Immunogenicity of Biological products

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Agenda

- Introduction
- Immunogenicity monitoring
  - Guidelines, white papers…
  - Risk management plan
  - Bioanalytical tools
    - Assays platform
    - Assays development/validation
- Cases study

Different denominations

- Biologics, biotechnology products, biological products, recombinant proteins, biopharmaceuticals, protein therapeutics, protein drugs, biotherapeutics…

different denominations may be encountered !

Official definitions of Biologicals

- **EMEA guidance**

  « biological/biotechnology-derived proteins...proteins and polypeptides, their derivatives and products of which they are components, e.g. conjugates »

- **ICH topic S6**

  « Products derived from characterized cells through the use of a variety of expression systems including bacteria, yeast, insect, plant and mammalian cells... proteins and peptides, their derivatives and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals »

- **Directive *2003/63/EC***

  Substance which is produced by or extracted from a biological source and that needs for its characterization and the determination of its quality a combination of physicochemical-biological testing, together with the production process and its control.

Biologicals vs small molecules

Small molecules

- Organic synthesis
- Low MW (Rule of <5kDa)
- Well-defined properties
- Purity standards well established
- Optimized by medicinal chemistry

Biologicals

- Produced by living host cells
- Complex production process that contributes to the definition of the drug substance (DS)
- High MW (usually from 5 to 150kDa and higher)
- Complex and poorly defined properties (eg, tertiary structures, glycosylation)
- Broad specifications that may vary during development, difficult to standardize
- Protein engineering required

Types of biotech-products

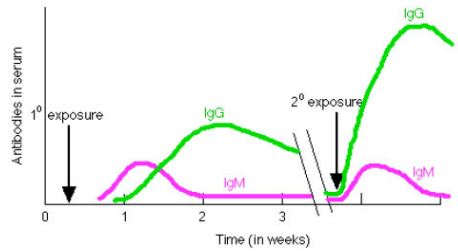
Hormones	Growth hormone, insulin (analogues) and erythropoietin
Blood products	Albumin, thrombolytics, fibrinolytics and clotting factors
Cytokines and growth factors	Interferons, interleukins and colony-stimulating factors
Antagonists/inhibitors	Soluble receptors
Monoclonal antibodies and related products	Mouse, chimeric or humanized Ab; whole molecule or fragment; single chain or bispecific; and naked or conjugated
Modified human proteins	Fusion (IgFc), polyethyleneglycol (PEG)ylation, liposome encapsulation and drug–toxin conjugate
Vaccines	Recombinant proteins or peptides,DNA plasmid and anti-idiotype
Gene-transfer products	Viral and non-viral vector-delivery systems and DNA–RNA chimaeras
Cell-based therapies	Autologous, allogeneic and xenogeneic
Tissue-engineered products	Cells, tissues, naturally occurring/synthetic biomaterials, extracorporeal and long-term implants

Cavagnaro JA. Preclinical safety evaluation of biotechnology-derived pharmaceuticals. Nat Rev Drug Discov 2002 Jun;1(6):469-75.

Immunogenicity of Biologicals

- It is assumed that most or all therapeutic proteins may induce an immunogenic response with production of Anti-Drug Antibodies (ADA) in patients.

- Many factors contribute to immunogenicity:
  - Foreign amino acid sequences
  - Aggregated, oxidated, deamidated product
  - Host cells proteins, manufacturing changes
  - Formulation, route of administration (SC > IP> IV) and frequency of dosing
  - Immune status, age, disease of patient



- This immunogenicity can be in some cases associated with serious adverse effects:
  - No observed effect or clinical event
  - Altered PK/PD (increased or decreased exposure)
  - Decreased efficacy (decrease exposure or neutralization of the product)
  - **Severe hypersensitivity reactions (HSR)**
  - **Cross-reactivity with endogeneous proteins, autoimmunity**

best case

worst case

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Monitoring is mandatory !

- Both biopharmaceutical industry and regulatory agencies keep on searching for more informative antibodies assays and antibody monitoring strategies.

- There is a need to assess /measure immunogenicity
  - It is a safety concern (risk-based)
  - Regulatory expectations are regularly increased

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Guidelines for Biologicals

Specific part dedicated to IMMUNOGENICITY

	QUALITY	SAFETY	EFFICACY
ICH	Q5E: Comparability of biotechnological/biological processes(2004)	**S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (1997)** M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials (2008)	
EMEA	Comparability of Medicinal Products containing Biotechnology-derived Proteins as Active Substance Quality Issues (2003)	**Immunogenicity assessment of biotechnology-derived therapeutic proteins (2008)** Requirements For First-in-man Clinical Trials For Potential High-risk Medicinal Products (Draft 2007) Comparability of Biotechnology-Derived Medicinal Products after a change in the Manufacturing Process - Non-Clinical and Clinical Issues (2007)	Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins (2007)
FDA	Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived (1996)	Nonclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (2007)	

White papers for immunogenicity

- AAPS Immunogenicity Sub-Committee (Biotech scientists and FDA representatives)
  - review ADA testing methods across biotech industry, summarize industry experience and publish recommendations for
    - assay design/optimization
    - validation
    - testing strategies

