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Immunogenicity testing for biotherapeutic products

Meenu Wadhwa, PhD

23 July 2017

Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

Immunogenicity testing for biotherapeutic products

Meenu Wadhwa

Disclaimer

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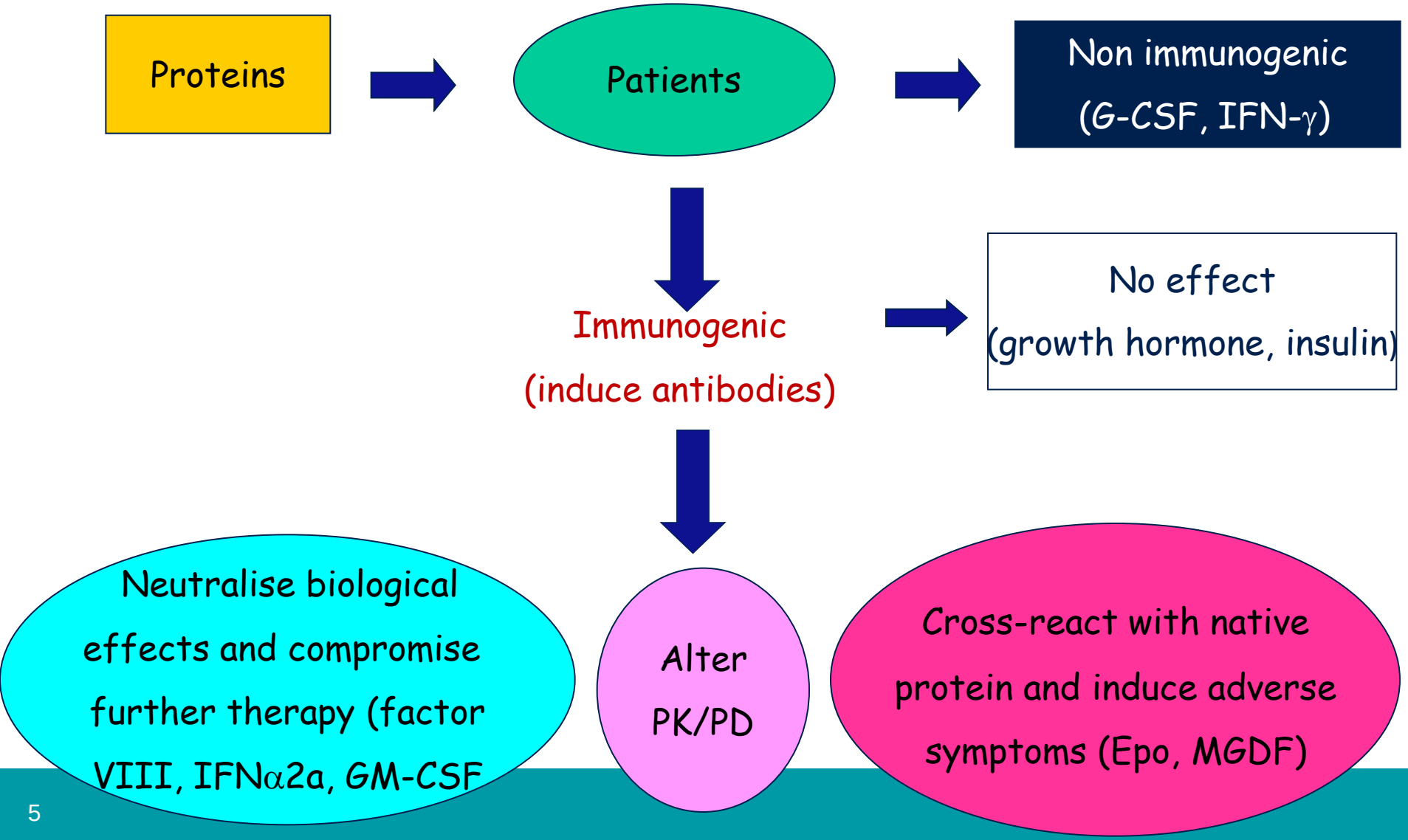
Unwanted Immunogenicity



Antibodies and Adverse Effects

The New England Journal of Medicine

VOLUME 346 FEBRUARY 14, 2002 NUMBER 7

PURE RED-CELL APLASIA AND ANTIERYTHROPOIETIN ANTIBODIES IN PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN

NICOLE CASADEVALL, M.D., JOELLE NATAF, M.D., BÉATRICE VIRON, M.D., AMIR KOLTA, M.D., JEAN-JACQUES KILADJIAN, M.D., PHILIPPE MARTIN-DUPONT, M.D., PATRICK MICHAUD, M.D., THOMAS PAPO, M.D., VALÉRIE UGO, M.D., IRÈNE TEYSSANDIER, B.S., BRUNO VARET, M.D., AND PATRICK MAYEUX, PH.D.

