Medicines & Healthcare products Regulatory Agency

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Confidence in Biological Medicines

The revised EU (CHMP) immunogenicity guidance on therapeutic proteins

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Disclaimer

The views expressed here are entirely my own and do not necessarily reflect the views of NIBSC or MHRA or any of the expert groups that I am associated with

European guidance for immunogenicity of therapeutic proteins[1]

- Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins (EMEA/CHMP/BMWP/14327/2006) – 2008 → **revision ongoing**

- Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use. (EMA/CHMP/BMWP/86289/2010) – 2012

- Biosimilarity guidelines

- [1]http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000043.jsp&mid=WC0b01ac05800240cb

Timeline

Public Consultation – Oct'15 to end Jan'16

EMA Workshop – Mar'16

Revision

Various working parties - currently

Guideline consistency group

Final Version (early '17)

Revised Guideline: Differences from original

- Original guideline – implemented April 2008

- **Revision** - Experience gained over the past 8 years
  - Aligned with the mAb immunogenicity guideline
  - Includes risk-based approach
- Condensed; some ‘general’; background information removed/ shortened.
- **Assays** – specific information included
- **Comparative immunogenicity – biosimilars**
- **Pharmacovigilance**
- **Integrated summary of immunogenicity**
- Annex 1- Text on assays replaced with 2 Tables on assays and a flow-chart with strategy

General and not prescriptive

Common platform - harmonise immunogenicity evaluation

Immunogenicity Guidance

- Initial sections of GL pertaining to scope, risk factors – same/similar wording
- Scope - Evaluation of immune response to a therapeutic protein
- Proteins and polypeptides, their derivatives, and products of which they are components, e.g. conjugates.
- Focus on biotechnology-derived proteins, “therapeutic proteins” . Coagulation factors excluded

Non-clinical assessment

- ‘Ongoing consideration should be given to use of emerging technologies (novel in silico, in vitro and in vivo assays) which might be used as tools during development or for a first estimation of risk for clinical immunogenicity. In vitro assays could be helpful in revealing cell-mediated responses.’

- Endogenous protein - Usually, safety risks would be predictable…. existing knowledge on the biological functions of the endogenous protein and **animal studies would not be required to confirm these safety risks.**

- **Only in absence of sufficient knowledge, and if theoretical considerations are suggestive of a safety risk, animal immunization studies with the therapeutic protein or the animal homolog may be considered** …... Any relevant experience on the consequences of induction of an immune response to the endogenous protein or its absence/dysfunction in animal models should be included.

- **Biosimilars - Comparison of ADA between a biosimilar and the reference product in an animal model is not recommended**.

Immunogenicity testing

- Key principles of immunogenicity assessment
  - the types of antibodies induced and their measurement
  - the impact of antibodies on clinical outcome are well established and remain the same
  - Adoption of a ‘multidisciplinary approach’

Risk-based Approach

For each product, identify and consider the ‘risk’ based on factors relating to the product and its intended use.
Conduct studies to address the risk and the severity of clinical consequences – patient and treatment-related factors

Case-by-case risk analysis warranted

Risk-based Approach

PERSPECTIVE

nature biotechnology

Shankar, G., Pendley, C., Stein, K.E. (2007) *Nat Biotechnol*, 25(5): 555-561

- General strategy to assign immunogenicity risk levels to biological drug products, and
- risk level–based ‘fit-for-purpose’ bioanalytical schemes for the investigations of treatment-related ADAs in clinical and nonclinical studies.’

Implied that risk can be determined solely by the product 'class’ and that the extent of testing can also be directly linked to this categorisation.

Table 3 Bioanalytical strategy for evaluating immunogenicity in clinical studies

Bioanalytical scheme for lower-risk products	Bioanalytical scheme for medium-risk products	Bioanalytical scheme for higher-risk products
Frequency of sampling within study:	Frequency of sampling within study:	Frequency of sampling within study:
More frequently early in the drug program and less frequently (baseline, end of study, and possibly longer term follow-ups) in phase 3 trials	More frequently early in the drug program and less frequently (baseline, end of study, and possibly longer-term follow-ups) in phase 3 trials	More frequently throughout all phases of clinical development
Assessment of ADAs:	Assessment ADAs:	Assessment of ADAs:
Detection of ADAs through screen and confirmatory immunoassays	Detection of ADAs through screen and confirmatory immunoassays	Detection of ADAs through screen and confirmatory immunoassays
Characterization of titer/relative concentration of ADAs	Characterization of titer/relative concentration of ADAs	Characterization of titer/relative concentration of ADAs. Consider real-time IR bioanalysis for the program.
Consideration of value of mapping ADA reactivity to distinct parts of the drug molecule	Consideration of value of mapping ADA reactivity to distinct parts of the drug molecule	Consideration of value of mapping ADA reactivity to distinct parts of the drug molecule. In the absence of a validated neutralizing antibody assay, the antigenic reactivity data could indicate neutralizing potential and preclude (to be decided for each patient) further treatment.
Characterization of neutralizing activity of ADA positives may be explored as necessary.	Characterization of neutralizing activity of ADA positives using a target binding inhibition–based neutralizing antibody immunoassay or a cell-based neutralizing antibody bioassay	Characterization of neutralizing activity of ADA positives using cell-based neutralizing antibody bioassay, and a target binding inhibition–based neutralizing antibody immunoassay if one is also available
	Consider testing NAb against the endogenous protein using a cell-based NAb bioassay.	If a neutralizing antibody assay is more sensitive than the screening immunoassay, all samples should also be tested using the neutralizing antibody assay.
	Clinical protocol design:	Clinical protocol design:
	Include clinical monitoring of endogenous factor deficiency, particularly for sole-activity factors/ replacement therapies and life-threatening diseases.	Include clinical monitoring of endogenous factor deficiency, particularly for sole-activity factors/ replacement therapies and life-threatening diseases.
		Consider sequential patient dosing rather than classic cohort model for first-in-human studies.

