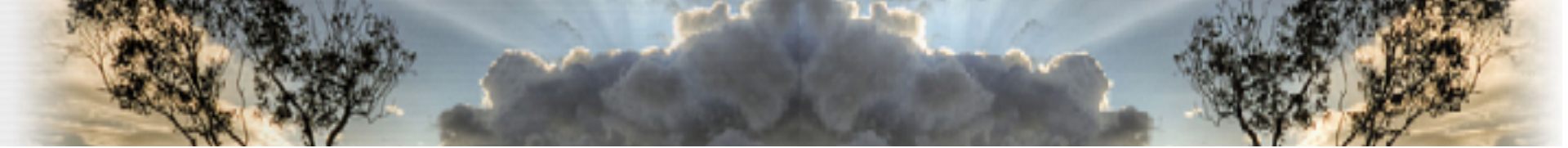


EBF Cyberconnect Events

Training Day: Managing the Practical Aspects of Immunogenicity

23-24 March 2021

Immunogenicity – an Introduction

Joanne Goodman, on behalf of the EBF

Content

- What is immunogenicity
- Impacts of immunogenicity
- Factors that influence immune responses
- Risk assessment
- Clinical assessment and correlation of data
- Assays
- Summary

Immunogenicity = immune response

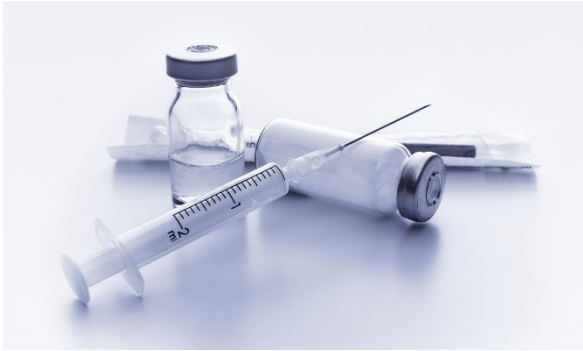
Ability of a foreign substance to provoke an immune response in humans or animals

WANTED
Immunogenicity

UNWANTED
Immunogenicity

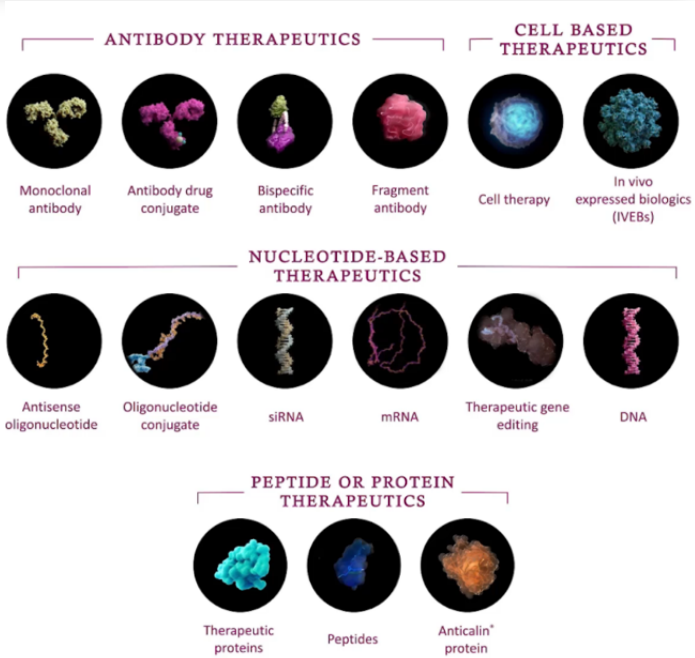
WANTED
Immunogenicity

- Typically related to vaccines
- Intentional immune response
- Injection of an antigen leads to an immune response against the pathogen
- Confers protection against future exposure or the effects of exposure



UNWANTED
Immunogenicity

- Associated with biotherapeutic drugs
- Some patients may mount an undesired immune response
  - Anti-drug antibody (ADA)
- Can impact the effect of the therapeutic
- May induce adverse effects
  - Hypersensitivity reactions