PRCA cases in Thailand, Korea - many marketed products

blood

2001 98: 3241-3248
doi:10.1182/blood.V98.12.3241

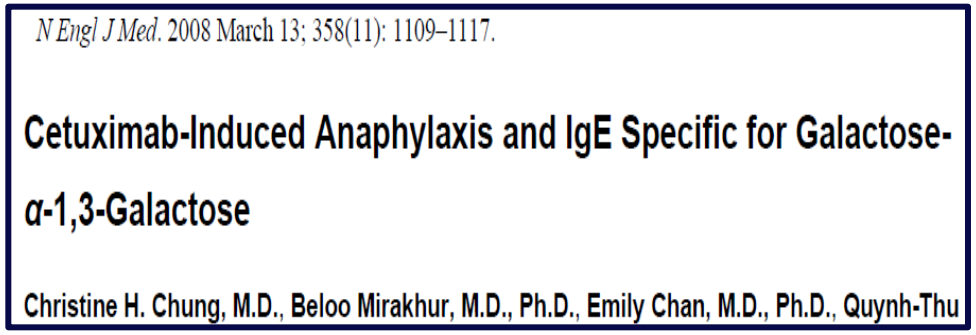
Thrombocytopenia caused by the development of antibodies to thrombopoietin

Junzhi Li, Chun Yang, Yuping Xia, Amy Bertino, John Glaspy, Michael Roberts and David J. Kuter

Cross-reactivity with endogenous protein

- MAb against EGFR – colorectal cancer, squamous cell carcinoma of head and neck
- 25/76 patients experienced hypersensitivity
- 17 had pre-existing IgE antibodies against gal-α-1, 3 gal present on Mab (expressed in murine myeloma cells)
- Cases clustered in different US states; IgE antibodies potentially due to tick bites etc

Product with same antigen as natural immunogen



N Engl J Med. 2008 March 13; 358(11): 1109–1117.

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

Christine H. Chung, M.D., Beloo Mirakhur, M.D., Ph.D., Emily Chan, M.D., Ph.D., Quynh-Thu

Clinical Impact

Efficacy – impaired clinical response

Safety – Infusion reactions, hypersensitivity reactions, serum sickness

– Cross-reactivity with an endogenous counterpart

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

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."

Product Name	Protein	Indication	% Patients with Immune Response
Intron A Roferon Pegasys PegIntron	IFN-α2a	Hepatitis C	7 25 9 1
Betaferon Avonex Rebif	IFN-β	Multiple Sclerosis	25 – 45 2 – 6 12 – 28
Eprex, Procrit Neorecormon, Aranesp	Epo	Anemia	Rare
Neupogen, Nivestim	G-CSF	Myeloregeneration, neutropenia	0-1.5 1.6
Leukine, Leucomax	GM-CSF	Myeloregeneration, immunostimulation	2 – 95
Proleukin	IL-2	Oncology	47–74
Mabthera	Anti-CD20	NHL SLE	0 65
Humira	Fully human anti-TNFα	RA	12 -28
Remicade	Chimaeric anti-TNFα	Crohn’s RA	61 12

Risk Factors

Product related:

Nature of the protein (molecular structure - primary sequence, novel epitopes, post-translational modifications e.g. glycosylation, oxidation)

Impurities, contaminants, formulation excipients, aggregates

Properties (immunomodulatory/ target..)

Treatment related:

Dose, route of administration, frequency of administration, duration of therapy, concomitant treatment

Patient related:

Age, gender, genetic make-up, immune status, disease/medical history, previous exposure

Unwanted Immunogenicity

Current Position

Testing for unwanted immunogenicity is integral to product development (clinical & post-marketing phase) for ensuring the clinical safety of a biotherapeutic and of a biosimilar

Animal data not predictive of immunogenicity in humans. *In silico* and T cell methods - clinical utility in prospective studies is lacking

Human clinical data needed

Every product needs to be evaluated for immunogenicity individually and an appropriate strategy adopted based on intended clinical use

Guidance – EMA, FDA, WHO

Immunogenicity testing

- Develop an integrated analysis strategy and study plan (incl sampling) relevant for the product (risk) and intended treatment to elucidate the clinical relevance of immunogenicity data

  - Carefully designed studies (clinical trials)
  - Antibody assays - evolve during development BUT VALIDATED assays for pivotal clinical trials and for post-marketing studies
  - Suitable positive controls; negatives;
  - Data interpretation/ threshold for +ve samples
  - Sampling points (incl baseline, post -treatment), frequency of sampling, sample volumes, processing/storage
  - Methods for assessing clinical response

