Meenu Wadhwa, PhD, UK

- Principal Scientist, National Institute for Biological Standards and Control, UK

GaBI Educational Workshops

First Latin American Educational Workshop on
Similar Biotherapeutic Products

20 January 2015, Sheraton Maria Isabel Hotel & Towers, Mexico City, Mexico

Immunogenicity testing for biotherapeutic products

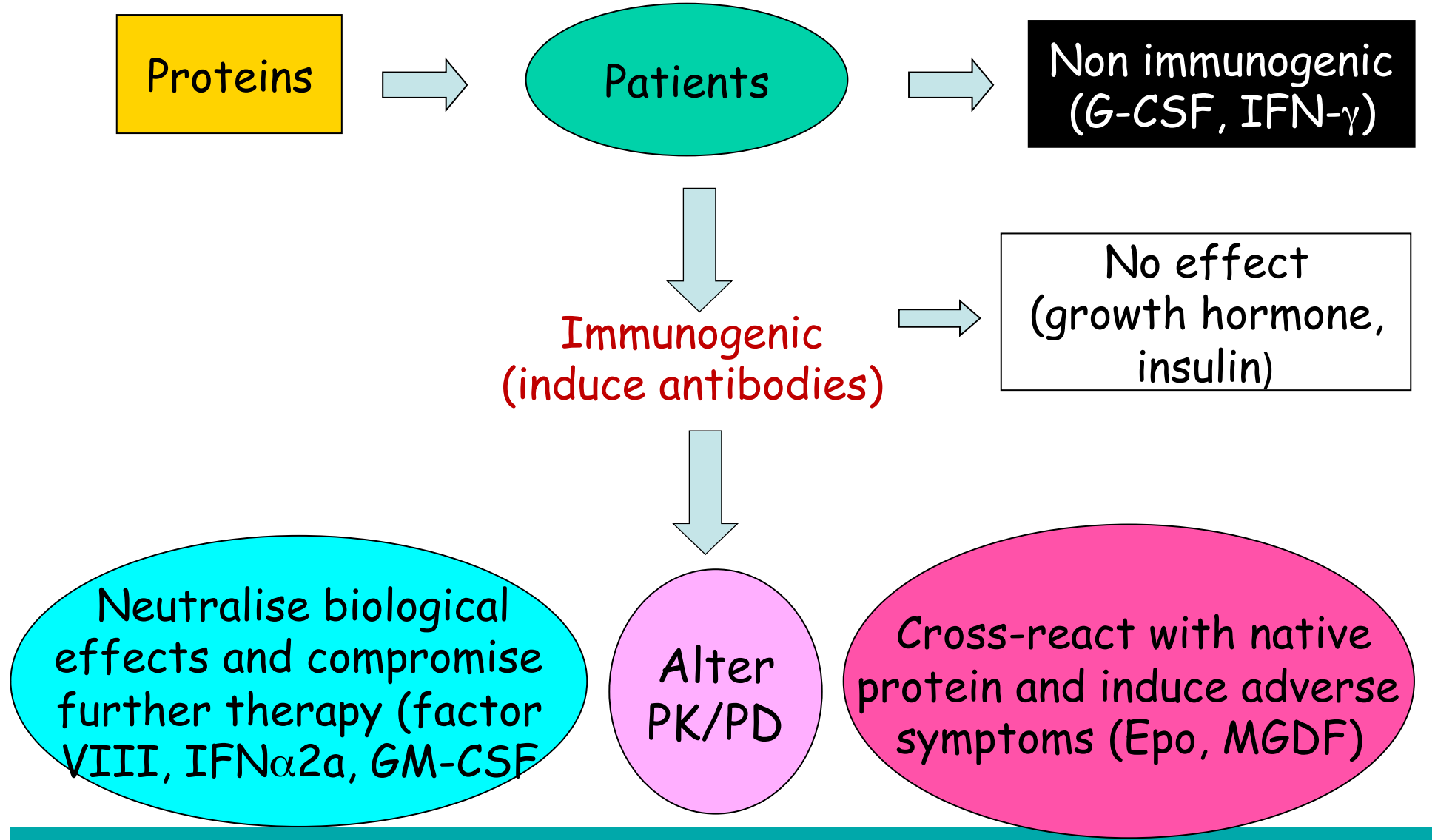
Meenu Wadhwa, PhD

20 January 2015

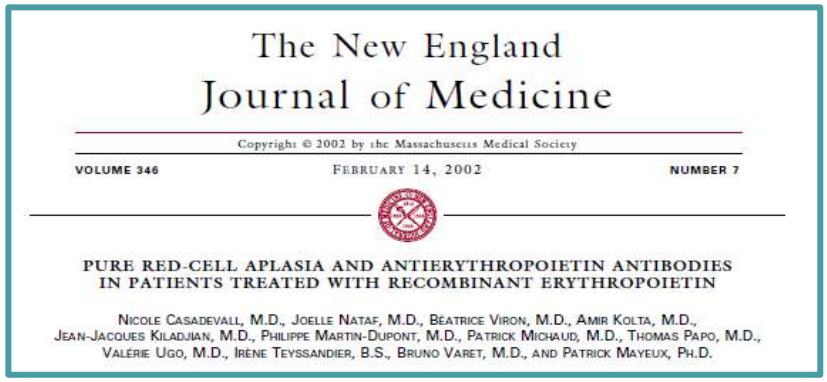
GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

PPCI
Pro Pharma Communications International

Unwanted Immunogenicity



Antibodies and Adverse Effects



The New England Journal of Medicine

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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.

Eprex: Formulation change (1999)
Cause: Leachates from uncoated stoppers (adjuvant).
Formulation/Containers: risk factors

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
Rituximab	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 Influencing Unwanted Immunogenicity

- Molecular structure, amino acid sequence, novel epitopes, glycosylation, degradation, oxidation, aggregation, deamidation
- Process related impurities, contaminants, host cell proteins