e.g., mAbs - TNFs, CD52, integrins

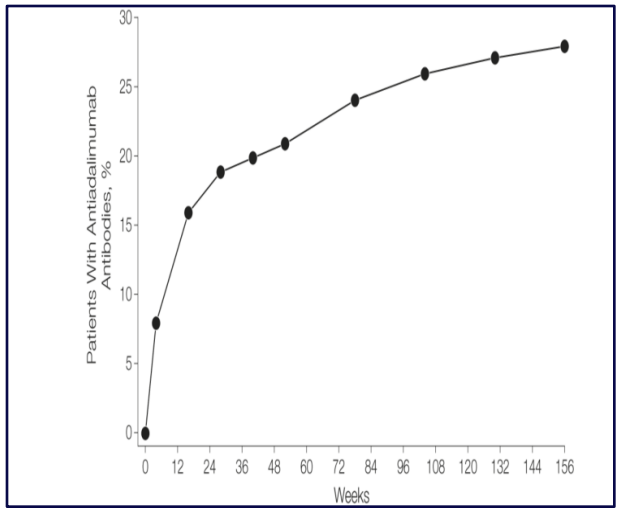
e.g., IFN-beta

e.g., EPO, TPO,

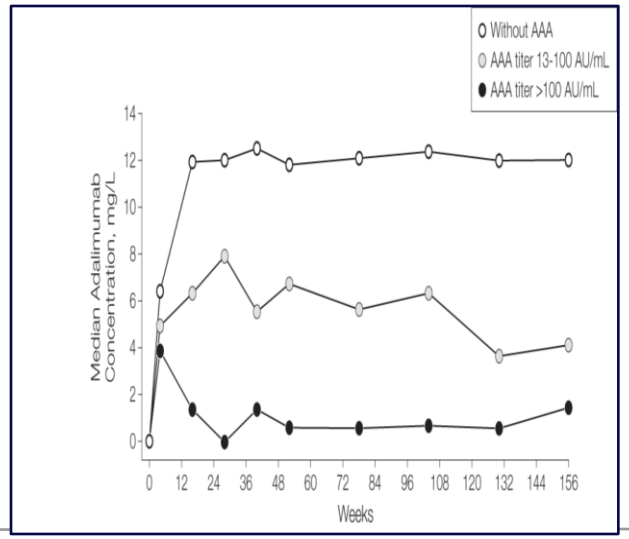
Antibodies and clinical impact

RA patients treated with Adalimumab over 3 years

A



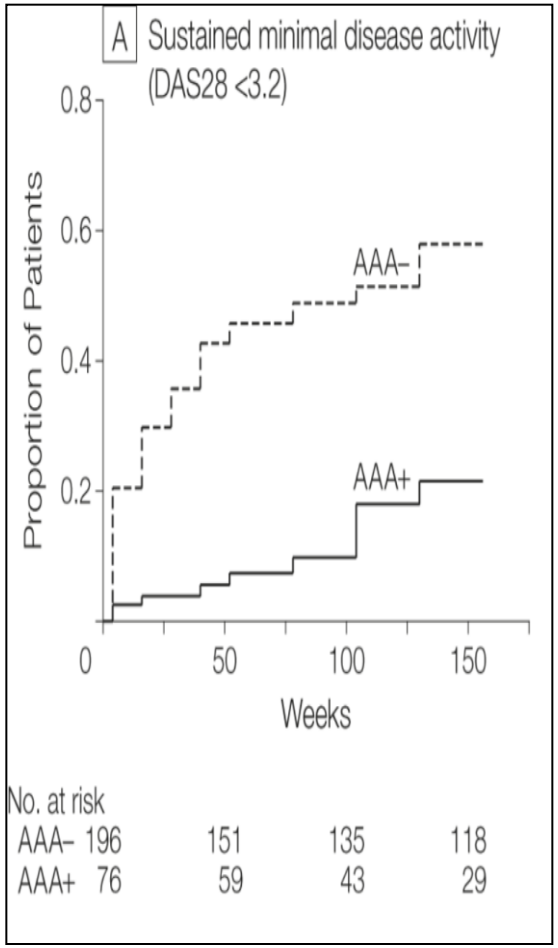
B



Ab -ve

Low Ab

High Ab



Abs develop within 24 weeks

diminish levels of therapeutic

compromise efficacy

Bartelds et al : **Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up** JAMA. 2011;305(14):1460-1468.

Examples

Tysabri (Natalizumab)- Humanized antibody that binds to the α4 subunit of α4β1 integrin; inhibits lymphocyte trafficking into tissues

RRMS patients, 2 years – Mono & Combination therapy (IFN-beta1a)

- ADA at baseline; every 12 weeks - Bridging ELISA and functional assay
- ADA incidence - 9%; 3% transient and 6% persistent - high incidence of infusion reactions, loss of clinical efficacy in Ab +ve relative to Ab -ve patients.
- Outcome - Patients with a suboptimal clinical response or persistent infusion-related adverse events should be tested for ADA

- Likely to missed if sampling as performed as proposed for **'low risk'** products: *Shankar et al (2009)*

Infliximab – anti-TNF and approved for RA and inflammatory disorders

- Immunogenic ; rates vary – disease, other medication

The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. Calabresi PA, Giovannoni G et al Neurology 2007, 69(14):1391-403

Clinical Impact

Neurology. 2013 Feb 6. [Epub ahead of print]

Fatal Neuroinflammation in a Case of Multiple Sclerosis with Anti-Natalizumab Antibodies.

Svenningsson A, Dring AM, Fogdell-Hahn A, Jones I, Engdahl E, Lundkvist M, Brännström T, Gilthorpe JD.

"significant neurological abnormalities … after… six infusions of natalizumab, …. extremely high titers of antibodies against the drug."

" death..from 'rebound neuroinflammation as a result of the development of natalizumab anti-drug antibodies."