- Determine clinical consequences . Assess how risk can be managed/mitigated

Key elements – low/high risk, assay capability, interference, clinical impact

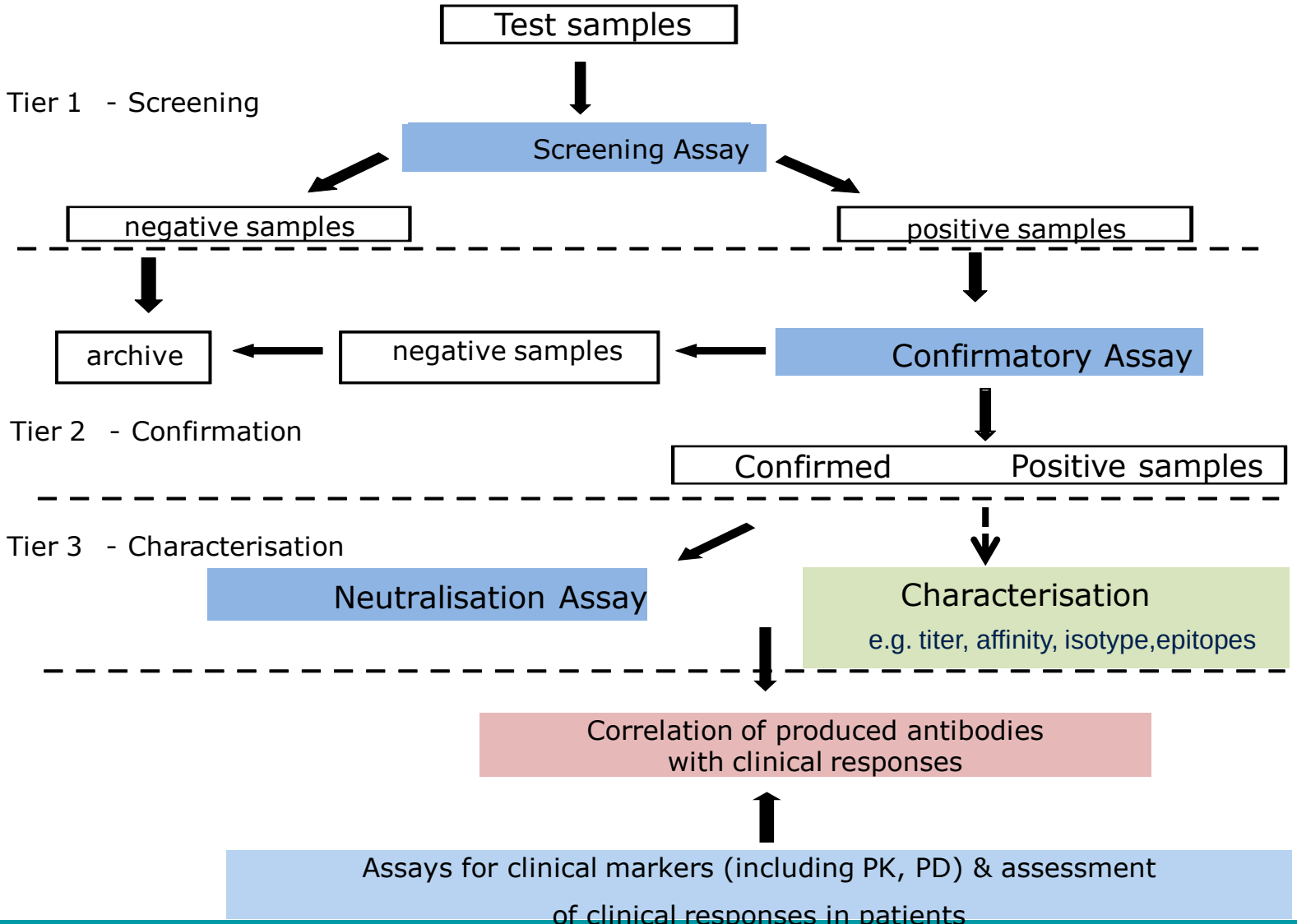
Planning of Studies

- **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

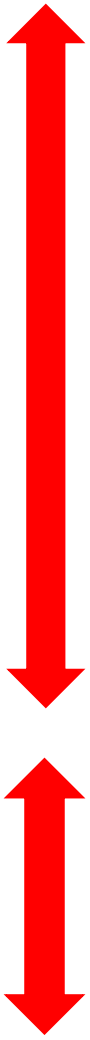
Strategy for Immunogenicity testing



Antibody assays

- ELISAs
  - direct format problematical for mAbs
  - Bridging formats; sensitive and robust
- Radioimmunoprecipitation assays (RIPA)
- Other technologies
  - Surface plasmon resonance (SPR),
  - Electrochemiluminescence (ECL),
  - AlphaLisa etc
- Bioassay
  - Cell-based
  - Non-cell-based

Choice dependent on the MOA



Screening

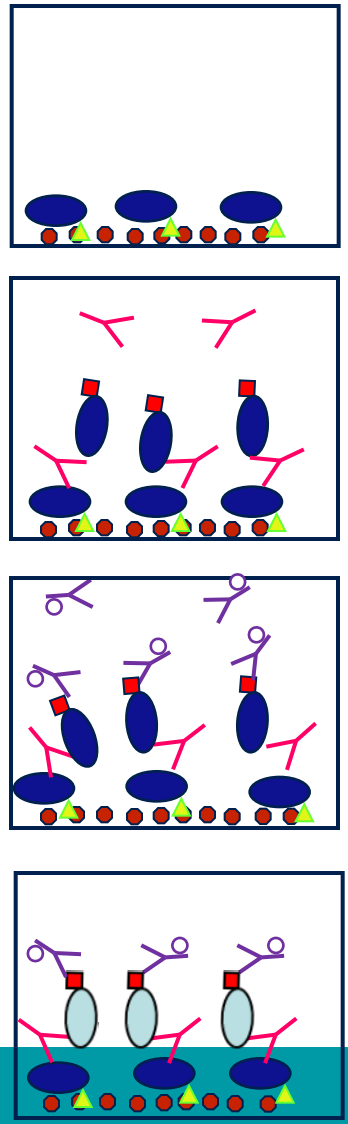
Principles vary

Neutralization

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



Streptavidin (●) plates
coat biotinylated antigen

add sample / control Ab
& DIG - antigen

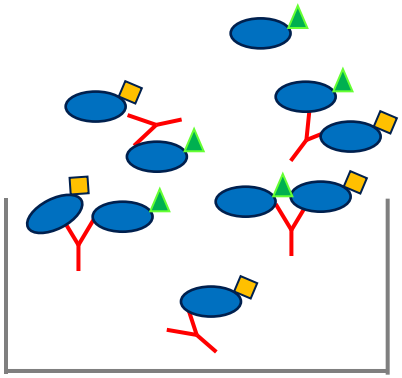
add anti-DIG Ab
AP conjugate

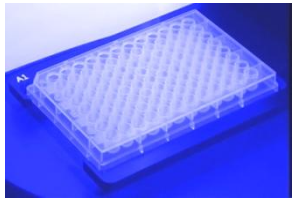
Add substrate
& measure OD

Bridging ECL assay

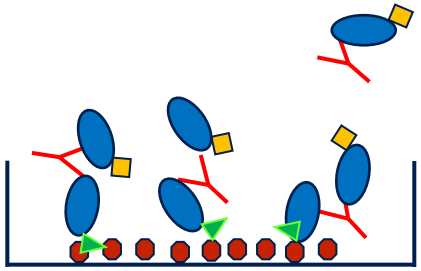
MSD platform

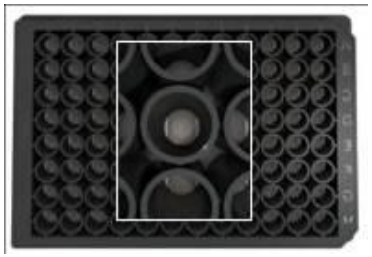
Mix sample / control Ab
biotinylated therapeutic ,
& sulfo-TAG therapeutic