- Formulation
- Protein properties e.g.,immunostimulatory/suppressive, redundant/non-redundant
- Dose, route, frequency of administration and duration of therapy
- Immune status, age, genetic profile, disease, treatment
- Previous exposure

Immunogenicity Risk

Any subtle change in the manufacturing process can significantly influence the immunogenicity of a product

An apparently small change in manufacture/ processing can substantially increase immunogenicity. Careful development of the biosimilar product to ensure the quality attributes are similar to the reference product is necessary. This applies to not only the product characteristics but also in terms of impurity profile, aggregates etc

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

- Consider the risks of immunogenicity. Develop an integrated immunogenicity analysis strategy and study plan (incl sampling) relevant for intended clinical use

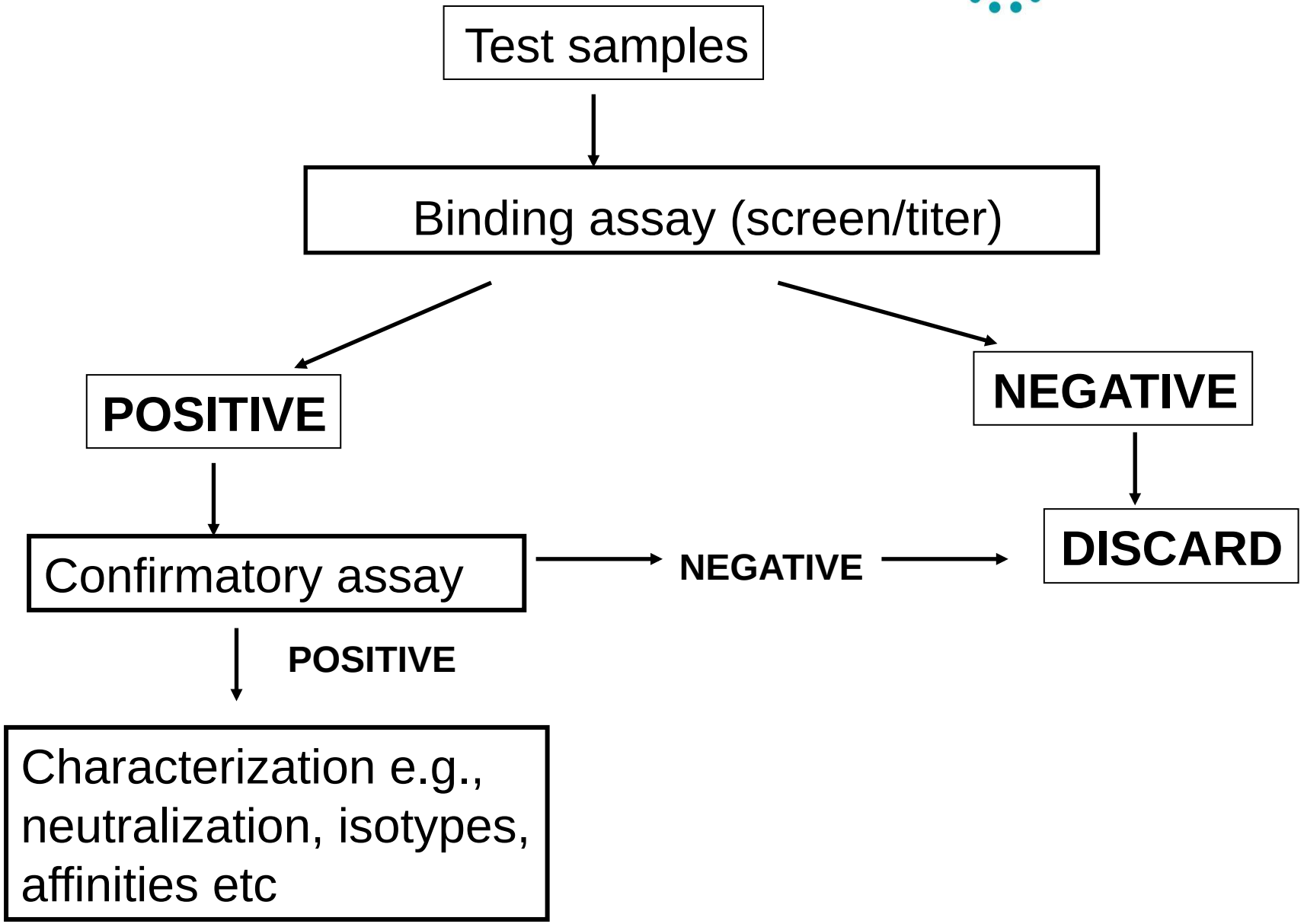
- Determine antibody incidence and magnitude, onset and duration of response
  - Test for antibodies using sensitive and valid assays (detect all antibodies, minimise matrix or residual product interference)
  - Test for therapeutic in samples