Actas Dermosifiliogr. 2009;100:103-12

CONSENSUS STATEMENT

Reactions to Infliximab Infusions in Dermatologic Patients: Consensus Statement and Treatment Protocol

L. Puig,[a] E. Sáez,[b] M.J. Lozano,[b] X. Bordas,[c] J.M. Carrascos,[a,d] F. Gallardo,[e] J. Luelmo,[f] M. Sánchez-Regaña,[g] M. Alsina,[h] and V. García-Patos[i] for the Spanish Academy of Dermatology and Venereology Psoriasis Working Group

with the administration of infliximab is the possibility of infusion reactions, which may be immediate or delayed; these reactions are related to the immunogenicity of this monoclonal antibody, leading to the production of anti-infliximab antibodies. Infusion reactions to infliximab are not usually anaphylactic (ie, they are not mediated by immunoglobulin E), and re-exposure of the patient using specific protocols to

Risk-based Approach

CORRESPONDENCE

A European perspective on immunogenicity evaluation

C. Schneider, M. Papaluca, P. Kurki (2009) Nature Biotech 27 : 507-508

'A standardized algorithm' - Inappropriate

Less intensive evaluation for 'low risk drugs' (regulatory concern)

Unexpected product characteristics likely to be missed (e.g., manufacturing or impurities)

Structure may imply 'low risk', but other attributes may pose 'higher risk' (e.g. novel expression system)

Consequences like infusion reactions are usually less severe than those for high risk – e.g., PRCA

BUT

Impact on benefit-risk assessment and associated with immunogenicity

Immunogenicity testing

- Identify Product Risk
- Develop an integrated analysis strategy and study plan (incl sampling) relevant for the product and intended treatment to elucidate the clinical relevance of immunogenicity data.
  - Carefully designed studies (clinical trials)
  - Antibody assays - likely to evolve & be refined during development BUT assays for pivotal clinical trials and for post-marketing studies are expected to be validated.
  - Suitable positive controls; negatives and plans for data interpretation/ determining threshold for +ve samples
  - Sampling points (incl baseline, post -treatment), frequency of sampling, sample volumes, processing/storage
  - Methods for assessing clinical response

Every product needs to be evaluated for immunogenicity individually and an appropriate strategy adopted for each development programme

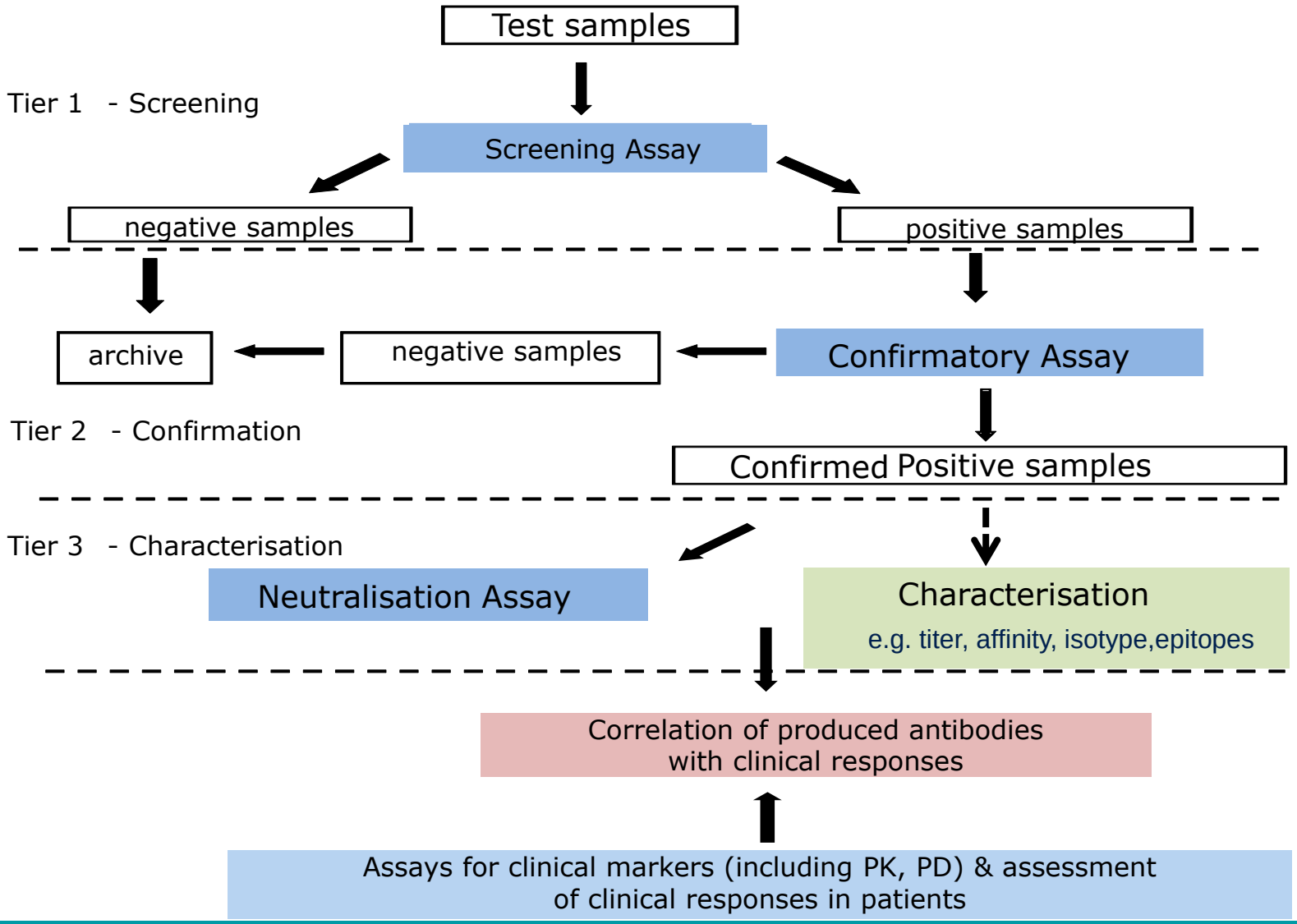
Planning of Studies

- **Sampling strategy for ADA – frequency, timing and analysis dependent on risk assessment**

- Schedule should be adapted individually for each product and designed to
  - consider the PK of the product and assay capability.
  - distinguish transient/persistent antibodies
  - include baseline
  - Also post-cessation sampling (long enough to allow conclusions to be drawn regarding a persistent immune response triggered by therapeutic or uncover an immune reaction that was suppressed by the therapeutic).