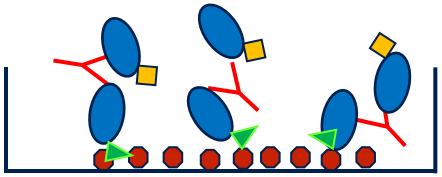


Transfer to streptavidin /
avidin ( ) - coated plates





Add read buffer (TPA)
Emission of light at 620 nm
following a voltage-stimulated
oxidation-reduction process.
Measure ECL counts



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

Some Considerations

- Pre-existing antibodies – Usually < 10%. If high incidence, investigate specificity; problematical from bioanalytical, efficacy & safety perspective

- Antibody detection can be impacted by
  - Matrix effects - 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.

  - Residual therapeutic/immune complexes
    - Some products (e.g. mAbs) persist or are given chronically at high doses
    - High levels of drug and/or immune complexes expected; false negative
    - Although a suitable positive control can be used, it does not reflect the situation with clinical samples (varying isotypes, affinities etc within/between patients over time).

- Corrective measures implemented on a case-by-case basis as appropriate
  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, prevent ADA binding → false negative

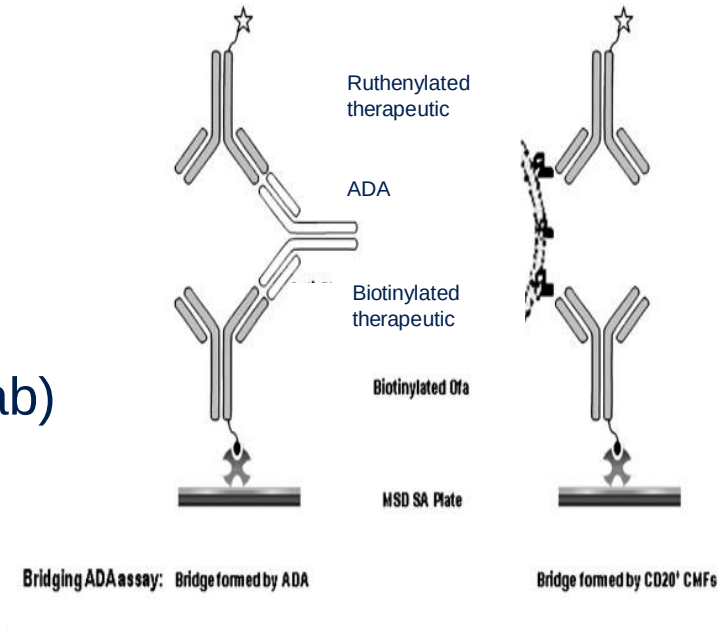
Membrane-bound target or multimeric soluble target may form bridge with therapeutic → false positive