- Determine the characteristics of the antibodies
  - Assess for neutralization activity and biological impact, isotype, affinity

- Determine clinical consequences & significance of immunogenicity (cross-reactivity with marketed products) and how the risk can be managed/mitigated

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

Tiered Scheme



Immunogenicity Testing: A Tiered Approach

Screening assays - for 'identification' of all antitherapeutic antibodies

- ELISAs - direct, bridging, other formats
- Radioimmunoprecipitation assays (RIPA)
- Surface Plasmon Resonance (SPR)
- Other technologies e.g., ECL, DELFIA, Gyrolabs

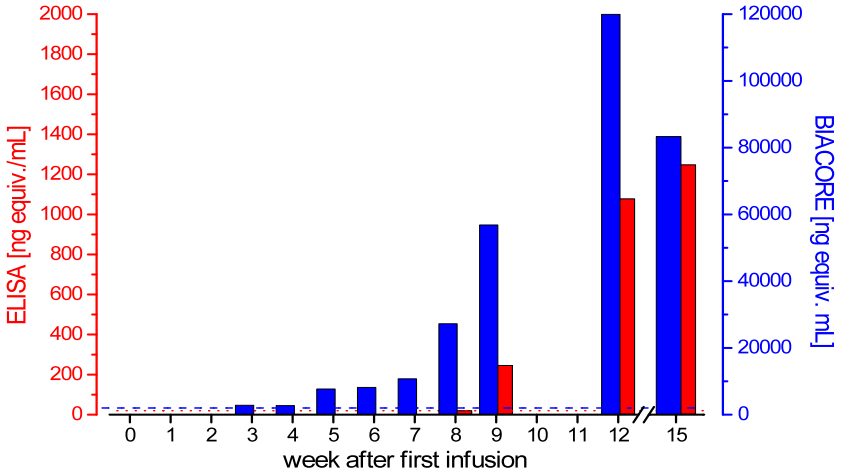
Confirmatory assays - for confirming antibodies

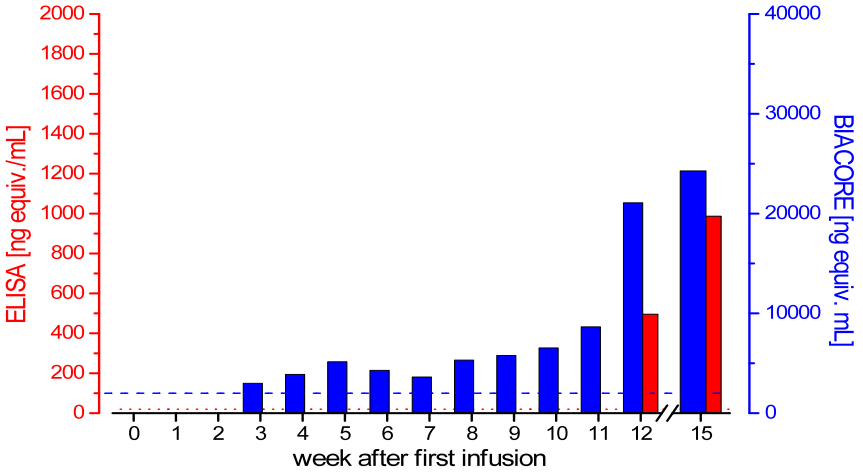
Other assays - for specificity of the antibodies