  - At early stages, frequent, sequential sampling (to assess the risk); based on knowledge, consider sampling
    - Less/more frequent sampling during long -term follow up
    - Real time (high risk)/retrospective (low risk) evaluation

Strategy for Immunogenicity testing



Assays are critical for evaluation of clinical immunogenicity

Antibody Assays

While assay design, strategy & extent of testing likely to vary between products, certain key elements -

Sensitivity - Sufficiently sensitive assays to detect clinically relevant levels of antibodies (important for screening)

Interference – Assay results should not be confounded by matrix/target interference or from residual product. Any interference needs to be evaluated and strategies to minimise/overcome this implemented

Assays with drug tolerance exceeding the levels of therapeutic expected in the samples.

Validated for ‘fitness of purpose’ i.e. clinical evaluation

Screening assays

- First step (mainstay)
  - Sensitive & capable of detecting all clinically relevant antibodies
- Several platforms and formats/detection systems.
  - All detect antigen-antibody interaction but differ in their scientific principles.
  - Moderate throughput and automated
  - Relative merits and weaknesses need to be considered when developing/selecting an assay for use.

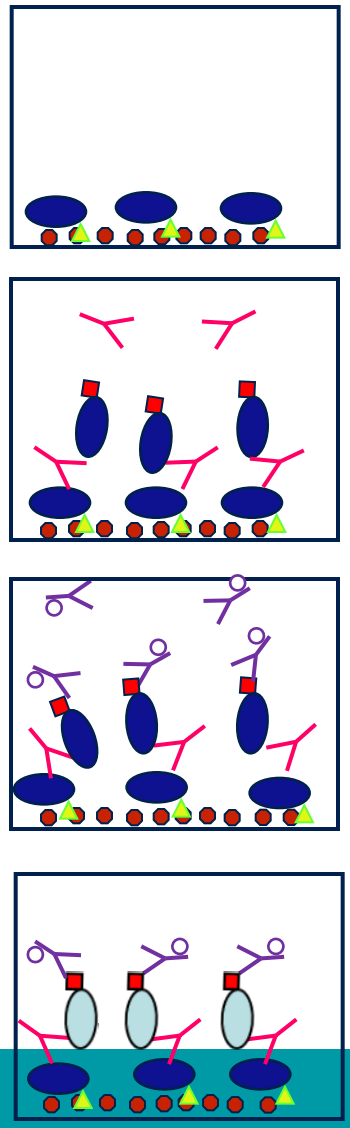
Technologies for antibody measurement are rapidly evolving and progress with this needs to be considered during assay selection.

Bridging ELISA Formats

- Popular – ease of use, throughput
- Dual arm binding
- No requirement of secondary antibody

- Requires labelled therapeutic - Labelling may alter epitopes.
- May fail to detect rapidly dissociating antibodies.
- Affected by therapeutic/target interference, matrix components e.g. rheumatoid factors
- Lacks sensitivity toward IgG4

Homogeneous



Some Considerations

- Pre-existing antibodies – if detected, investigate the reactivity (problem from bioanalytical, efficacy & safety perspective)

- Antibody detection can be impacted by

  - Matrix effects can cause false positive or negative results.
    - Examples - soluble target, Fc receptors, complement components or complement receptors, disease specific factors such as rheumatoid factors should be evaluated & corrective measures implemented on a case-by-case basis as appropriate.

  - Residual therapeutic/immune complexes/target
    - Options proposed.

    - Technology limitations may not always allow a fully tolerant assay to be developed, however, assessment needs to be performed using the best possible assay and the approach taken properly justified

- Approaches taken must be validated for effectiveness and adopted on a case-by-case basis based on their suitability and according to needs.

Target interference

Monomeric soluble target can bind therapeutic (labelled and/or immobilized) and prevent ADA binding → false negative

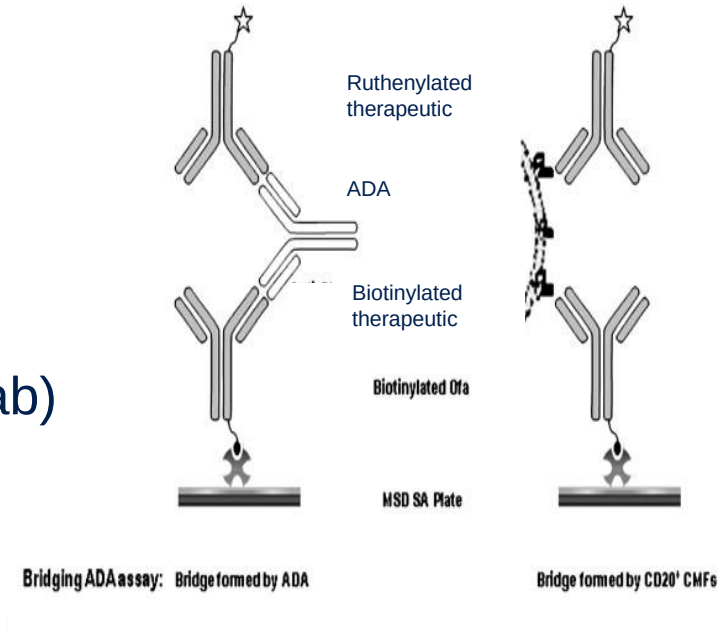
Membrane-bound target or multimeric soluble target may form bridge with therapeutic (labelled and/or immobilized) → false positive