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 in sample

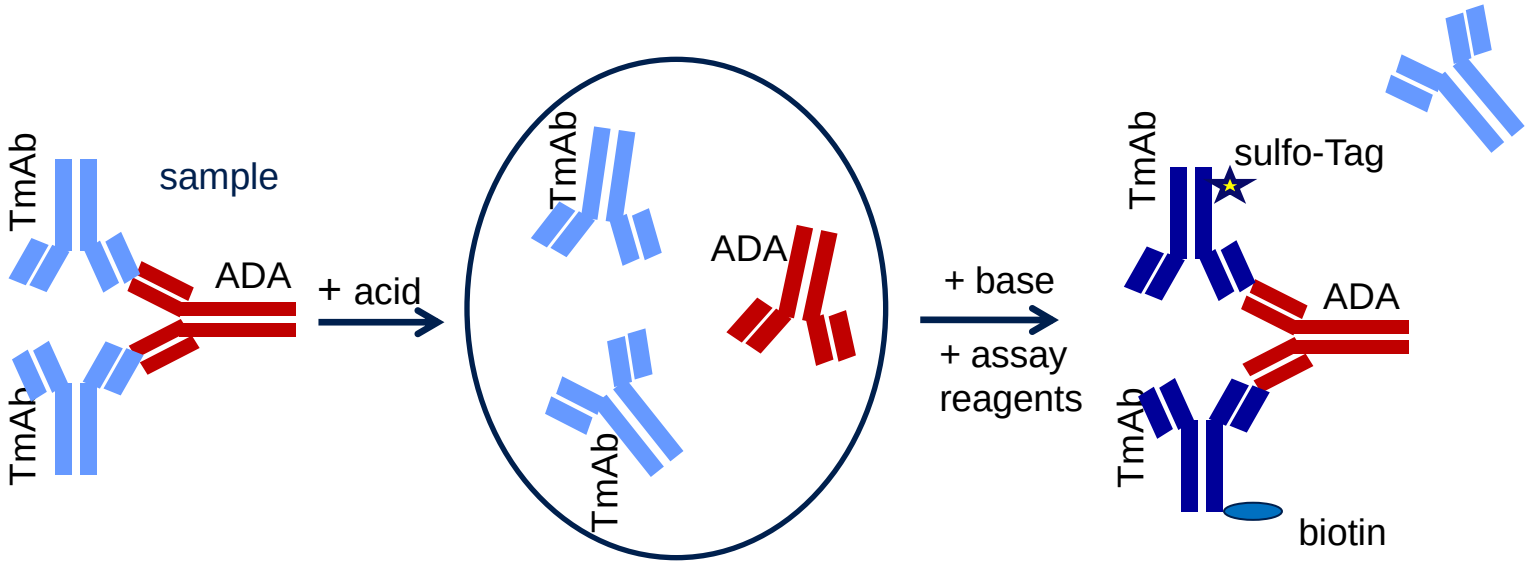


Adapted from Chen K. et *al*, 2013,JIM 394:22-31

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

Comparison of Platforms

Technology platforms	Sensitivity (ng/mL)	Drug tolerance (Drug:ADA [a])	Pros	Cons
Solid-phase ELISA	10	20:1	Generic reagents and instrumentation	Low drug tolerance
Gyros	4–20	100:1	Assay automation Assay time <2 h	Sole technology provider Fluorescent label stickiness
AlphaLISA	20	100:1	Homogeneous assay without wash steps	Sole technology provider Pipetting under restricted light conditions Hook effect
MSD ECL	10	100:1	Fewer steps than ELISA	Sole technology provider
Solution ELISA	25	200:1	Improved drug tolerance Generic reagents and instrumentation	Requires high quality streptavidin plates

[a] Drug:ADA ratio was determined at 100 ng/mL of positive control ADA and calculated using the molar values of Drug and ADA.

Screening Assay is the first step (mainstay)

Sensitive & capable of detecting all clinically relevant antibodies

Mikulskis *et al*, 2011,JIM 365: 38-49;

Testing is challenging

- No perfect assay for antibody screening. Each assay has its own relative merits and weaknesses
- May need to evaluate more than one assay platform, assay/ assay conditions dependent on therapeutic

- Assays qualitative (no reference standard); controls needed
  - Positive: for development, defining sensitivity, tolerance.
    Hyperimmunised sera - affinity purified, mAbs, anti-idiotypic abs
  - Negative: for threshold/cut-off for ‘discrimination’.
    Healthy sera, diseased /baseline sera, irrelevant antibody

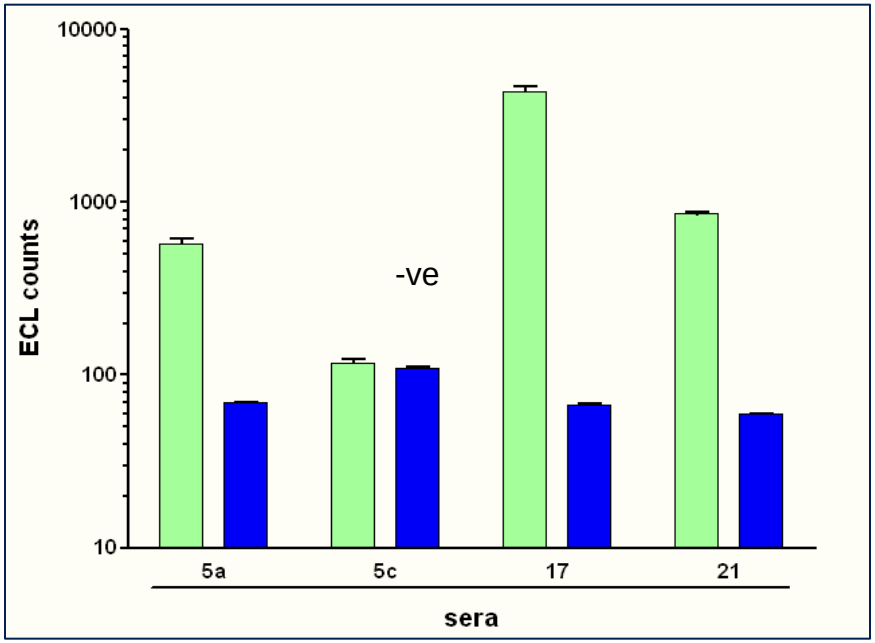
- Clear criteria for discriminating +ves from –ves

- Regulatory obligation to validate assays

Target : Measure Polyclonal response

Confirmatory Assays

- For eliminating false positive samples post initial screen.
- Spike sample with excess antigen and compare with unspiked sample

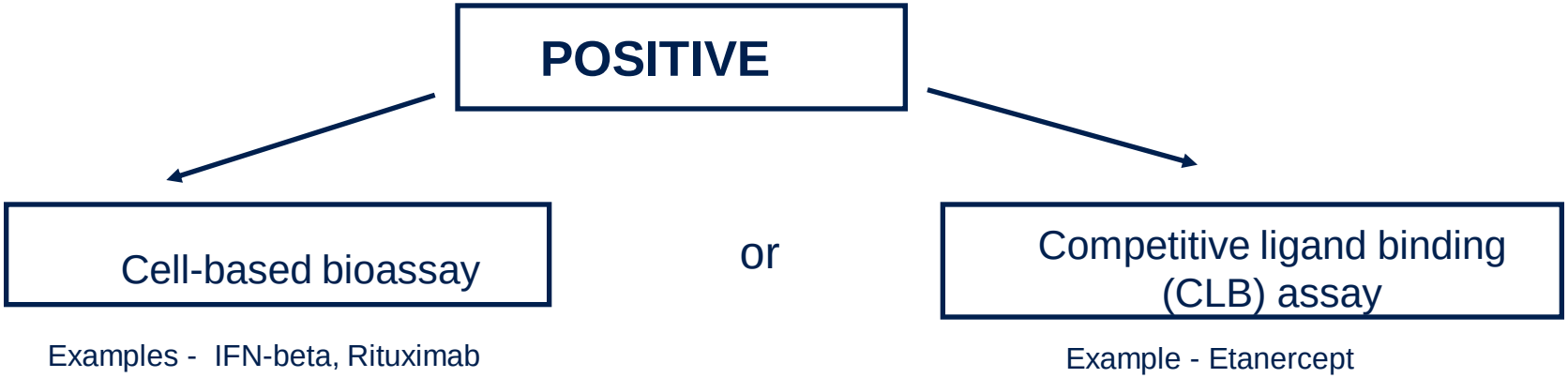


Unspiked (green bars) and spiked samples (blue bars).