- Different « White papers » as recommendations for immunogenicity evaluation

Recommendations for ADA assays

ELSEVIER

Journal of Immunological Methods 289 (2004) 1–16

JIM Journal of Immunological Methods

www.elsevier.com/locate/jim

Standardization

Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products

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Received 14 March 2004; accepted 14 June 2004

Recommendation for validation of ADA assays

Recommendations For The Validation Of Immunoassays Used For Detection Of Host Antibodies Against Biotechnology Products

Gopi Shankar[1], Viswanath Devanarayan[2], Lakshmi Amaravadi[3], Yu Chen Barrett[4], Ronald Bowsher[5], Deborah Finco-Kent[6], Michele Fiscella[7], Boris Gorovits[8], Susan Kirschner[9†], Michael Moxness[10], Thomas Parish[11], Valerie Quarmby[12], Holly Smith[13], Wendell Smith[14], Linda A. Zuckerman[15] & Eugen Koren[16,*]

J Pharm Biomed Anal. 2008 Dec 15

Risk Management Plan (RMP)

- Even if providing a background and data with
  - project attributes, difference between product and endogenous counterpart, Literature reference (e.g. knockout animals)
  - preclinical animal data
    - how animal modeling reflects clinical situation
    - how « good » is the assay for immune monitoring

- Both FDA and EMEA want a risk management plan for immunogenicity in submission dossier
  - RMP provides an immunogenicity risk class designation for the compound and recommends an immunogenicity testing strategy for non-clinical and clinical studies
  - RMP is a dynamic process and requires periodic evaluations with updates with relevant information

RMP « immunogenicity part »

- Classify the biological regarding its risk category
- Risk assessment must be carried out in collaboration with toxicologists, clinicians, PK and assay experts
- The greater the risk, the more extensive and more frequent Ab testing and characterization should be applied
- Recommendation for routine monitoring of changes in clinical response and linking immunological findings to clinical events
- Immunogenicity as part of all clinical trials
- Evaluate in all patients
- Analyse AE and possible link to unwanted immune response

- Introduction
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Bioanalysis support for immunogenicity monitoring

- Development and validation of different assays for
  - Binding ADA (anti-Drug Antibody) evaluation in preclinical and clinical studies
  - ADA Characterization
  - + PK assay (complementary assay to ADA assay)

- Using different technologies
  - Select the more appropriate assay (regarding specificity, sensitivity, high throughput method…)

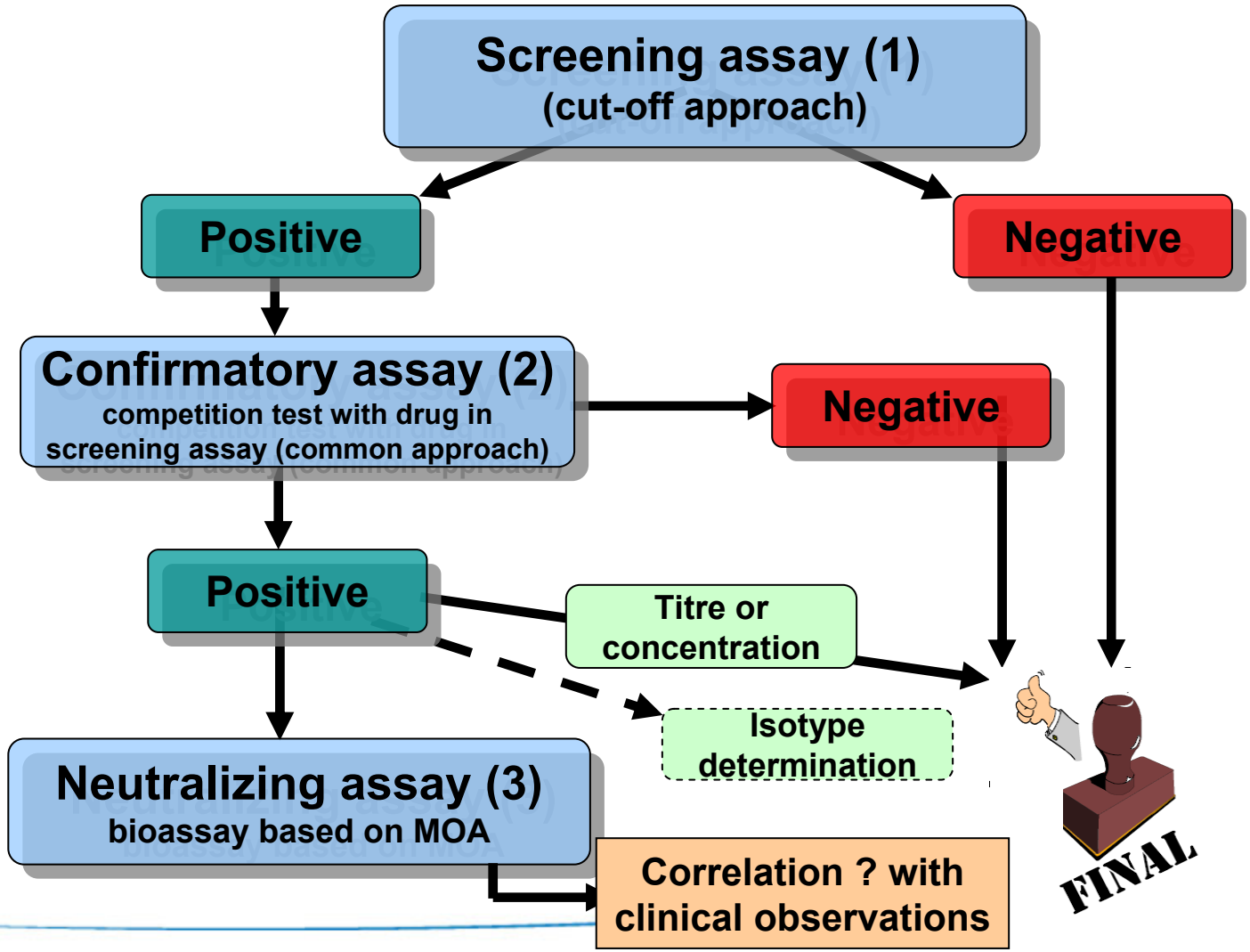
3 assays are expected for immunogenicity evaluation