Mitigation: Deplete target - dissociate & affinity capture with Ab
Block drug target interaction - sol receptor , another Ab

Rituximab:
Immunodepletion – beads coated with another anti-CD20 Ab or added antibody; Ultracentrifugation;
Specificity check - bi-confirmation step (spike another anti-CD20 Ab +/- Rituximab)

Bevacizumab :

VEGF



Adapted from Chen K. et *al*, 2013,JIM 394:22-31

Therapeutic/Drug interference

Some products (e.g. mAbs) persist or are given chronically at high doses

- High levels of drug and/or immune complexes expected
- Drug in sample will compete with immobilized /labelled drug (bridging) for binding to ADA ⟹ False negative

Drug tolerance assessment during development & validation

- Surrogate positive control (PC) antibody at different levels spiked in control sera with different amounts of therapeutic (reflecting levels expected – PK data)
- PC may not fully reflect the nature of the clinical samples (varying isotypes, affinities etc within/between patients over time).

Requirement

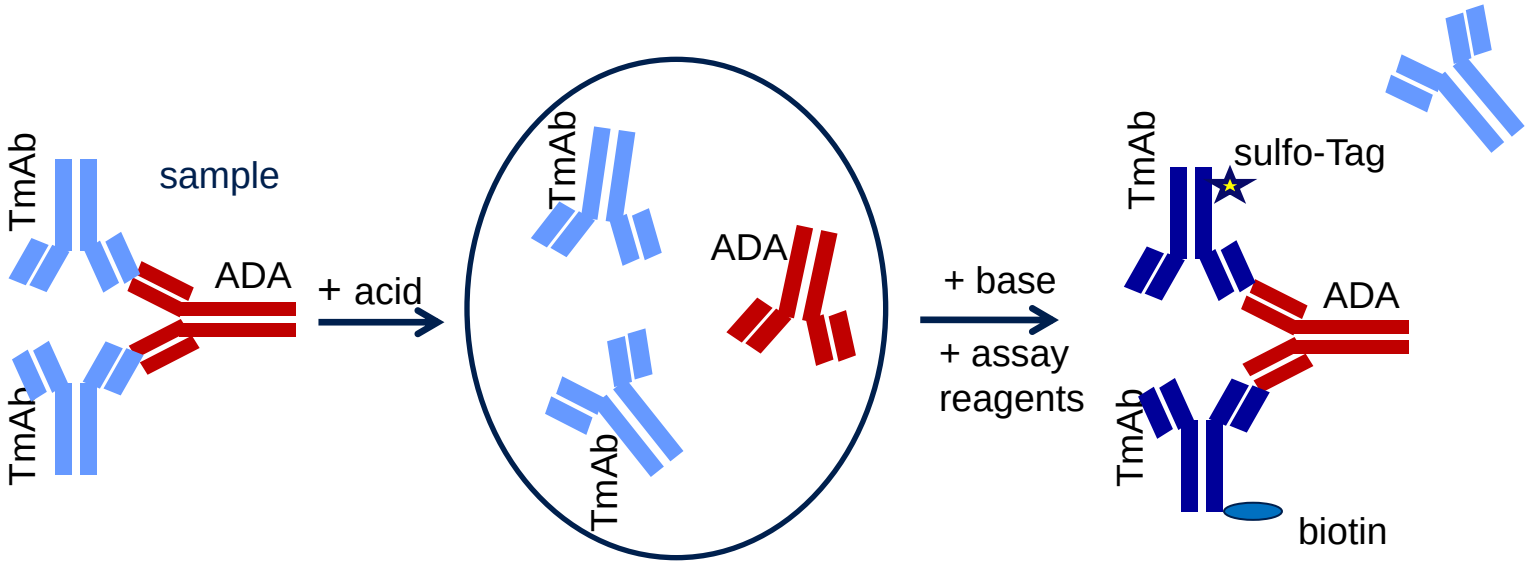
Optimal mitigation strategy which does not impact ADA detection

Assays with drug tolerance exceeding the levels of therapeutic expected in the samples.

Problem of residual therapeutic

- Samples with no/low therapeutic (e.g. washout); increase sample dilution and/or increase incubation times, increase conjugate concentration
- Acid treatment (e.g. acetic acid 300 mM). Optimize incubation period and pH

Acid dissociation (AD):