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

Reporting Of Data

- Screening Cut-point (CP) to distinguish positive from negative samples i.e. the assay threshold at or above which samples are defined as +ve
  - Determined statistically based on the level of binding using healthy (& diseased) sera & inclusion of a false-positive rate;

- Confirmatory cut-point for eliminating false positives is the level of signal inhibition at or above which a sample is judged to have specific antibody
  - Derived by testing negative controls (e.g. drug-naïve samples) in the absence and presence of therapeutic

- Titer determination – informative as it can be linked to ADA of clinical impact.
  - Titer is the maximal sample dilution providing a signal above the screening cut-point

‘Industry standard – harmonised approach’

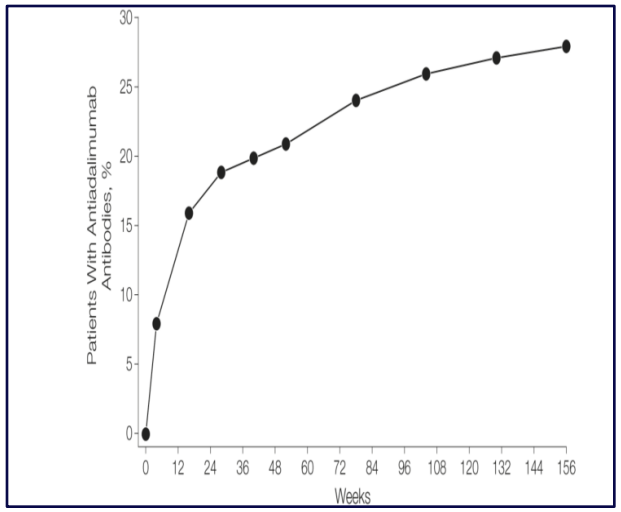
Clinical Impact of ADA

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

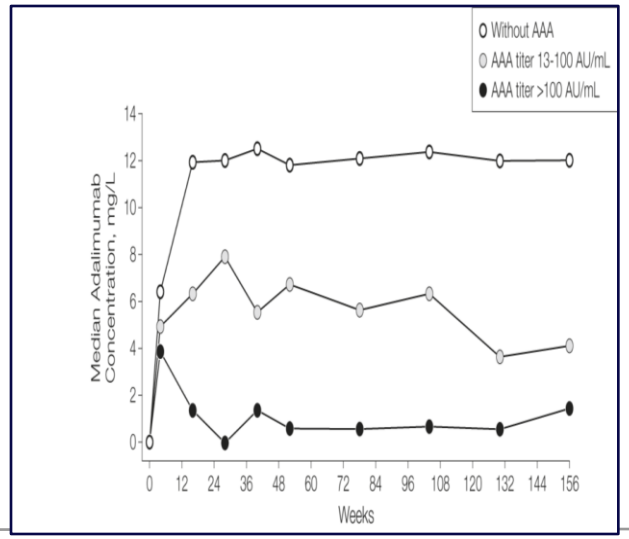
Antibodies and clinical impact

RA patients treated with Adalimumab over 3 years

A



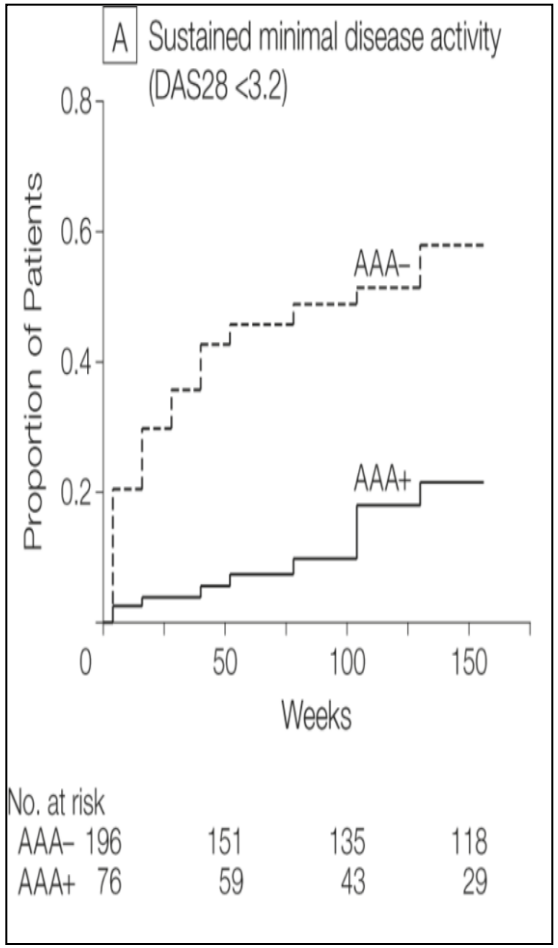
B



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.