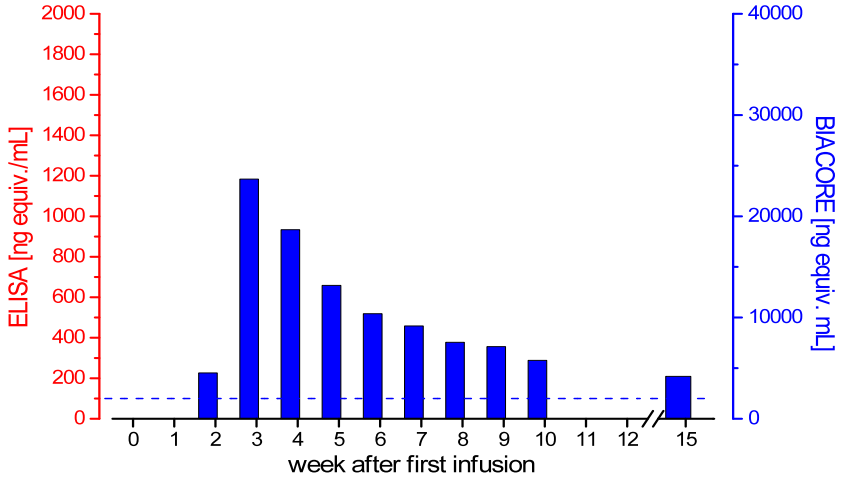
Neutralization assays - for discriminating neutralizing & non-neutralizing antibodies.

- Cell- based assay or
- Non-cell-based ligand binding assay

Data from Biacore vs ELISA Therapeutic mAb





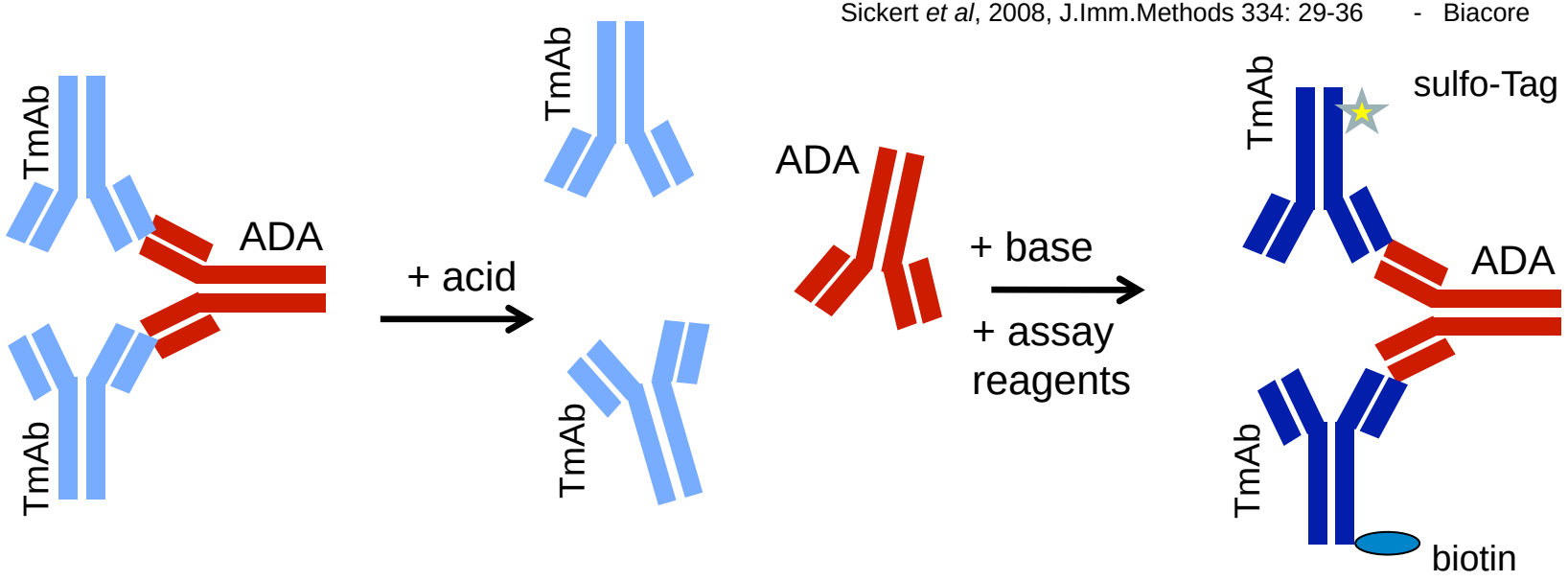


Data from Ulrich Kunz, (Boehringer Ingelheim)