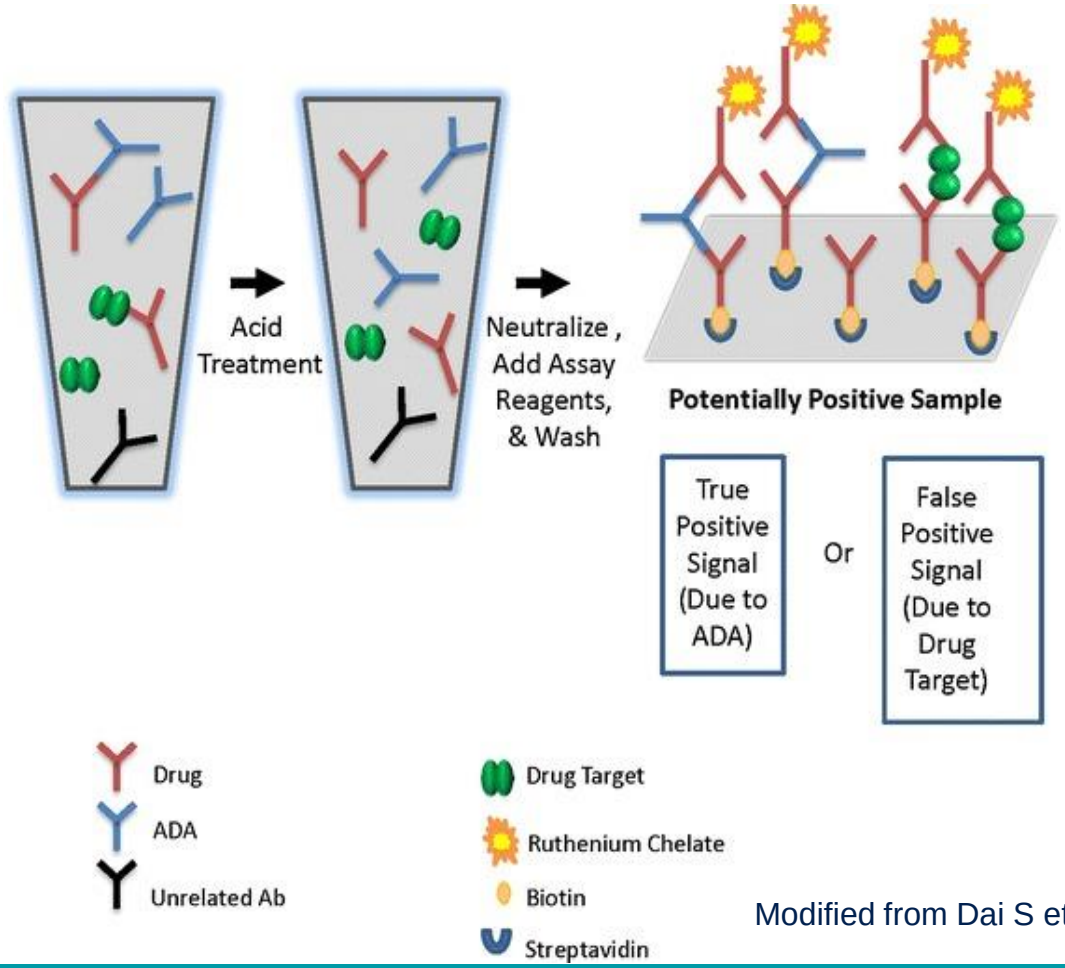
Associated risks:

- ADA Denaturation due to low pH treatment (may not be seen with PC at development)
- **Acid - dissociation cannot be universally applied to improve capability of ADA assays**
  Potential release of soluble target from therapeutic: target complexes → target interference

Lofgren *et al*, 2006,JIM 308:101-108; Bourdage *et al*, 2007, JIM 327:10-17; Smith *et al*, 2007, Reg.Tox.Pharm.49:230-237: Dai S. *et al*, 2014, AAPS J 16:464-477

Drug/Target Interference

Acid dissociation : Potential release of soluble target from therapeutic: target complexes → target interference



Seen with NGF during immunogenicity assessment of an NGF antibody, Fulranumab

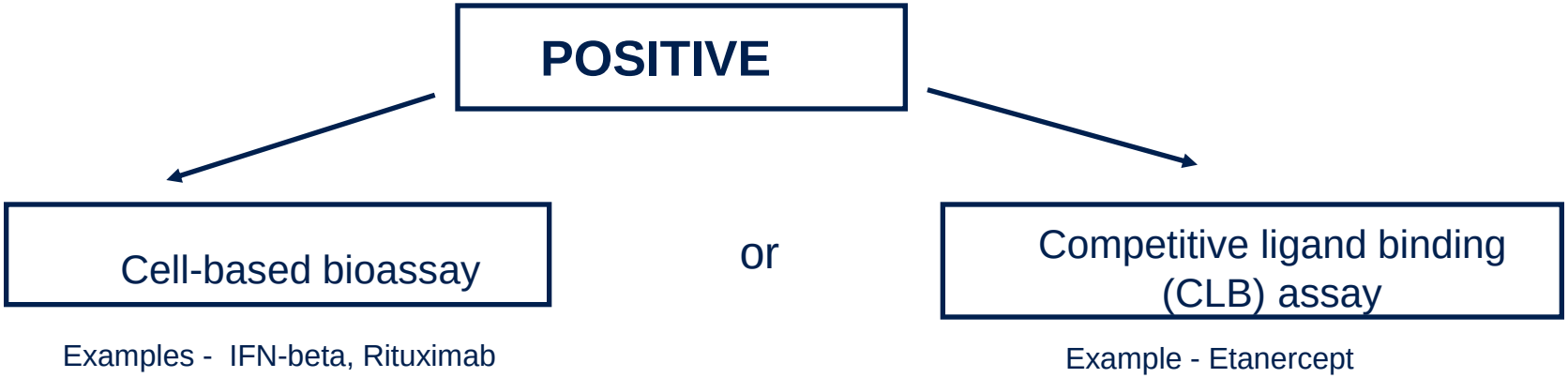
ECL assay
Chelate – highly stable, multiple excitation cycles: signal amplified, Large dynamic range, highly sensitive, better drug tolerance, less susceptible to matrix effects e.g., RF etc

Modified from Dai S et al. AAPS J. 2014 May; 16(3): 464–477

Neutralizing Antibody Assays

- Determination of the neutralizing potential is essential and deviation needs a strong justification.

- Any sample containing NAbs against the therapeutic reduces or abolishes the bioactivity of a known amount of the therapeutic.