Biosimilars : Comparative Immunogenicity

- Historical data cannot be used to compare different products
- Study with appropriate design, duration based on product (chronic – min 6 m);

- Sufficient size (not statistically powered) ⟹ allows conclusion on ADA & any impact on PK, efficacy and safety

- 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)
  - The consequences of immunogenicity also must be compared.

Biosimilars : Comparative Immunogenicity

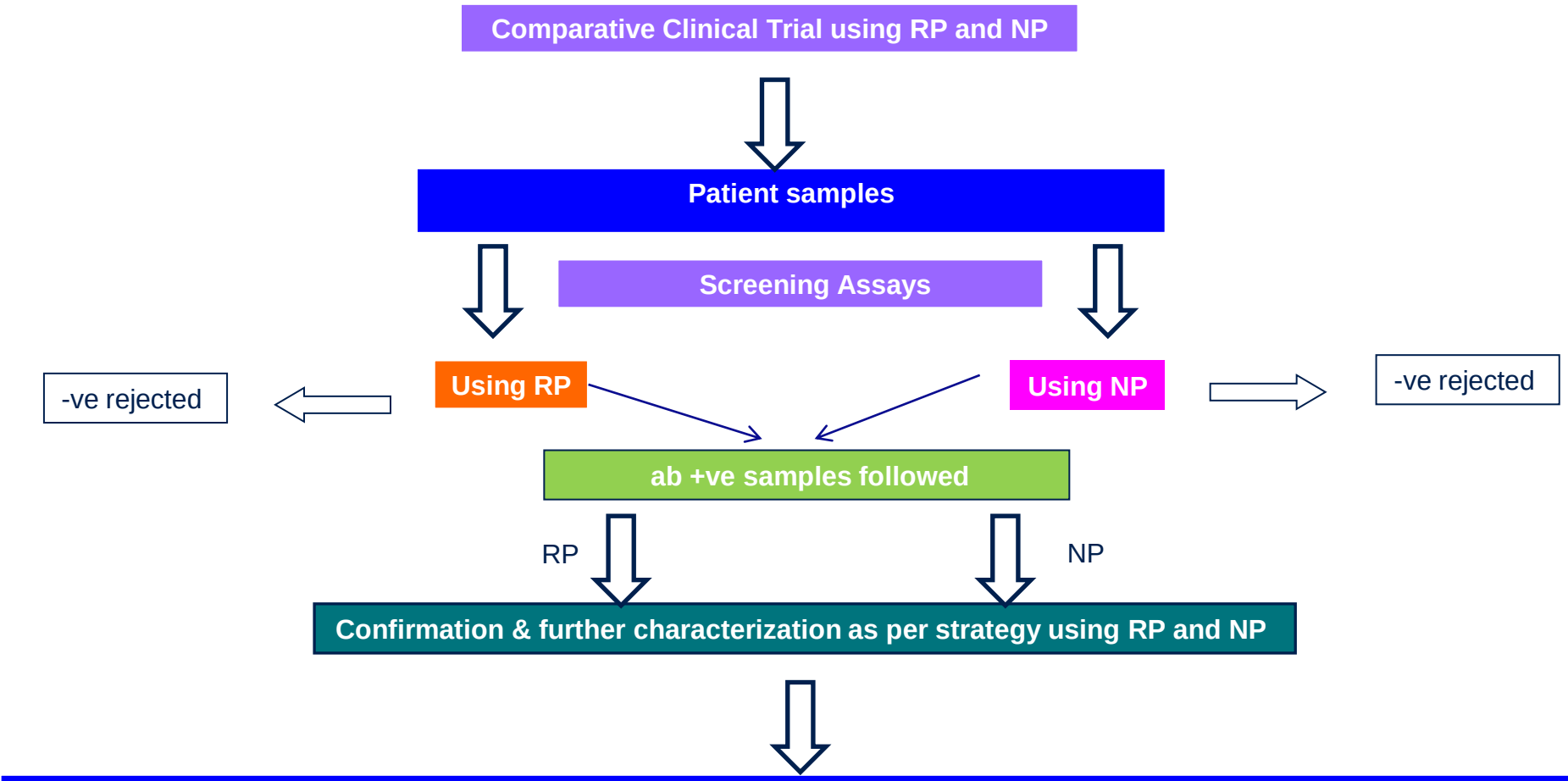
One assay/Two assay

- Positive controls for both products; State-of-art assays
- Ideally using administered therapeutic product (true immunogenicity)

- Challenging - develop/validate 2 assays with similar sensitivity and specificity
- 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

- Single assay - employing ‘biosimilar’ for both arms (relative)
- Conservative & risks under-estimating the immunogenicity of reference product
- Adoption of this approach minimises variability
- Any differences will question ‘comparability’ unless no clinical impact. Exploring the root cause of the differences important e.g., new epitopes

Relative Immunogenicity



Any association of antibodies with infusion reactions, hypersensitivity reactions etc

Biosimilars: Comparative Immunogenicity

Proper assessment is reliant on assay and assay execution

Expectation –

- Assays are properly validated and executed
- Antigenic equivalence shown
  - Similar criteria for antibody +ve samples (cut-point)
  - Similar drug tolerance
  - Similar sensitivity
- Similar antibody incidence, titre, onset, neutralization

Differences – Root-cause analysis, comparability paradigm

- Clinical consequences are not worse than the reference product

In the EU, for products approved to date, both approaches have been accepted

What is required?