Residual therapeutic

Principle of acid dissociation: (AD)

Patton *et al*, 2005, J.Imm.Methods 304: 189-195 - bridging ELISA
Sickert *et al*, 2008, J.Imm.Methods 334: 29-36 - Biacore



Acid dissociation or a variation to remove the therapeutic & prevent immune complex formation.
Other options e.g., sample dilution, wash-out samples.
Possibly a strategy which involves a combination of above approaches

Lofgren *et al*, 2006,JIM 308: 101-108; Bourdage et al, 2007JIM 327: 10-17; Smith *et al*, 2007, Reg.Tox.Pharm.49:230-237

Lofgren *et al*, 2006,JIM 308: 101-108; Bourdage et al, 2007JIM 327: 10-17; Smith *et al*, 2007, Reg.Tox.Pharm.49:230-237

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.

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

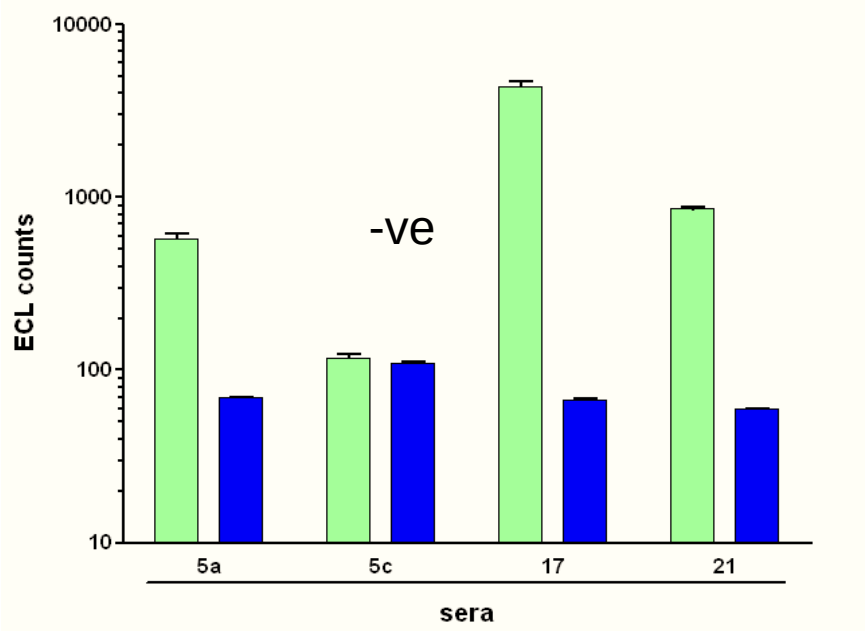
- Assays qualitative (no reference standard); controls needed
  - Positive: for development, defining sensitivity, tolerance.
    Hyperimmunised sera - affinity purified, mAbs, anti-idiotypic abs
  - Negative: for threshold/cut-off for ‘discrimination’
    Healthy sera, diseased /baseline sera, irrelevant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

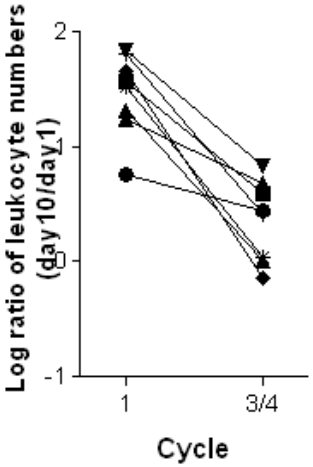
Neutralization Assay

Assessment of neutralizing activity in a functional assay is important.

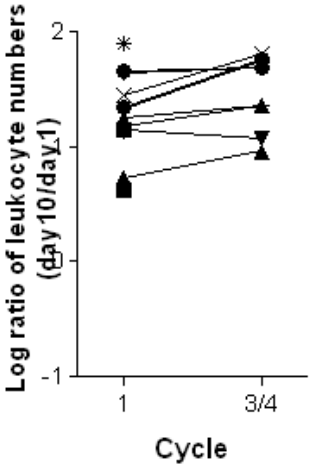
Any sample containing NAbs against the therapeutic reduces or abolishes the bioactivity of a known amount of the therapeutic.