Functional biological system to assess if the Abs detected by the binding assay have neutralizing activity

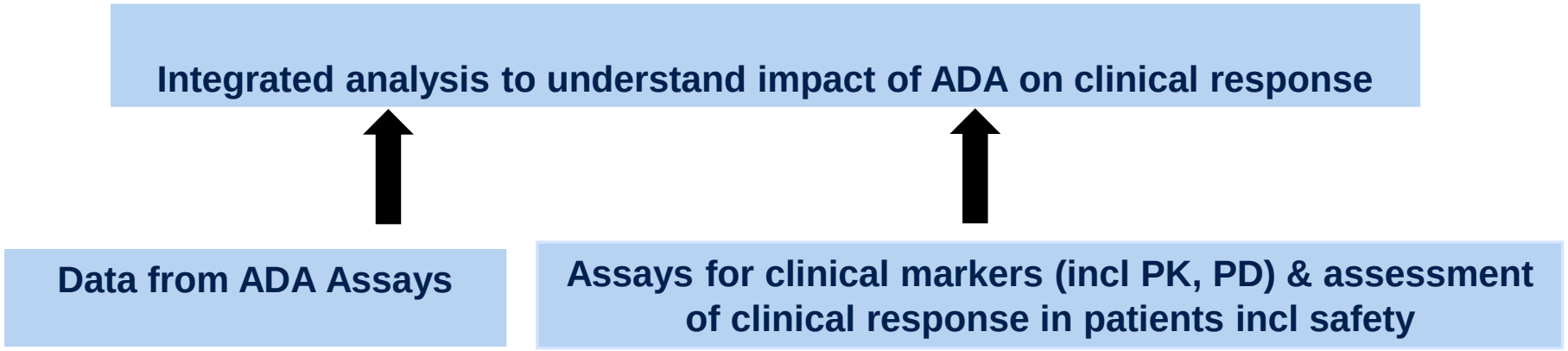
Competitive assay which detects Abs that prevent therapeutic from binding to target

Assay format dependent on risk assessment, sensitivity, MOA – soluble vs cell surface receptor, multiple active sites

Information Required: Antibody Assays

- Assay choice, sensitivity, specificity
- Minimal required sample diluton
- Pre-existing antibodies
- Matrix effects
- Drug tolerance (vs. drug concentration in the sample)
- Target interference (e.g. soluble receptors)
- Non-specific binding factors (matrix effects)
- Positive control antibody
- Selection of cut points
- Appropriate assay validation e.g., precision, robustness, intra assay/inter-assay variability
- Sampling schedule

Clinical Impact of ADA



Validated assays required for pivotal clinical trials and post-authorisation studies. Fit for purpose ADA assays for demonstrating clinical correlations of ADAs.

Biosimilars : Comparative Immunogenicity

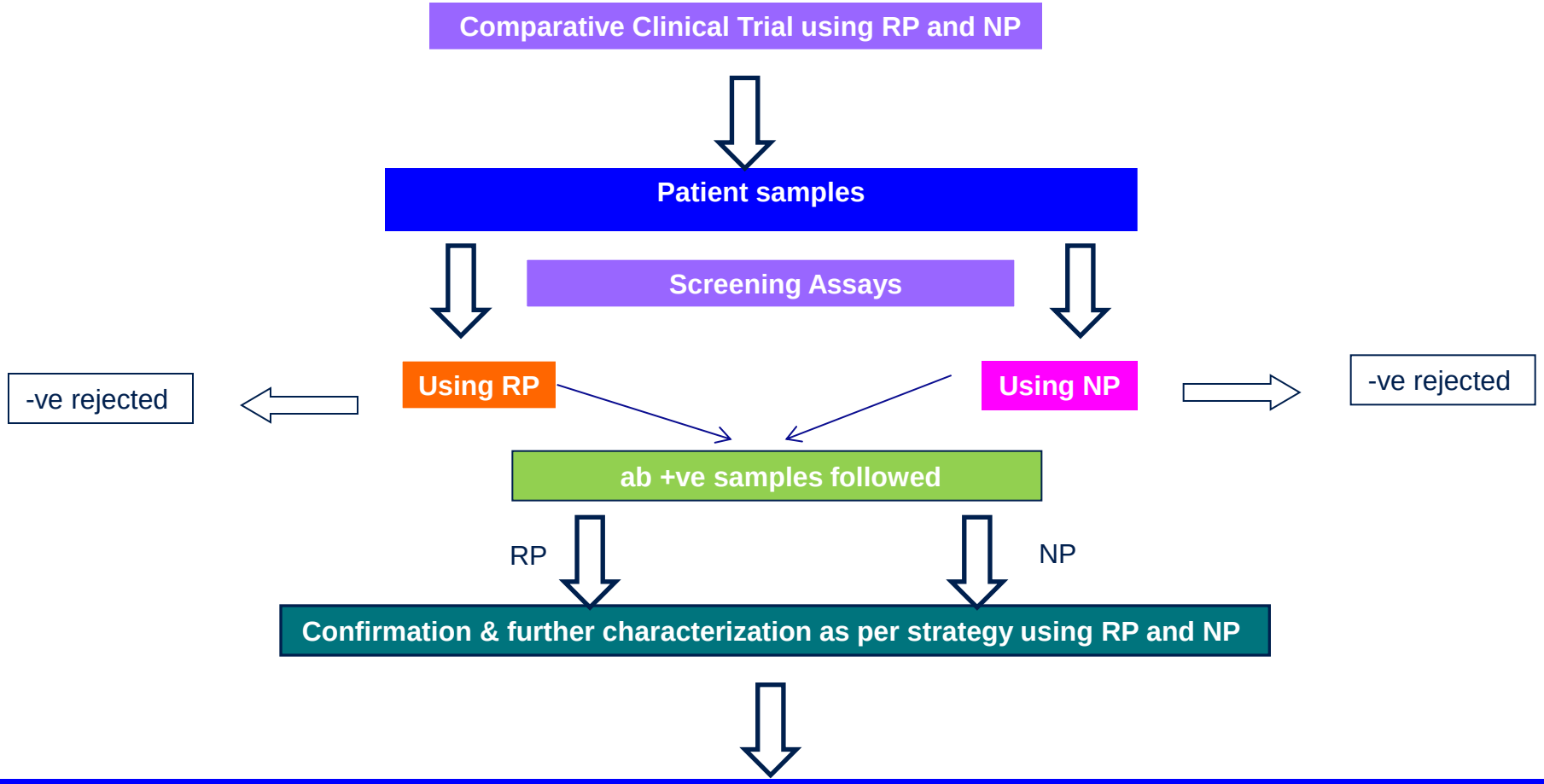
- Study - well designed, duration based on product (chronic – min 6 m);
- Sufficient size (not statistically powered) ⟹ allows conclusion on ADA & any impact on PK .
- Sensitive, homogeneous and clinically relevant patient population (ideally naïve). Extrapolation perspective
- Head-to-head studies
  - Same assay format
  - Same sampling points (baseline, sequential, treatment end) determined by product (PK, wash-out, post- termination)
  - Sampling when therapeutic levels are low (prior to administration)