- Minimum Requirements

1. Screening with cut-off approach
2. Confirmatory
3. Characterization
   - (Titration, Neutralization, Isotyping)

- Screening assays are the first pass at detecting anti-drug antibodies.
- Since it is expected that 5% false positives will be detected, a confirmatory assay is used to discount the false positives.
- All confirmed positive samples must be titrated and assessed for their neutralizing activity (isotyping may be required in some cases).

Process for immunogenicity monitoring



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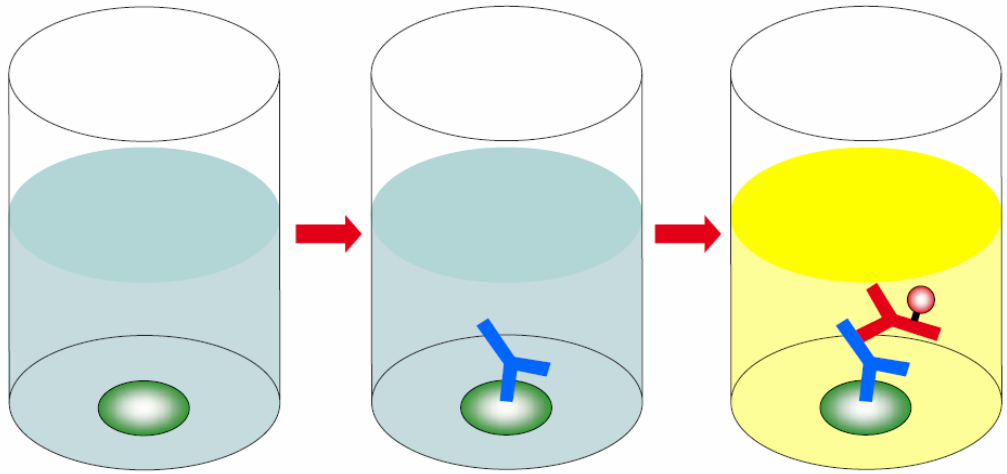
Assays technologies

- Antibody binding to the biological (ADA) can be monitored by:
  - Radio-Immunoprecipitation (RIP)
  - Direct / indirect ELISA
  - Bridging ELISA
  - Electrochemiluminescence (ECL)
  - Surface plasmon resonance (Biacore)
  - Magnetic bead LC/MS
- Bioassays investigating neutralizing effects of the antibodies

Direct ELISA

- Assay principle



Pros:

- ❖ Sensitivity
- ❖ Commercially available secondary antibodies
- ❖ High throughput

Cons:

- ❖ Source of the positive control has to be the same as that of the anti-drug antibodies
- ❖ Specificity (unspecific binding to matrix components)
- ❖ Can miss low-affinity antibodies due to the high number of washing steps

Bridging ELISA

Format-1

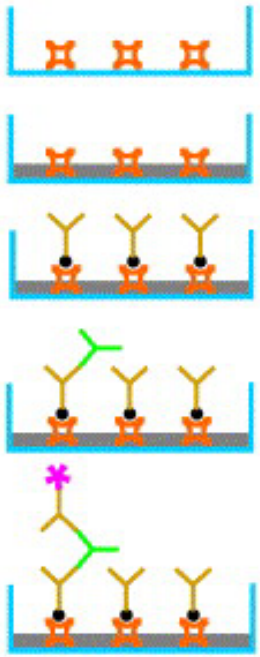
(1) Streptavidin is coated on the plate

(2) Coated plate is blocked

(3) Biotin-conjugated drug binds to streptavidin

(4) ADA in test sample binds to biotin-conjugated drug

(5) Enzyme-conjugated drug binds to ADA and produces signal after substrate is added.



Format-2

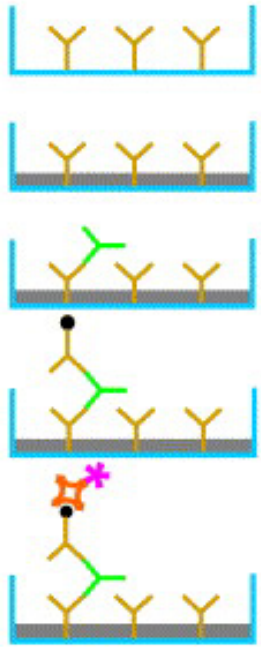
(1) Drug is coated on the plate

(2) Coated plate is blocked

(3) ADA in test sample binds to drug

(4) Biotin-conjugated drug binds to ADA

(5) Enzyme-conjugated streptavidin binds to biotin-conjugated drug and produces signal after substrate is added.