Cell-based bioassays often used. Data may correlate with clinical response. However, non-cell based competitive ligand binding (CLB) assays may be the method of choice. Likely to be dictated by the mode of action

Neutralising



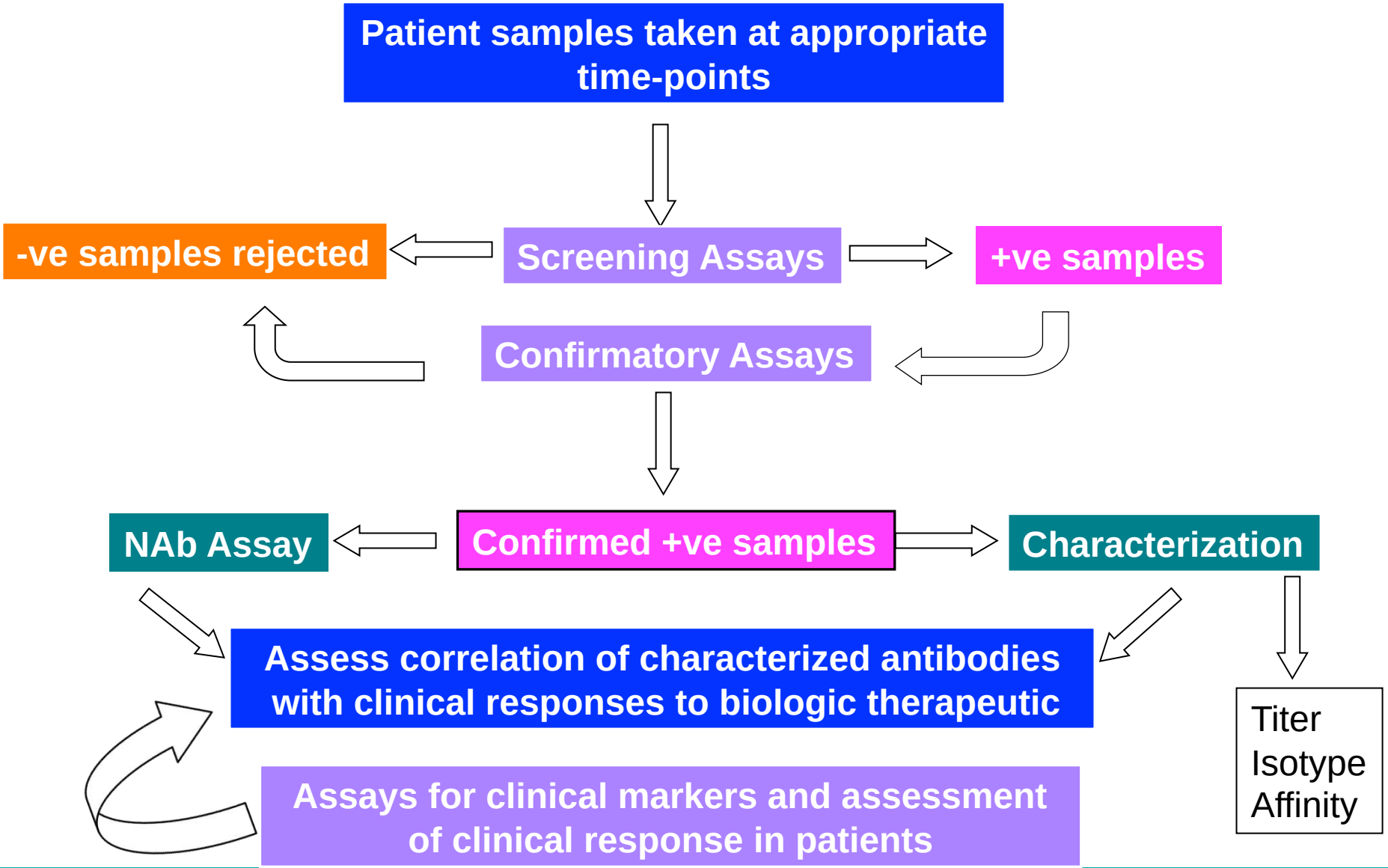
Non-Neutralising



Effect of GM-CSF antibodies on expansion of leukocytes

Wadhwa et al (1995) Clin. Exp. Immunol 104, 351-358

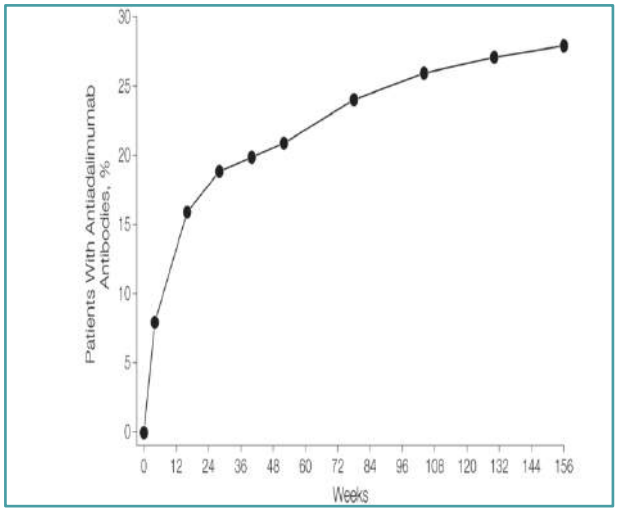
Strategy for Immunogenicity Assessment



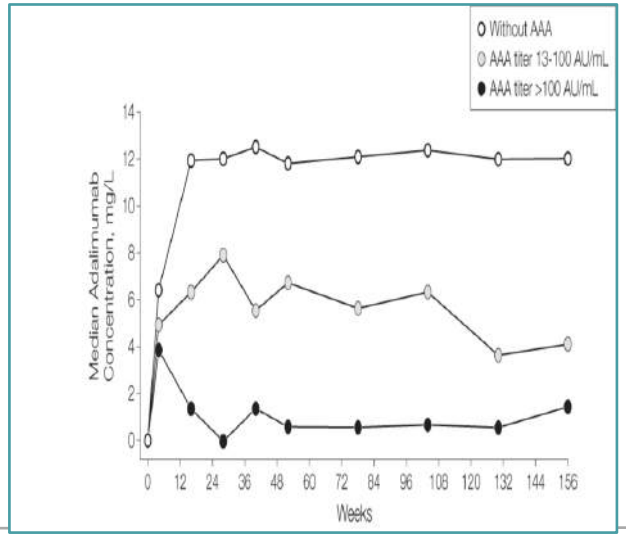
Antibodies and clinical impact

RA patients treated with Adalimumab over 3 years

A



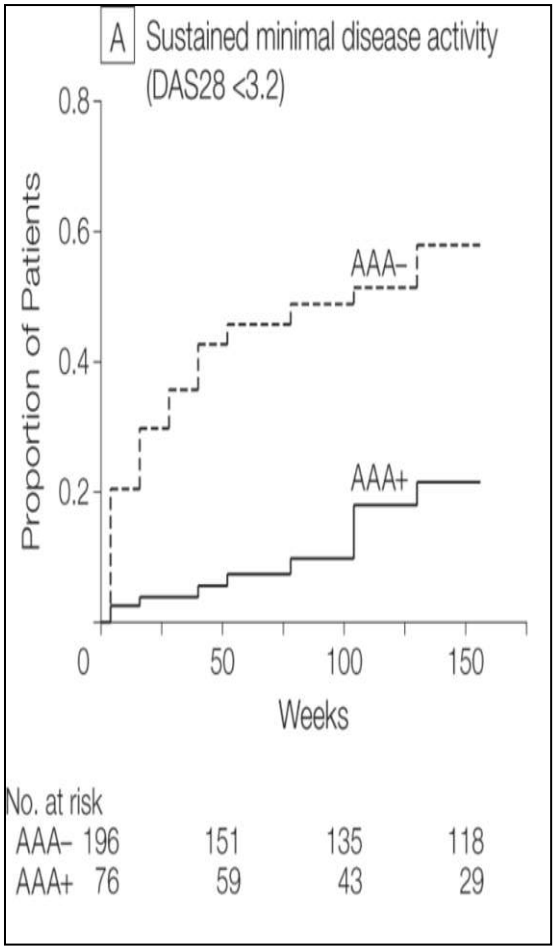
B



Ab -ve

Low Ab

High Ab



Abs develop within 24 weeks

diminish levels of therapeutic

compromises efficacy