Biosimilars : One/Two Antigen Assay

- Positive antibody controls against each product; State-of-art assays

- **Two antigen Assay** - using both products reflects true immunogenicity
- Challenge - Developing & validating two assays (incl cut-point)
- Cross- testing (each control with respective conjugated reagents and vice versa) for similar assay performance, i.e. comparable dose response curves, sensitivity, drug tolerance and no bias in recognition for one antigen vs the other
- Use same sera and same statistical method for deriving cut-point .

Relative Immunogenicity



Provide information regarding immunogenicity profile of each product – antibody types, kinetics of antibody development, cross-reactivity. Assess correlation of characterized antibodies with clinical responses to biologic therapeutic

Any association of antibodies with infusion reactions, hypersensitivity reactions etc

NP – biosimilar

Biosimilars : One/Two Antigen Assay

- One Antigen assay - Employing biosimilar as the antigen for both treatment arms. In principle, will detect antibodies to both BUT is conservative & not a TRUE comparison. Ensures most optimal detection of biosimilar ADA (but risks under-estimating immunogenicity of reference product).

- Adoption of this approach minimises variability

- Requires cross-testing of both positive controls for comparable sensitivity & drug tolerance. Check reactivity of each control with respective conjugated reagents and vice versa to demonstrate comparability

- Differences will question the comparability paradigm unless no clinical impact. Exploring the root cause of the differences important e.g., potential new epitopes

Summary of the immunogenicity program

Multidisciplinary exercise. Data dispersed in MAA.

- Recommended to include
  - an integrated summary of immunogenicity in the application, including a risk assessment to support the selected immunogenicity program.
  - this summary in chapter 2.7.2.4 Special Studies or, if more detailed, in chapter 5.3.5.3 of the CTD. The summary should be concise and contain links to the appropriate chapters of the application.
- Summary with risk assessment to evolve throughout the lifecycle of the product and may be used to support applications at various steps of product development.

Summary of the immunogenicity program

- The risk assessment may suggest a low risk of immune-mediated adverse effects. Nevertheless, it is expected that immunogenicity is studied with validated assays according to the scheme in Annex 1. Deviation from this scheme, e.g. omission of the testing for neutralizing ADAs, e.g. in case of single-dose clinical trials for low-risk therapeutic proteins, must be justified.

- The risk assessment may have an impact on additional characterization of the immune response (e.g. isotyping and epitope mapping), frequency of sampling, timing of the analysis, and selection of the target population.

Analysis of risk factors

Previous experience of the product/product class
- does the product have an endogenous counterpart
- do animal models provide useful data of potential consequences of immunogenicity (e.g. elimination of an endogenous protein)
- are there known antigenic sites of the molecule
- attempts to reduce the immunogenicity of the product before and during clinical trials

Physicochemical and structural aspects
- Are there new potentially immunogenic structures, e.g. sequences that are foreign to human
- Expression construct and the posttranslational profile e.g. non-human glycosylation patterns/glycans
- Stability and impurities (e.g. presence of aggregates (as visible or sub-visible particles)
- Formulation and packaging, e.g. potential impurities and leachables

Does the route and/or the mode of administration raise concerns

Patient- and disease-related factors
- State of the immunological tolerance
  - prone to autoimmune reactions
  - lack of immunological tolerance, e.g. defects in genes coding for endogenous proteins
  - concomitant immunomodulative therapy
- Pre-existing immunity
  - “natural” antibodies
  - antibodies due to previous therapy with related substances

The risk-based immunogenicity program

- Assay strategy
  - Rationale for the choice of assays
    - screening, confirmation, and titration
    - neutralizing
    - other, e.g. immunoglobulin class, sub-class
  - Specificity and sensitivity of the selected assays in the context of the particular product
    - selection of the positive control(s)
    - determination of the threshold for ADA-positivity
  - Drug and target tolerance of the assay
  - Matrix interference in different populations

- Approach to immunogenicity in clinical trials
  - Sampling for immunogenicity testing
  - Justification for the length of the follow up
    - on-treatment
    - off-treatment, post-exposure
  - Pharmacokinetics
    - Possible ADA-interference on the assays of product concentration
    - Drug trough levels in relation to drug tolerance of the ADA assay
  - Pharmacodynamics, efficacy and safety trials
    - how the program aims to reveal the incidence, persistence and clinical significance of potential ADAs
    - hypersensitivity, autoimmunity, loss of efficacy
      - definitions and symptom complexes[1]
      - analysis of clinical correlations of ADAs
- How the risk assessment influenced the immunogenicity program

Immunogenicity results

Immunogenicity in clinical trials (relative immunogenicity in case of manufacturing changes and biosimilars)

- (Relative) incidence of ADAs, including neutralising ADAs
- (Relative) titres and persistence over time
- Further characterisation if appropriate, e.g. immunoglobulin classes, cross-reactivity with related therapeutic or endogenous proteins
- (Relative) impact of ADAs on pharmacokinetics, pharmacodynamics, efficacy and safety
- Impact of pre-existing antibodies on pharmacokinetics, pharmacodynamics, efficacy and safety

Conclusions on the risk(s) of immunogenicity

Impact of the immunogenicity on the benefit/risk

Tools to manage the risk

- Identification of risk groups
- Is there a safe level or type of immunogenicity
- Pre-medication, co-medication
- De-immunisation
- Risk detection and mitigation tools

How to link adverse events to immunogenicity post-marketing (risk management plan)

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Thankyou!