Legend

Dong Geng 2004 J. Pharm. and biomedical analysis

Bridging ELISA

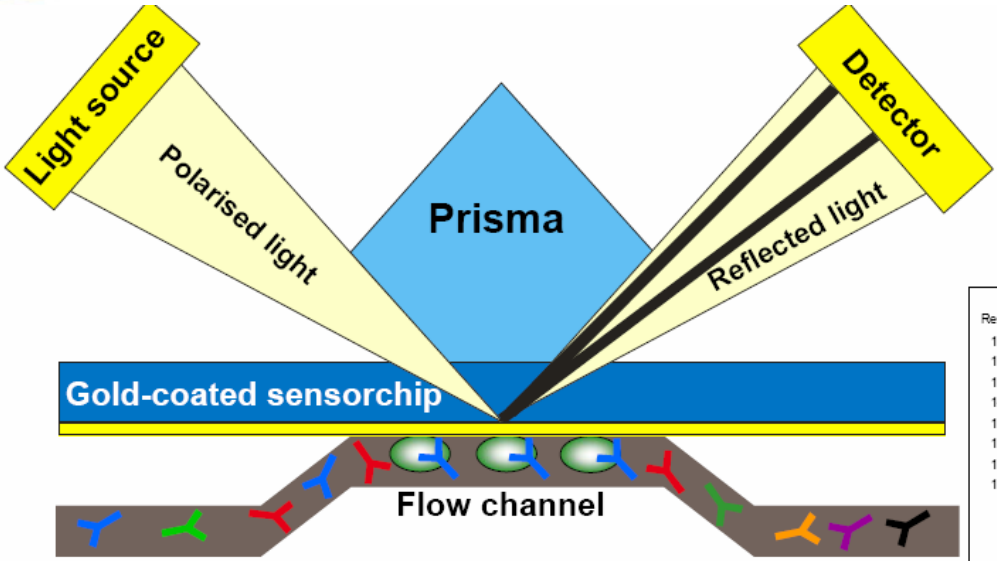
Pros:

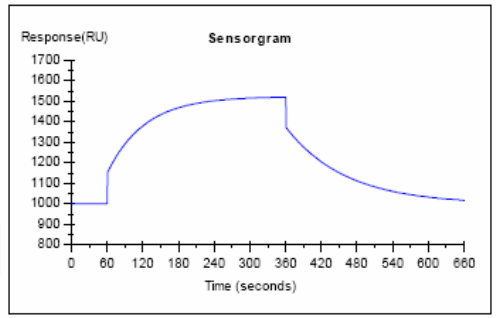
- ❖ High throughput
- ❖ Specificity (two-fold binding of drug required for signal)
- ❖ Possibility to use any positive control from different origin since format is species independent
- ❖ Same format can be used for both pre-clinical and clinical !

Cons:

- ❖ Sensitivity (special orientation of coated drug required) may be limitant
- ❖ Detection of low-affinity antibodies may be restricted
- ❖ Biotinylation might mask/denature epitopes recognized by anti-drug antibodies

Biacore for isotyping, confirmatory..





- Drug coated to sensorchip
- Injection of plasma / serum containing anti-drug antibodies
- Enhance the signal with anti-species Ab

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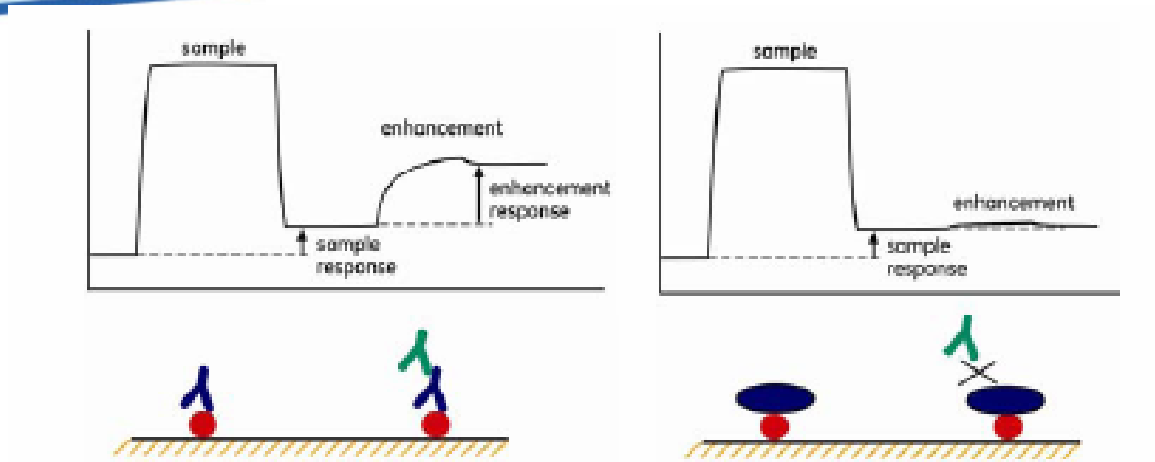
Biacore for isotyping, confirmatory..



