate more operational challenges
- When making the decision to calculate in-study cut points
  - Useful to visualise data in the form of histograms or boxplots
  - Use of a statistical assessment of the difference of the means (ANOVA) and variances (Levene's test)

Visualisation of disease state data



Myler *et al.* (2021)

- Baseline samples for six oncology populations

1. Use a common SCP for all disease states apart from DS-5
2. If a single SCP is preferable for all populations, the most conservative SCP from the other populations can be applied to DS-5

Pre-existing antibodies can cause challenges in cut point setting

- Present before treatment
- Specific or cross-reactive with a protein or glycan epitopes to the biotherapeutic drug
- May occur due to structurally similar dosed products or environmental exposures to non-human proteins
- Higher signal responses in baseline samples compared to majority of drug naïve samples
- Some modalities may inherently have pre-existing ADA (e.g. AAV, PEG etc.)
- ADA at baseline (“prevalence”) may have clinical consequences – “treatment boosted”

1. Assay approaches such as increased dilution
2. Removal of pre-existing samples from cut point assessment
3. Data may be handled by outlier methods and data transformation to bring close to normality (e.g. <20% of baseline samples)
4. Spike enough drug to eliminate the pre-existing signal
   - Would remove confirmatory tier and screen positives would move directly to the titration tier

Summary

- Cut points are needed to evaluate the cut off in the assay - there is no standard curve and the assays are not quantitative
  - Screening tier (positivity/negativity)
  - Confirmatory tier (confirming specificity)
  - Titration tier (magnitude and duration of response)
  - Neutralisation
- Cut points are statistically set to take through a number of false positives and the rate will differ moving through the different tiers
- Cut points are set pre-study with drug naïve samples
- Cut point pre-study may not be suitable for in-study implementation or pre-existing antibodies may pose challenges
- On-going industry discussions on how to handle cut points and the best statistical approaches

Acknowledgements

EBF FW organizing committee

EBF Steering Committee

EBF community for the continued discussions

Contact Information

Questions: info@e-b-f.eu

European Bioanalysis Forum vzw

www.e-b-f.eu