- Risk Assessment
- Choice of methods and justification,
- Strategy of testing (Screening, Confirmatory, Neutralization),
- Validated assays (reports);

Methods

- Antibody incidence and the titre (incl pre-existing)
- Kinetics of response i.e., onset, duration - transient/persistent, persistence after treatment cessation? How long?
- Neutralizing capacity of the antibodies (yes/no and titre)
- Any Impact on PK, PD etc (for pre-existing too)
- Any Impact on Efficacy, Safety etc (for pre-existing too)

Data

In some cases, further characterization

- Determine isotype, epitopes

Data

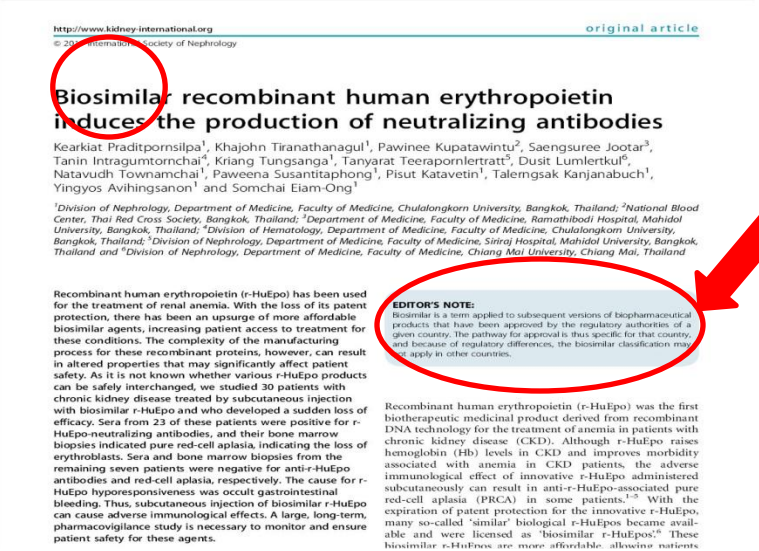
Antibodies for host cell proteins if appropriate.

Immunogenicity of Biosimilars

- In the EU, approved biosimilars have very similar immunogenicity profiles as the reference product.
- Example –
- Indication Remsima Remicade (reference)

  AS 37.5% 36.1%

  RA 55.6% 54.3%
- Immunogenicity likely to be higher for non-innovator products (in developing countries) but these are NOT biosimilars



http://www.kidney-international.org original article

© 20[illegible] International Society of Nephrology

Biosimilar recombinant human erythropoietin induces the production of neutralizing antibodies

Kearkiat Praditpornsilpa[1], Khajohn Tiranathanagul[1], Pawinee Kupatawintu[2], Saengsuree Jootar[3], Tanin Intragumtornchai[4], Kriang Tungsanga[1], Tanyarat Teerapornlertratt[5], Dusit Lumlertkul[6], Natavudh Townamchai[1], Paweena Susantitaphong[1], Pisut Katavetin[1], Talemgsak Kanjanabuch[1], Yingyos Avihingsanon[1] and Somchai Eiam-Ong[1]

[1]*Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand;* [2]*National Blood Center, Thai Red Cross Society, Bangkok, Thailand;* [3]*Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand;* [4]*Division of Hematology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand;* [5]*Division of Nephrology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand and* [6]*Division of Nephrology, Department of Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

Recombinant human erythropoietin (r-HuEpo) has been used for the treatment of renal anemia. With the loss of its patent protection, there has been an upsurge of more affordable biosimilar agents, increasing patient access to treatment for these conditions. The complexity of the manufacturing process for these recombinant proteins, however, can result in altered properties that may significantly affect patient safety. As it is not known whether various r-HuEpo products can be safely interchanged, we studied 30 patients with chronic kidney disease treated by subcutaneous injection with biosimilar r-HuEpo and who developed a sudden loss of efficacy. Sera from 23 of these patients were positive for r-HuEpo-neutralizing antibodies, and their bone marrow biopsies indicated pure red-cell aplasia, indicating the loss of erythroblasts. Sera and bone marrow biopsies from the remaining seven patients were negative for anti-r-HuEpo antibodies and red-cell aplasia, respectively. The cause for r-HuEpo hyporesponsiveness was occult gastrointestinal bleeding. Thus, subcutaneous injection of biosimilar r-HuEpo can cause adverse immunological effects. A large, long-term, pharmacovigilance study is necessary to monitor and ensure patient safety for these agents.

EDITOR'S NOTE:
Biosimilar is a term applied to subsequent versions of biopharmaceutical products that have been approved by the regulatory authorities of a given country. The pathway for approval is thus specific for that country, and because of regulatory differences, the biosimilar classification may not apply in other countries.

Recombinant human erythropoietin (r-HuEpo) was the first biotherapeutic medicinal product derived from recombinant DNA technology for the treatment of anemia in patients with chronic kidney disease (CKD). Although r-HuEpo raises hemoglobin (Hb) levels in CKD and improves morbidity associated with anemia in CKD patients, the adverse immunological effect of innovative r-HuEpo administered subcutaneously can result in anti-r-HuEpo-associated pure red-cell aplasia (PRCA) in some patients.[1–5] With the expiration of patent protection for the innovative r-HuEpo, many so-called 'similar' biological r-HuEpos became available and were licensed as 'biosimilar r-HuEpos'.[6] These biosimilar r-HuEpos are more affordable, allowing patients

Misleading definition

Worldwide consensus - A biosimilar is a biotherapeutic accepted by a regulatory pathway which requires biological and clinical comparison with the original licensed product. The 'biosimilars' described in this paper are NOT real biosimilars.

Conclusions

Immunogenicity is a problem for all biologicals (incl biosimilars)

There is no fit for purpose recipe for immunogenicity evaluation.
A case-by-case approach

Assessment requires an optimal strategy and well-validated and executed methods

Risks need to be considered and managed for patient benefit