Pros:

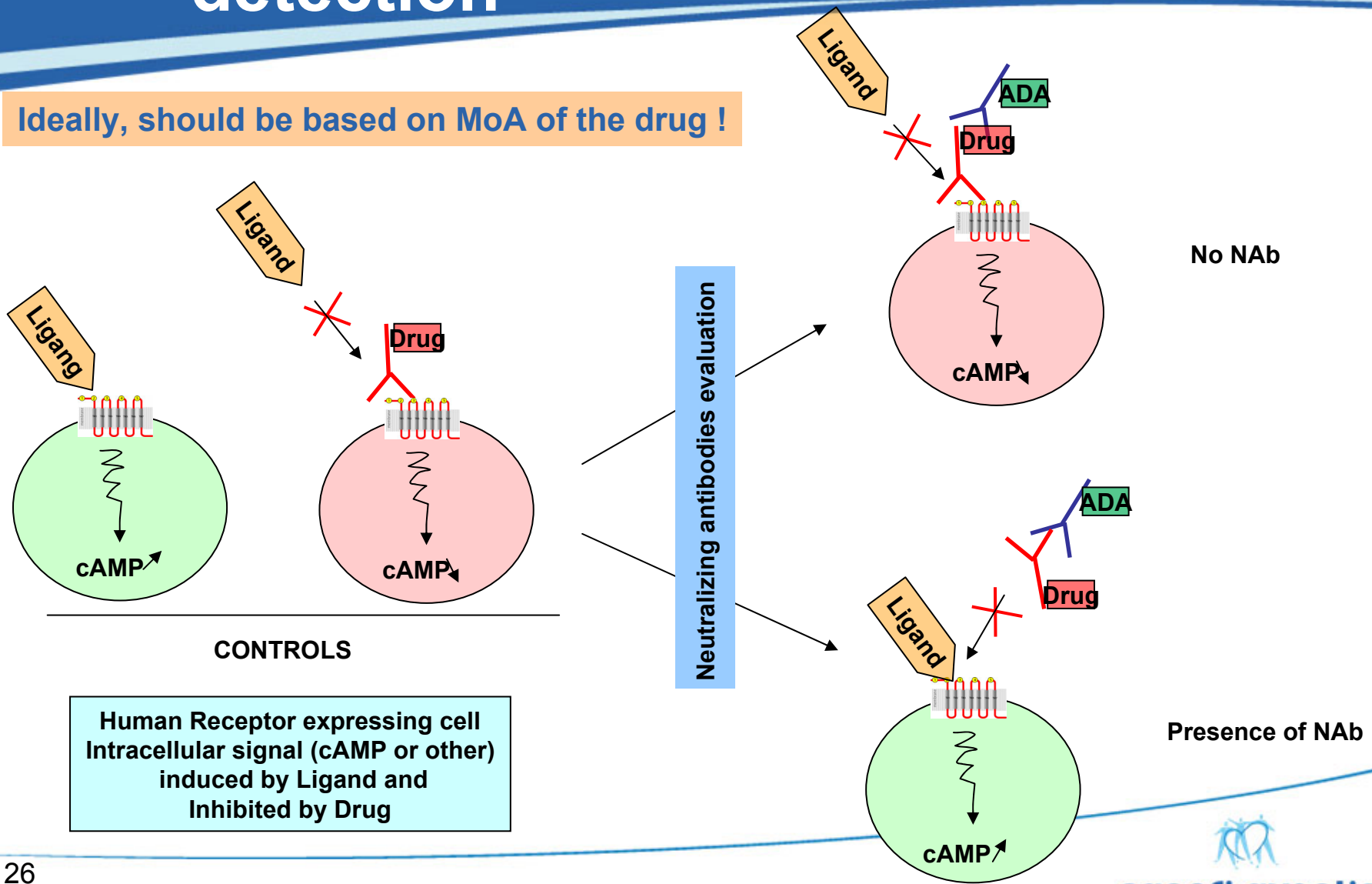
- ❖Large dynamic range
- ❖Secondary reagents not mandatory/ not species dependent
- ❖Detection of low affinity antibodies
- ❖Sensorgrams include information about affinity of anti-drug antibodies
- ❖Easy procedure for isotyping (IgG, IgA, IgM, IgE)

Cons:

- ❖Masking of binding epitopes by chemical coupling
- ❖Less sensitive than ELISA (May be superior to ELISA in detection of low affinity ADAs in certain circumstances)
- ❖Time consuming, usually not adapted for high throughput screening
- ❖Costs (specific equipment)

Bioassay for Neutralizing Ab detection

Ideally, should be based on MoA of the drug !



