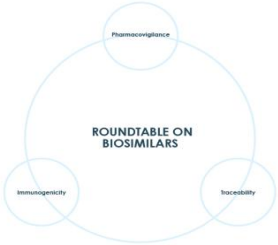


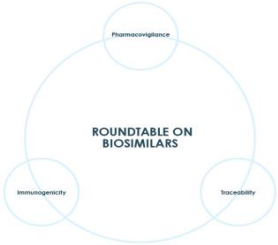

# Robin Thorpe, PhD, FRCPath, UK

- Former Head of Biotherapeutics Group, National Institute for Biological Standards and Control, UK

GaBI Scientific Meetings

ROUNDTABLE ON BIOSIMILARS
Pharmacovigilance, Traceability, Immunogenicity

15 November 2016, Real Academia Nacional de Farmacia, Madrid, Spain




# Evaluation of immunogenicity of biosimilars

Robin Thorpe, PhD, FRCPath, UK

15 November 2016

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

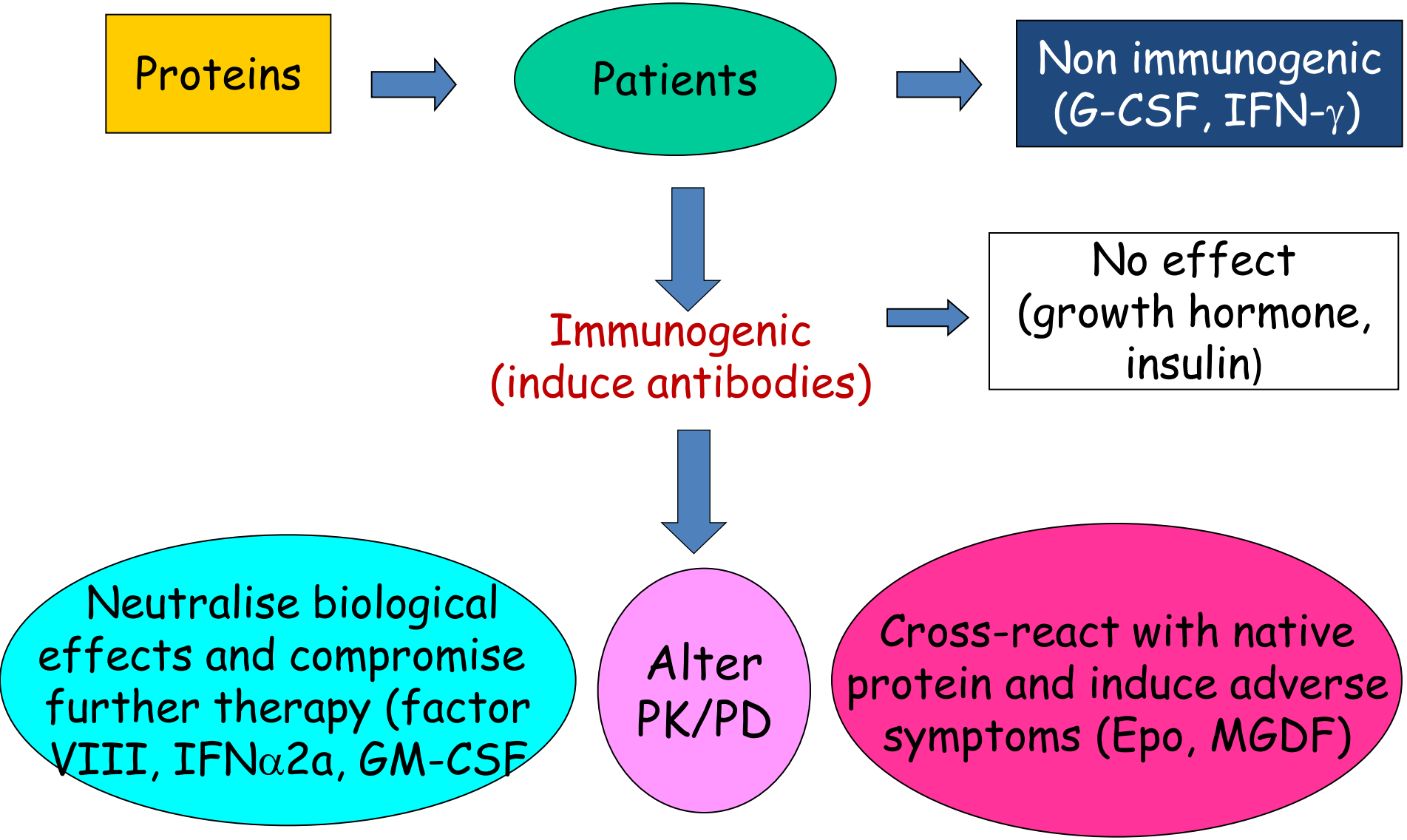
Universidad de Alcalá

PPCI
Pro Pharma Communications International

# Evaluation of Immunogenicity of Biosimilars

**Robin Thorpe PhD., FRCPath.**
**Email: rt7184@gmail.com**
**‘Phone +44 1438 715263**

# Unwanted Immunogenicity



# Unwanted Immunogenicity

- Biological products can induce antibodies with different characteristics:
  - Non-neutralizing (binding) antibodies against active (and/or inactive) product-related substance(s).
  - Binding antibodies against contaminants.
  - Neutralising antibodies.
  - Mixtures of the above.
- Different assays are needed for detection and measurement of these antibody types.

# Examples of therapeutics and their immunogenicity profile

| Product name | Protein | Indication | % Patients with immune response |
|---|---|---|---|
| ReFacto | Factor VIII | Hemophilia A | ~ 30% |
| Intron A | Interferon α | Hepatitis C | 7% |
| Roferon A | | | 25% |
| Pegasys | | | 9% |
| Pegintron | | | 1% |
| Betaseron | Interferon β | Multiple Sclerosis | 10-45% |
| Avonex | | | |
| Rebif | | | |