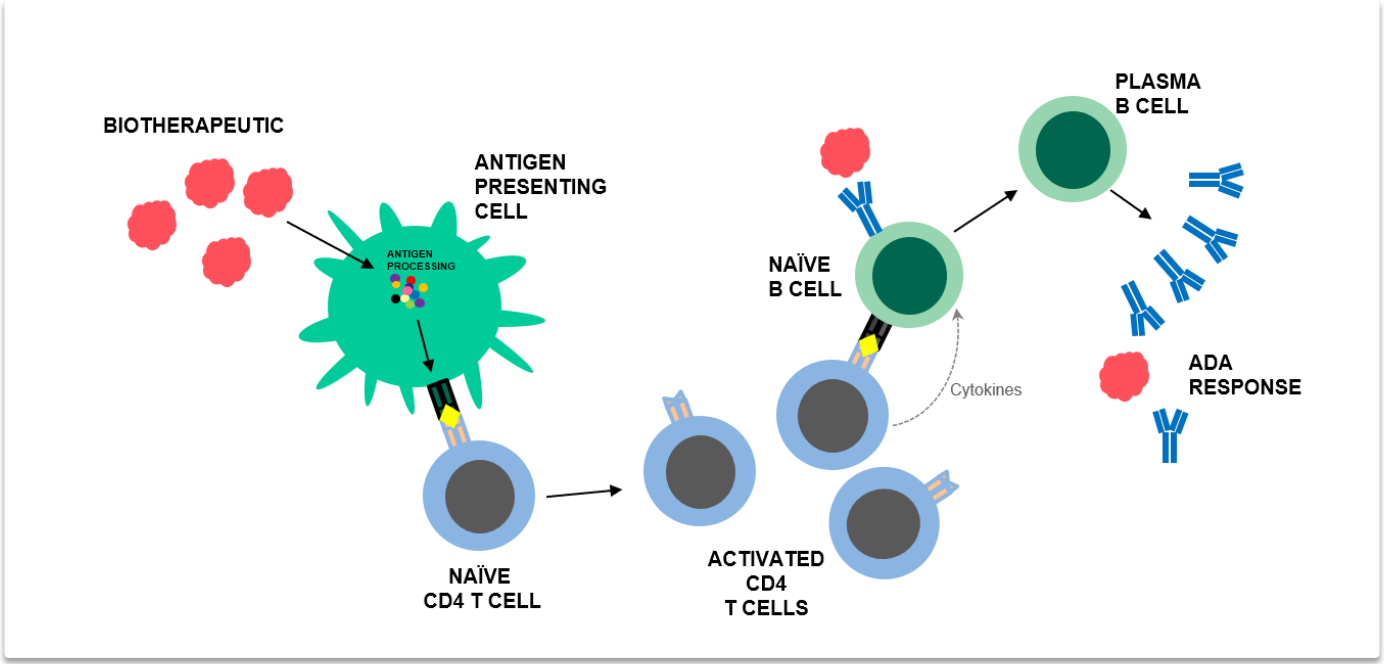


Differences between NCE and NBE drugs

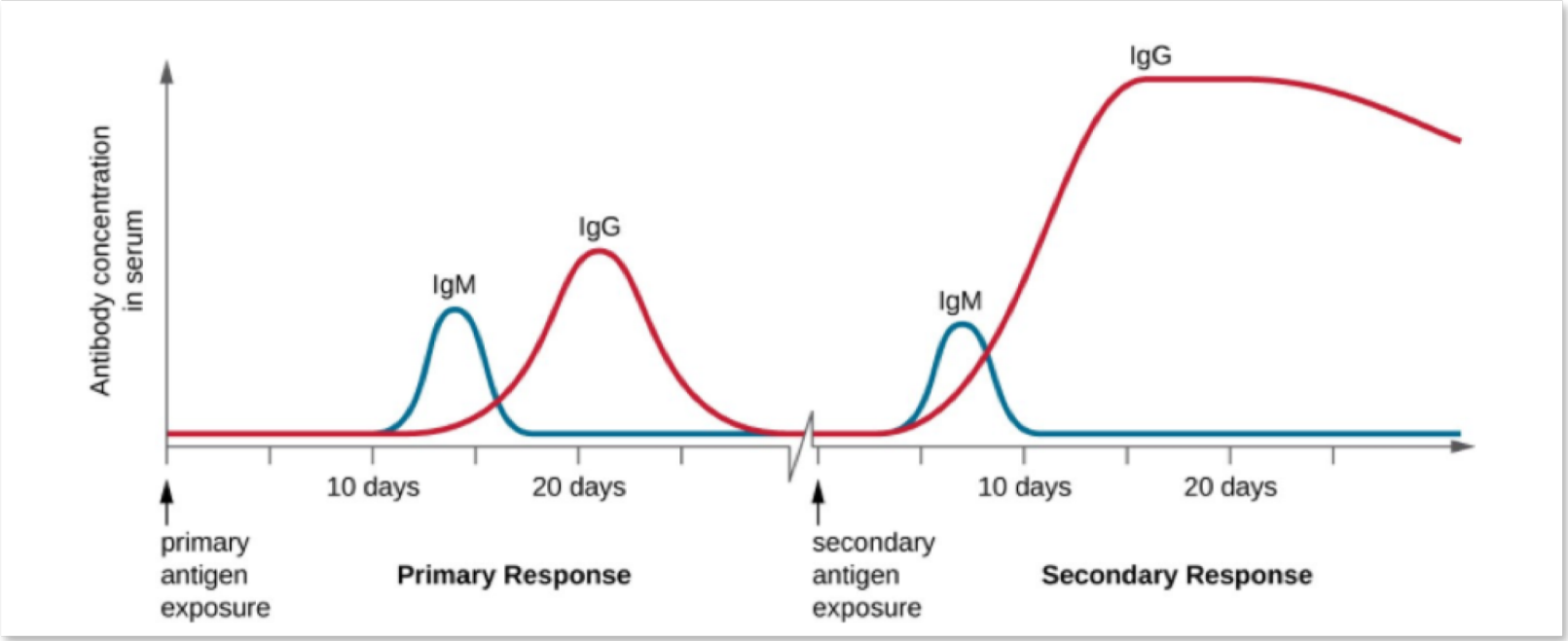
Small Molecules (NCE)	Large Molecules (NBE)
Chemically synthesised	Large, complex molecules or mixture of molecules
Combine specific chemical ingredients in an ordered process	Manufactured in a living system such as a microorganism or in plant or animal cells Often modified by glycosylation or PEGylation Host cell proteins
Short half-life (hours)	Many biologics act through FcRn receptor allowing recycling back to the cell surface – can mean long half-life (days/weeks)

Such characteristics increase the potential to induce an antibody-mediated immune response