Human Receptor expressing cell
Intracellular signal (cAMP or other) induced by Ligand and Inhibited by Drug

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Cut-off definition
Screening assay

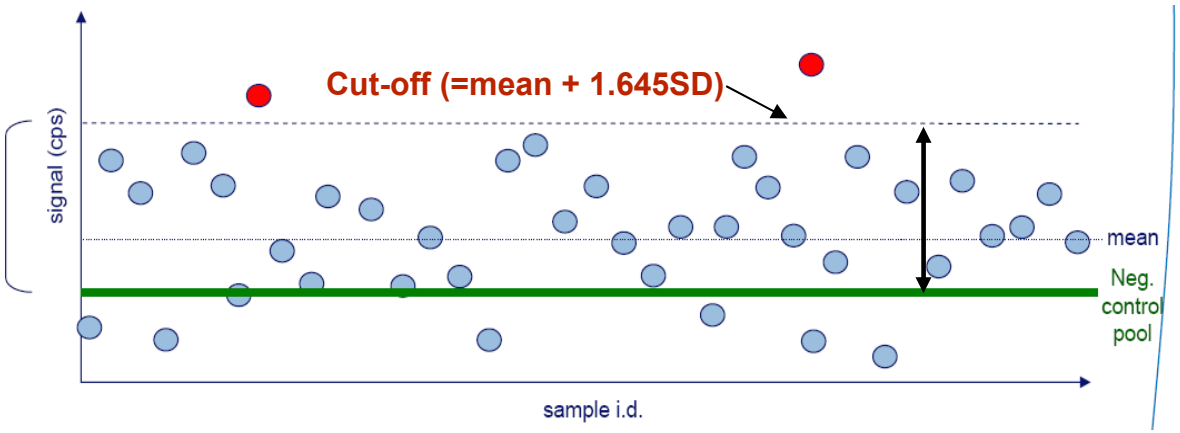
- Defined as the level of response of the ADA assay at and above which a sample is defined to be « reactive » (« potential positive ») for the presence of ADA and below which it is probably negative

- One of the main validation item for ADA assay

- Is established by a statistical evaluation of responses for a set of samples (~50-100) representative of naïve animals / subjects (negative for ADA)

Determination of the cut-off

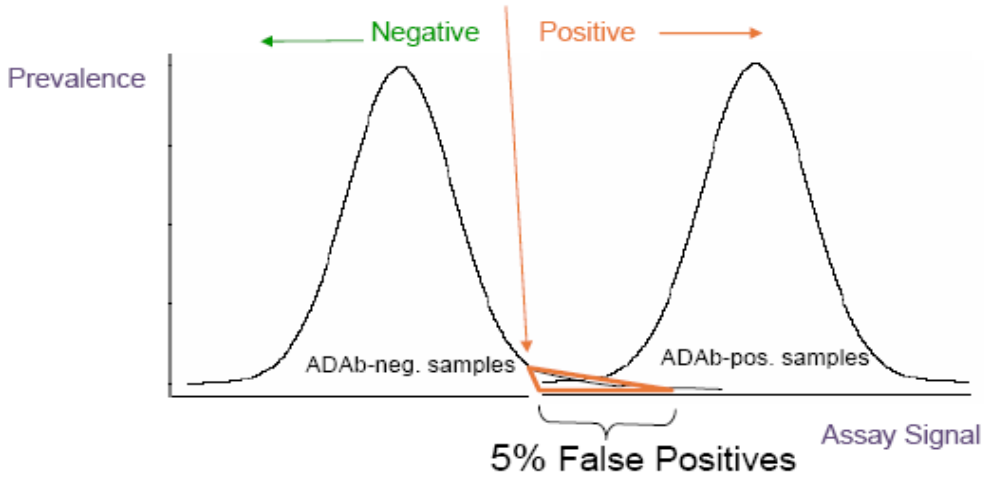
- Qualitative assay with cut-off approach statistically determined providing 5% false positive rate (mean + 1.645 SD for normal distribution or 95th percentile)



- Normalisation factor (NF)
  - = relative response Cut-Off / relative response of negative control pool
- For each plate calculation of Normalised Cut-off
  - = NF x relative response of negative control

5% false positive rate is recommended

It is more appropriate to have false positive than false negative (when using a risk based approach)



- White Paper recommends Mean + 1.645 SD rule
  - **~5%** of neg population should test positive
  - Hopefully - no false negatives