| Eprex | Erythropoietin | Anemia | Non immunogenic<br>Some cases of pure red cell aplasia with Eprex |
| Aranesp | | | |
| Epogen | | | |
| Procrit | | | |
| Leukine | Granulocyte macrophage colony stimulating factor | Oncology | 2.3% (neutralizing antibodies) |
| Neupogen | Granulocyte colony stimulating factor | Oncology | Non immunogenic |
| Neulasta | | | |
| Enbrel | TNF receptor II human Ig Fc fusion | Rheumatoid arthritis | 16% |
| Proleukin | Interleukin-2 | Oncology | 74% |

# 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.

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

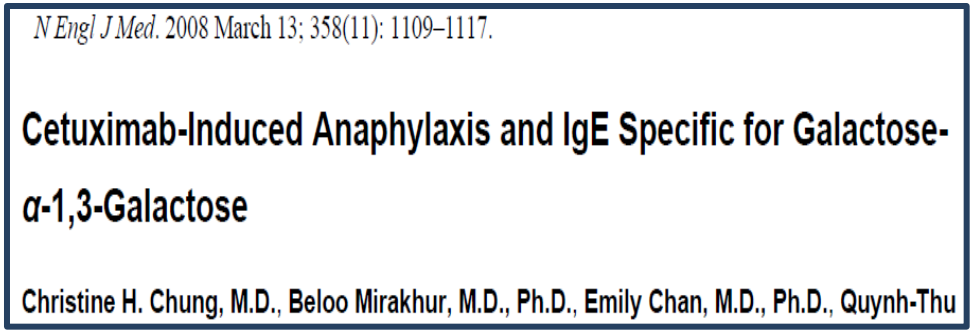
**Thrombocytopenia caused by the development of antibodies to thrombopoietin**

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."

# ‘Biosimilar’ EPO is Immunogenic?

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



## 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], Talerngsak 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

Under the generic drug paradigm of the Thai Food and Drug Administration, 14 biosimilar r-HuEpos were licensed by 1 January 2009. These products came from various countries such as Argentina, China, South Korea, and India.

The number of cases using ‘biosimilar’ r-HuEpos have increased enormously because of their more affordable prices. With their usage, adverse effects of the less than identical therapeutic agents have started to increase.

Many clinicians in Thailand were starting to see an increase in PRCA cases which raised an important issue whether the immunogenicity of biosimilar therapeutic agents were indeed equivalent to the innovative r-HuEpo.