Immune response



Immune responses on exposure to an antigen

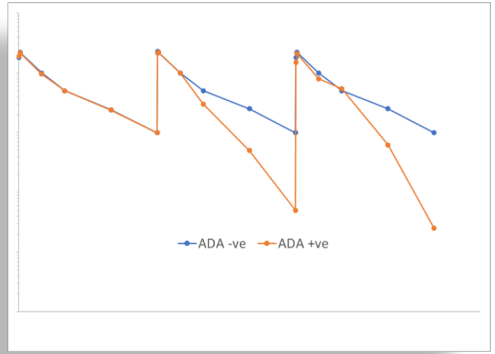


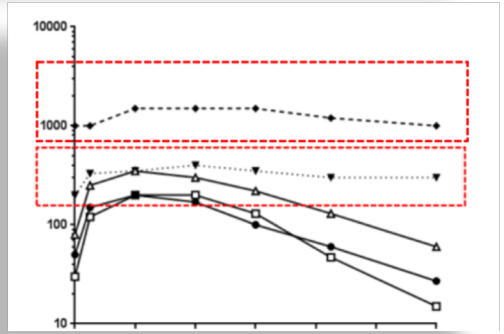
https://courses.lumenlearning.com/microbiology/chapter/b-lymphocytes-and-humoral-immunity/

Potential impact of unwanted immunogenicity of biotherapeutics

ALTERED DRUG EFFICACY

- **Clearing antibodies**: reduced PK, increased clearance, reduced exposure
- **Sustaining antibodies**: increased PK, reduced clearance, prolonged exposure, increased target distribution
- **Neutralising antibodies (nAb):** drug binds at or near target binding site, reduced Pk, reduced PD, possible toxicity