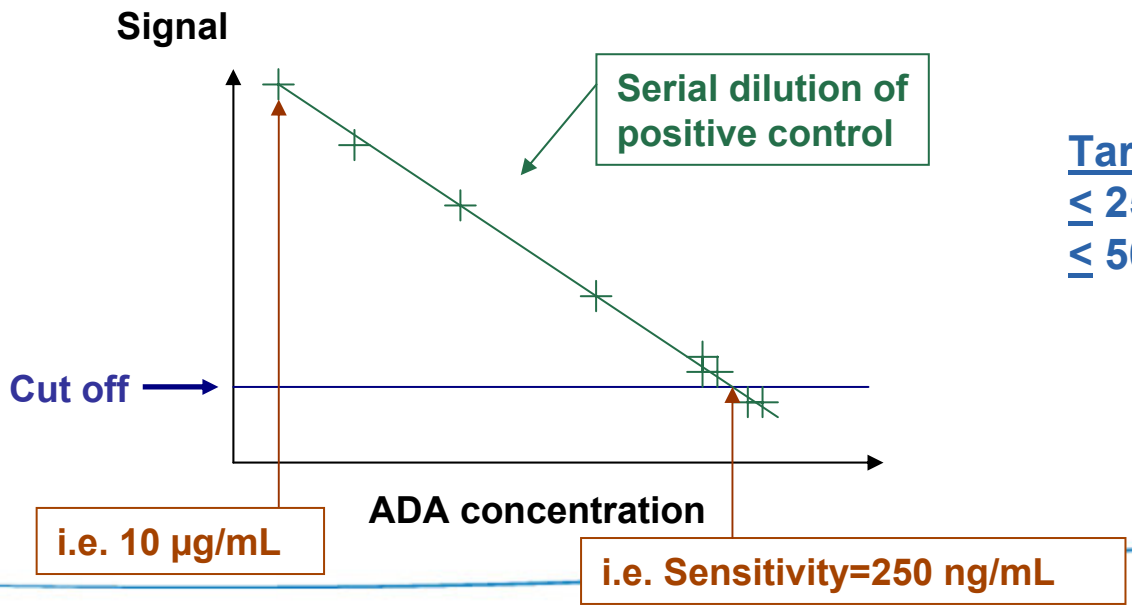
Some other recommendations for cut-off…

- Use samples from an appropriate population for the cut-off determination
- Start with healthy subject plasmas then re-define cut-off with individual patient plasmas as soon as available (clinical program progresses beyond Phase I or target disease population is available)
- It is recommended to use at least 50 (15-20 for animal) different naives human samples for cut-off determination
- It is established on 3 independent runs

ADA assay sensitivity determination

Sensitivity

- Defined by the lowest concentration at which a positive control antibody preparation provides a positive signal
  - Providing sensitivity of the assay
- Sensitivity is highly dependant of the positive control (affinity, avidity, etc)
- Sensitivity of the assay must be expressed in concentration limits (mass of ADA / volume unit)



Target Sensitivity:
≤ 250ng/mL for human
≤ 500-1000ng/mL for animal

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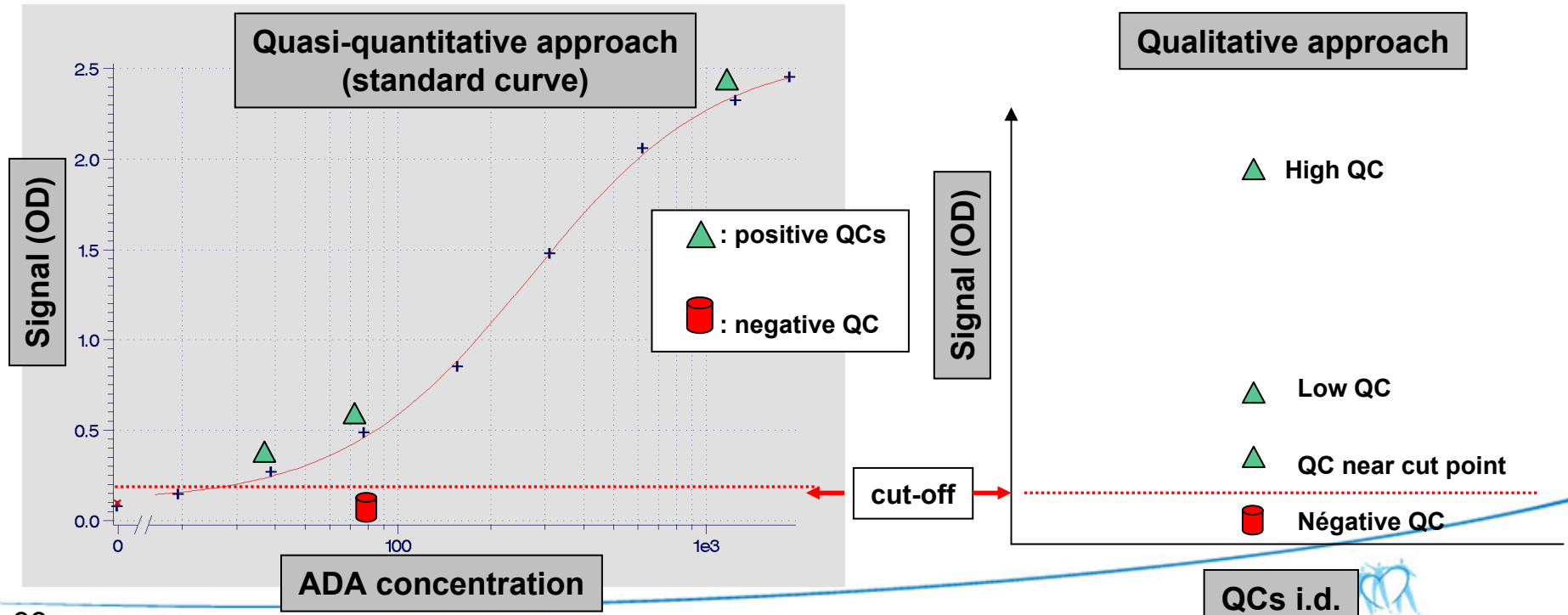
ADA assay system of controls