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.

# Unwanted Immunogenicity- The Most Challenging Issues

- It is impossible to predict
  - the incidence of unwanted immunogenicity
  - the characteristics of the immune response
  - the clinical consequences & significance of such immunogenicity
- THE ABOVE NEED TO BE ASSESSED IN APPROPRIATE STUDIES

# Factors Influencing Unwanted Immunogenicity

## Product and Patient related

- Molecular structure, novel epitopes, glycosylation, degradation, oxidation, deamidation
- Product impurities
- Formulation
- Aggregation
- Protein – biological properties e.g., immunostimulant
- Dose, route, frequency of administration and duration of therapy
- Immune status, age, genetic profile, disease, treatment
- 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

European Medicines Agency

London, 13 December 2007
Doc. Ref. EMEA/CHMP/BMWP/14327/2006

**COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)**

# GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

| | |
|---|---|
| **DRAFT AGREED BY BMWP** | July 2006 |
| **ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION** | January 2007 |
| **END OF CONSULTATION (DEADLINE FOR COMMENTS)** | July 2007 |
| **AGREED BY BMWP** | October 2007 |
| **ADOPTION BY CHMP** | December 2007 |
| **DATE FOR COMING INTO EFFECT** | April 2008 |


| | |
|---|---|
| **KEYWORDS** | *Immunogenicity, unwanted immune response, biotechnology derived proteins, immunogenicity risk factors, assays, clinical efficacy and safety, risk management* |

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 September 2015
EMEA/CHMP/BMWP/14327/2006 Rev. 1
Committee for Medicinal Products for Human Use (CHMP)

# Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins

Draft

| | |
|---|---|
| Draft agreed by Biosimilar Medicinal Products Working Party (BMWP) | August 2015 |
| Adopted by CHMP for release for consultation | 24 September 2015 |
| Start of public consultation | 01 October 2015 |
| End of consultation (deadline for comments) | 31 January 2016 |

This guideline replaces 'Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins' (EMEA/CHMP/BMWP/14327/2006).

Comments should be provided using this template. The completed comments form should be sent to BMWP.secretariat@ema.europa.eu


| | |
|---|---|
| **Keywords** | ***Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay strategy, binding antibodies, neutralising antibodies, risk factors, safety, efficacy, pharmacokinetics, risk management, integrated summary of immunogenicity*** |



30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
**Telephone** +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
**Send a question via our website** www.ema.europa.eu/contact

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 May 2012
EMA/CHMP/BMWP/86289/2010
Committee for Medicinal Products for Human Use (CHMP)

# Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.

| | |
|---|---|
| Draft agreed by Similar Biological Medicinal Products Working Party | October 2010 |
| Adoption by CHMP for release for consultation | November 2010 |
| End of consultation (deadline for comments) | May 2011 |
| Final agreed by BMWP | March 2012 |
| Adoption by CHMP | 24 May 2012 |
| Date for coming into effect | 1 December 2012 |

*Disclaimer: This guideline is intended as an addendum to Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins EMEA/CHMP/BMWP/14327/2006 and should be read in conjunction.*


| | |
|---|---|
| **Keywords** | **Immunogenicity, monoclonal antibodies, similar biological medicinal products, clinical use, assay strategy.** |



7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
**Telephone** +44 (0□20 7418 8400 **Facsimile** +44 (0)20 7418 8416
**E-mail** info@ema.europa.eu **Websi□e** ww□.ema.europa.eu