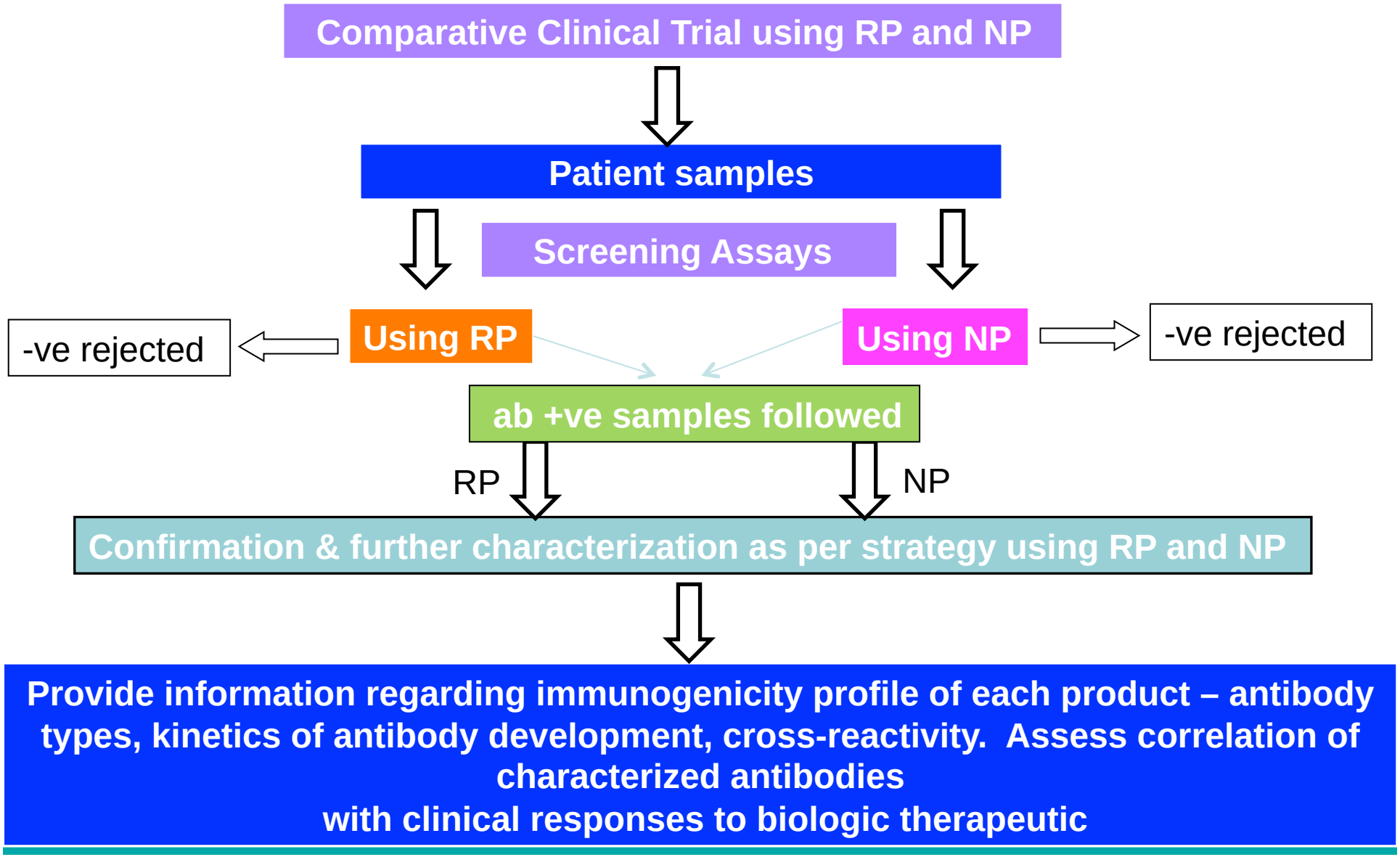
Bartelds et al : **Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up** JAMA. 2011;305(14):1460-1468.

Biosimilars : Comparative Immunogenicity

- Innovator vs biosimilar product immunogenicity
- Historical data cannot be used for comparisons across different products/ studies
- Design studies to demonstrate whether immunogenicity of the two products is similar or significantly different.
- Select a homogeneous and clinically relevant patient population.
- For extrapolation, consider suitability of patient population.
- Head-to-head studies using same assays & sampling strategy.
- Assays based on administered therapeutic product. Cross-reactivity studies
- Post-approval monitoring for pharmacovigilance necessary

Similar antibody incidence, titres, neutralization, clinical consequences.
Lower immunogenicity does not preclude biosimilarity
Assessed in context of totality of evidence

Relative Immunogenicity



Conclusions

Systematic evaluation of immunogenicity is important

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

Risks need to be considered and managed for patient benefit

Guidelines

EMA

•Guideline on Immunogenicity Assessment of Biotechnology Derived Therapeutic Proteins EMA/CHMP/BMWP/14327/2006

•Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use. EMA/CHMP/BMWP/86289/2010

FDA

Guidance for Industry (Draft)

•Assay development for immunogenicity testing of therapeutic proteins. December 2009

•Immunogenicity Assessment for Therapeutic Protein Products . February 2013