- **Establish a suitable system of control to ensure the validity of results**
  - A QC set must be define and a recommended one would include: Negative QC / QC near the cut point / LOW QC / HIGH QC
  - Define acceptance criteria for QCs
  - QC set will be used to validate each run



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ADA assay validation

- Cut-off determination
- Sensitivity and Specificity
- Free Drug interference
- Matrix effect
- Stability of positive control and incurred samples (when available)
  - 24h @37°C, -20°C (-80°C) for months
  - Freeze/thaw cycles
- Dilution and parallelism
- Co-medications…

Main challenges of immunogenicity monitoring

- Matrix effect, impact on sensitivity
- Interference of residual drug in samples
  - Proteins, particularly Mab, have long ½ life (2-3 weeks)
  - May lead to underestimation of ADA
  - Need optimization of assay to reduce interference
  - Design studies so that late time-points are collected for monitoring ADA
- ADA follow-up
  - from preclinical to late clinical phase III and then post registration to ensure long term safety and efficacy

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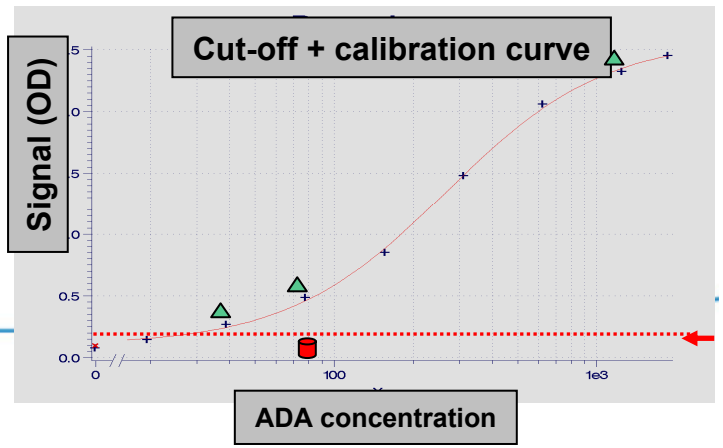
Case study 1 – Therapeutic Protein X

- Drug = Tetramer protein (monomer of 34 kDa) produced in yeast
- One cycle treatment: 5 i.v. administrations over a week
- Immunogenicity evaluation:
  - A first qualitative assay was validated for ADA monitoring
    - Direct ELISA: Protein X coated plate / plasma incubation / anti-hIg -HRP
    - Cut-off determination for each assay
    - Assay for IgA, IgM, IgG detection

- FDA required <u>two dictinct and quantitative assays</u> for IgG and IgE anti-protein X detection
  - Mab anti-protein X was chimerized into human IgG and human IgE
  - Development and validation of 2 « semi-quantitative » assays

Case study 1 - IgG assay

- IgG assay format: Direct ELISA
  - Protein X coated plate / unknown plasma or chimeric Ab spiked plasma (Std) / anti-hIgG –HRP
- N cut-off determination on 100 healthy subject plasmas
- N cut-off confirmation on 50 patient plasmas
  - N cut-off = 1.95
- Clinical samples analysis
  - Qualitative approach with cut-off determination for each plate
  - + Semi quantitative approach with validated calibration range: 0 to 10 µg/mL (with QCs at 0.6 / 1.8 / 5 and 10µg/mL)



Cut-off = 1.95 X response of pool negative

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Case study 1 – IgE assay

- IgE assay format: EIA
  - anti-hIgE coated plate / unknown plasma or chimeric IgE Ab spiked plasma (Std) / Drug X conjugated to biotin / streptavidin-HRP
- N cut-off determination on 100 healthy subjects plasmas
- N cut-off confirmation on 50 patient plasmas
  - N Cut-off = 1.46
- Clinical samples analysis
  - qualitative with cut-off determination for each plate
  - + semi quantitative with calibration range 0-20ng/mL with LLOQ of 2ng/mL

Case study 2 - Protein AVIDIN

SSR29261: extractive avidin purified from hen egg proteins

This protein may be used as neutralizing agent for biotinylated anticoagulant molecule

Thanks to the very high affinity between Biotin and Avidin : Kd = 10^{-15}

Case study 2 - Immunogenicity risk evaluation

Characteristics	Risk category
Ig anti-avidin antibodies **High MW (~64kDa) exogeneous (extracted from hen egg) protein**	**High**
I.V. administration	**Low-medium**
Low frequency of dosing (in routine) ***Use only for neutralization of biotin-anticoagulant in case of over-bleeding or to stop anticoagulant for surgery (= one or two occasions)***	**Low**
Allergic reaction and IgE anti-avidin antibodies ***Presence of natural anti-avidin antibodies in human, risk of hypersensitivity reaction ?***	**Medium**
Neutralizing anti-avidin antibodies ***High affinity of avidin for biotin (Kd :10^{-15}) compared to Ag-Ab (mean Kd :10^{-9})***	**Very low**

Conclusions

- Immunogenicity monitoring is mandatory for all biological products (peptides, proteins, Abs, conjugates..)
- Process strategy (screening + confirmatory + characterisation) is well defined
- But each biological is unique and requires specific assays for ADA and NAb detection
- Validation of ADA assay is challenging and is not standardized
- Immunogenicity = laboratory observations + clinical observations + PK + safety + efficacy..

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