# Immunogenicity Testing: A Tiered Approach

**Screening assays - for 'identification' of <u>all</u> antitherapeutic antibodies**

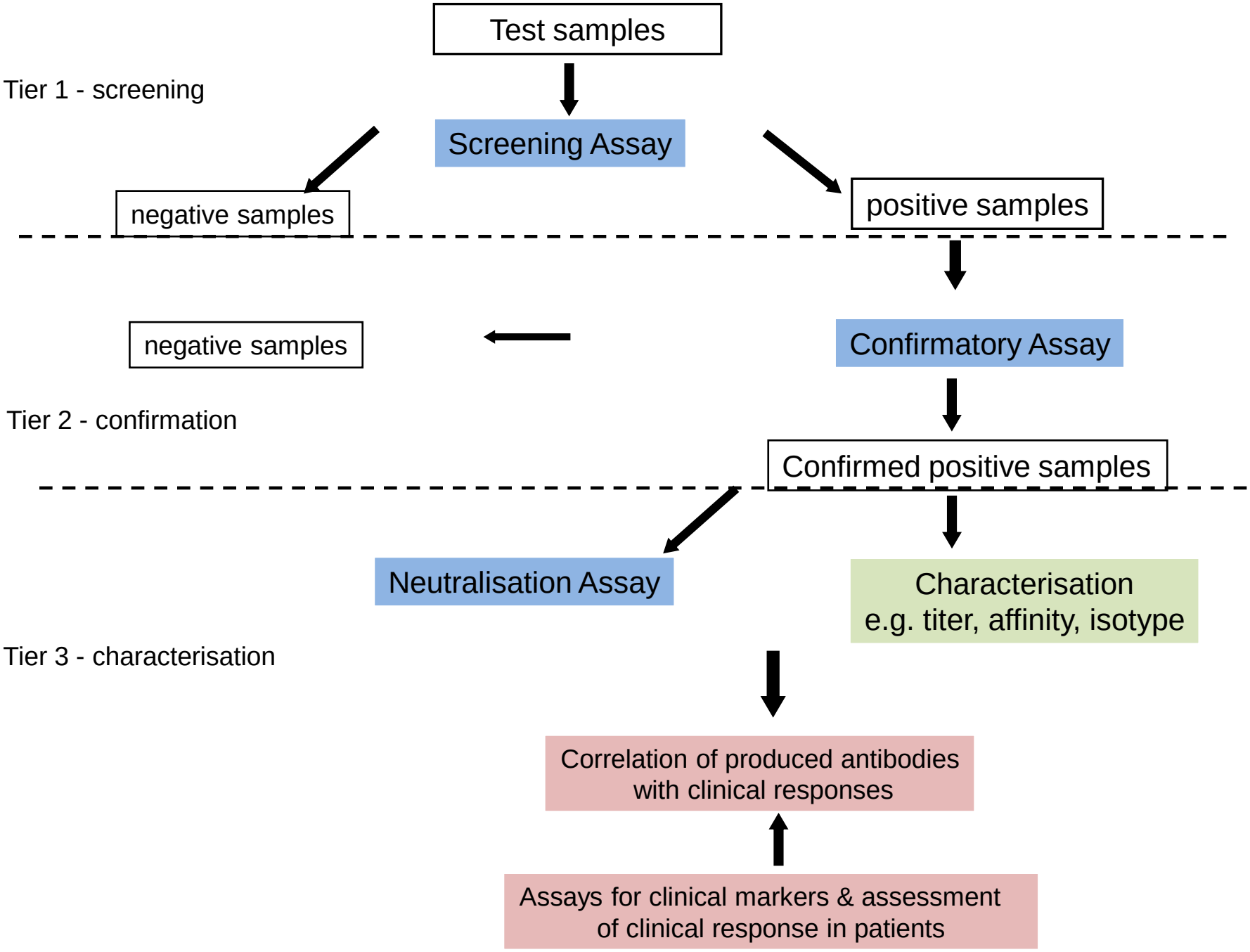
- ELISAs - direct, bridging, other formats
- Radioimmunoprecipitation assays (RIPA)
- Surface Plasmon Resonance (SPR)
- Other technologies e.g., ECL, DELFIA, Gyrolabs

**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


Test samples
Tier 1 - screening
Screening Assay
negative samples
positive samples
negative samples
Confirmatory Assay
Tier 2 - confirmation
Confirmed positive samples
Neutralisation Assay
Characterisation
e.g. titer, affinity, isotype
Tier 3 - characterisation
Correlation of produced antibodies
with clinical responses
Assays for clinical markers & assessment
of clinical response in patients

# Testing is challenging

- So far, there is 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, define sensitivity, tolerance. Hyperimmunised animal sera - affinity purified, mAbs, anti-idiotypic antibodies
  - Negative: for setting threshold/cut-off for 'discrimination'

    Pre-therapy sera, irrelevant antibody, healthy sera (single/pooled).

- Regulatory obligation to validate assays

Target : Measure Polyclonal response (antibodies of varying avidities, isotypes)

# Immunogenicity Assessment Strategy Design and Interpretation

- **Studies need to be carefully and prospectively designed to ensure all procedures are in place prior to initiation**
  - **Selection, assessment, characterization and validation of assays**
  - **Identification of appropriate sampling points, duration of testing**
  - **Sample volumes and sample processing/storage**
  - **Selection of statistical methods for analysis of data**
- **This applies to all assays**
- **Strategy needs to be established on a case-by-case basis – product, patients, expected clinical parameters**
  - **In chronic use – sequential sampling for a year**
  - **In view of variability of antibody responses, adequate numbers of patients needed**
- **However, unwanted immunogenicity may occur at a level, which is not detected in studies pre-approval so assessment post-approval, as part of pharmacovigilance surveillance is needed**

## Biosimilars as Biologicals

- As is clear from the EMA definition, Biosimilars are Biologicals. They differ from innovator Biologicals in the regulatory process used for their approval.
- As Biosimilars are ‘scientifically’ Biologicals they should be regarded as such when immunogenicity is being considered.
- There is no reason to treat approved Biosimilars any differently from all Biologicals (including innovator products) from the immunogenicity perspective.

# Biosimilars: Comparability Concept

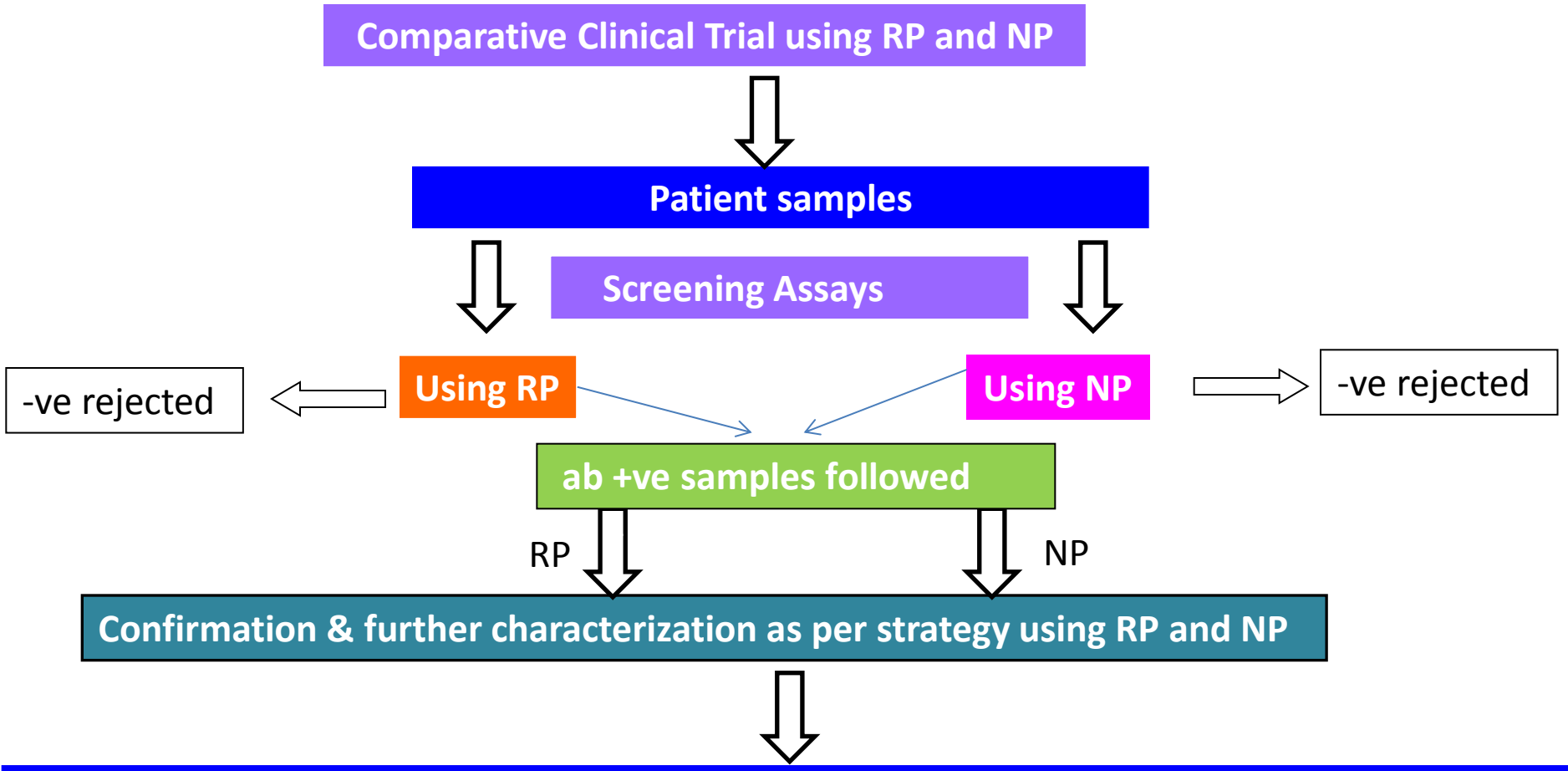
Comparability studies are needed to generate evidence substantiating the similar nature, in terms of quality, safety and efficacy, of the new similar biological medicinal product and the chosen reference medicinal product authorised in the Community.

# Comparative Immunogenicity

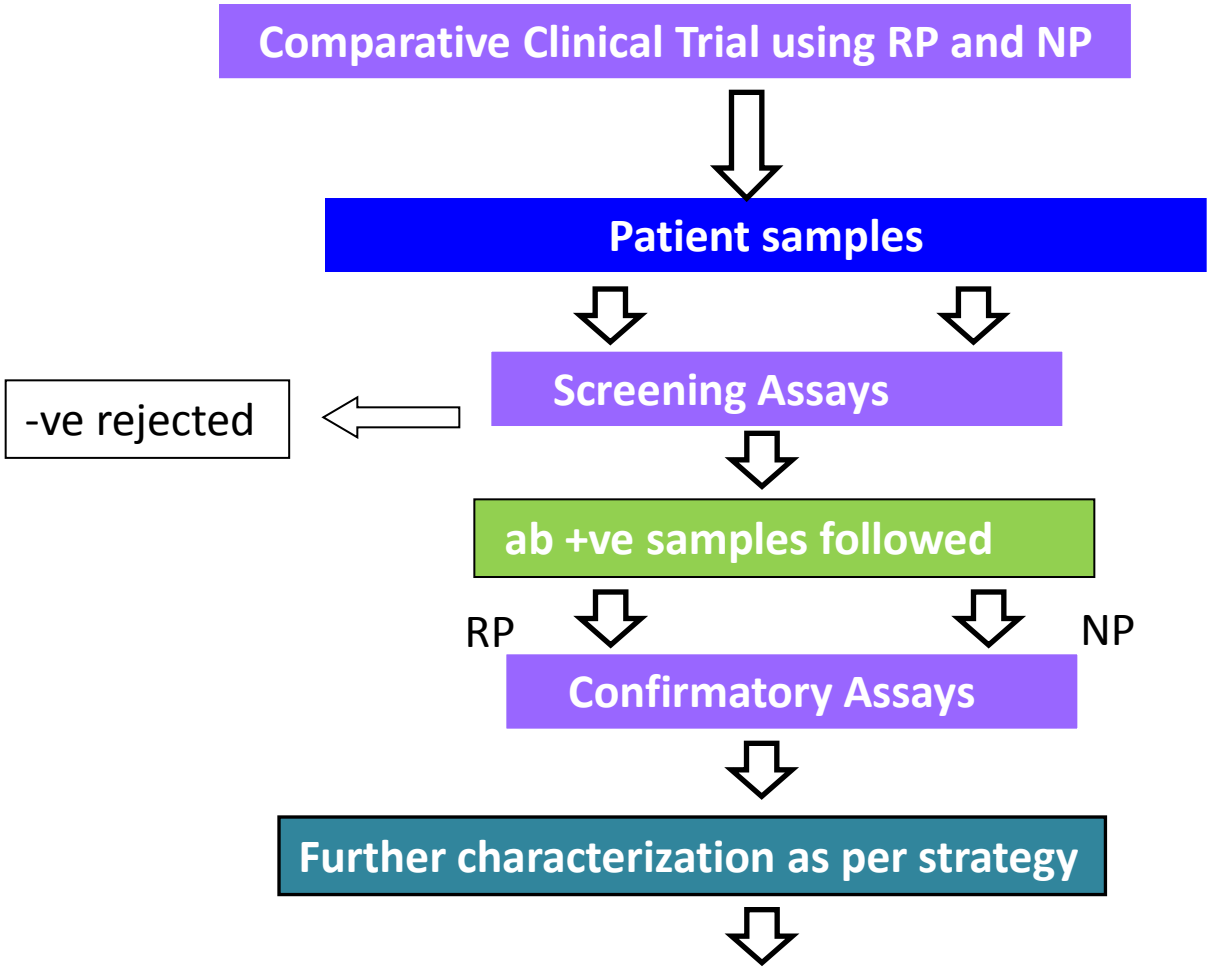
- Compares immunogenicity of different products ;

  Studies need to be designed to demonstrate whether the immunogenicity of the products is the same or significantly different.
- This is likely to affect the design of the studies & their interpretation.
- For this, a homogeneous and clinically relevant patient population should be selected. Head-to-Head studies needed. Same assays & sampling strategy should be used.
- The consequences of immunogenicity also must be compared.
- Post-approval assessment may be necessary, usually as part of pharmacovigilance surveillance.

# Relative Immunogenicity



# Relative Immunogenicity




If +ve ab controls for both RP and NP comparable in assay using NP as antigen & satisfy certain criteria, an assay using only NP is possible for both arms. However, if expression systems different, this may not be the right approach

**Provide information regarding immunogenicity of each product – titres etc, kinetics, cross-reactivity. Assess correlation of characterized antibodies with clinical responses to biologic therapeutic**

# Antibody Frequency for Biosimilar
## (presubmission studies)

| Biosimilar | Ab frequency | Reference | Ab frequency |
|---|---|---|---|
| Omnitrope (SC) | 0/51 (0.0%) | Genotropin | 1/44 (2.3%) |
| Valtropin (SC) | 3/98 (3.4%) | Humatrope | 1/49 (2.0%) |
| Binocrit (IV) | 2/314 (0.6%) | Erypo | 3/164 (1.8%) |
| Silapo (IV)<br>Silapo (SC) | 0/305 (0.0%)<br>0/323 (0.0%) | Erypo<br>Erypo | 0/304 (0.0%)<br>0/230 (0.0%) |
| Ratiograstim (SC) | 7/356 (2.0%) | Neupogen | 2/134 (1.5%) |
| Zarzio (IV / SC)<br>(Phase 1, crossover) | 0% | Neupogen | 0% |
| Nivestim | 3/183 (1.6%) | Neupogen | 0/95 (0.0%) |
| Bemfola | 0/249 (0%) | Gonal-f | 0/123 (0%) |
| Insulin Marvel § | T1DM: 25/114 (21.9%)<br>T2DM: 14/131 (10.7%) | Humulin | T1DM: 16/114 (14.0%)<br>T2DM: 17/136 (12.5%) |
| Remsima - AS<br>- RA | 37.5%<br>55.6% | Remicade | 36.1%<br>54.3% |

Data from EPARs at *www.ema.europa.eu*

§ Application withdrawn.

Table courtesy of Martina Weise

# Immunogenicity Studies: Biosimilars

The immunogenicity of the marketed product does not influence the need for comparative immunogenicity studies.

However, if the immunogenicity profiles of marketed and biosimilar products are significantly different, they can be considered DISSIMILAR

# Conclusions

- Immunogenicity issues occur all along the life cycle of a product and particularly when :
  - a new therapeutic protein is developed and used for various clinical indications
  - a change is introduced e.g. process, formulation, storage conditions etc
  - a biosimilar product is proposed
- Assessment requires:
  - an optimal antibody testing strategy
  - validated methodologies and reference standards

# Biosimilars: Unwanted Immunogenicity

Quote from EMA BMWP chairmen:

**‘Unwanted Immunogenicity is the biggest challenge for the approval of Biosimilars’**

# Acknowledgements

Meenu Wadhwa
Chris Bird
Isabelle Cludts

Colleagues of the BMWP