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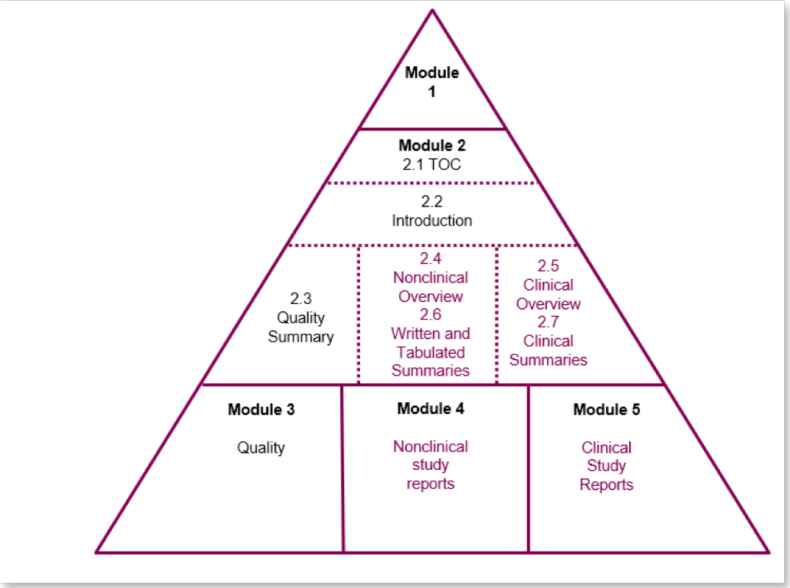
SAFETY CONCERNS

- Injection reactions
- Infusion reactions
- Anaphylaxis
- Cytokine release syndrome
- Hypersensitivity
- Immune complexes
- Vasculitis and tissue deposition
- Neutralisation of non-redundant endogenous protein leading to loss of physiological function

Immunogenicity assessment is a critical part of biotherapeutic drug submissions and registration



Common technical document

Immunogenicity

Formation of anti-adalimumab antibodies is associated with increased clearance and reduced efficacy of adalimumab. There is no apparent correlation between the presence of anti-adalimumab antibodies and the occurrence of adverse events.

In patients with polyarticular juvenile idiopathic arthritis who were 4 to 17 years, anti-adalimumab antibodies were identified in 15.8% (27/171) of patients treated with adalimumab. In patients not given concomitant methotrexate, the incidence was 25.6% (22/86) compared to 5.9% (5/85) when adalimumab was used as add-on to methotrexate. In patients with polyarticular juvenile idiopathic arthritis who were 2 to < 4 years old or aged 4 and above weighing <15 kg, anti-adalimumab antibodies were identified in 7% (1/15) of patients, and the one patient was receiving concomitant methotrexate.

In patients with enthesitis-related arthritis, anti-adalimumab antibodies were identified in 10.9% (5/46) of patients treated with adalimumab. In patients not given concomitant methotrexate, the incidence was 13.6% (3/22), compared to 8.3% (2/24) when adalimumab was used as add-on to methotrexate.

Patients in rheumatoid arthritis studies I, II and III were tested at multiple time points for anti-adalimumab antibodies during the 6 to 12 month period. In the pivotal trials, anti-adalimumab antibodies were identified in 5.5% (58/1053) of patients treated with adalimumab, compared to 0.5% (2/370) on placebo. In patients not given concomitant methotrexate, the incidence was 12.4%, compared to 0.6% when adalimumab was used as add-on to methotrexate.

In patients with paediatric psoriasis, anti-adalimumab antibodies were identified in 5/38 subjects (13%) treated with 0.8 mg/kg adalimumab monotherapy.

In adult patients with psoriasis, anti-adalimumab antibodies were identified in 77/920 subjects (8.4%) treated with adalimumab monotherapy.

In adult plaque psoriasis patients on long term adalimumab monotherapy who participated in a withdrawal and retreatment study, the rate of antibodies to adalimumab after retreatment (11 of 482 subjects, 2.3%) was similar to the rate observed prior to withdrawal (11 of 590 subjects, 1.9%).

In patients with moderately to severely active paediatric Crohn's disease, the rate of anti-adalimumab antibody development in patients receiving adalimumab was 3.3%.

In patients with Crohn's disease, anti-adalimumab antibodies were identified in 7/269 subjects (2.6%).

In adult patients with non-infectious uveitis, anti-adalimumab antibodies were identified in 4.8% (12/249) of patients treated with adalimumab.

Humira (adalimumab) EPAR

Immunogenicity may be multi-factorial

Product

- Structure (human/chimeric/non-human, modifications, T cell epitopes)
- Product quality (isoforms, impurities)
- Glycosylation, PEGylation, oxidation, deamination
- Formulation
- Mode of action, immunomodulatory properties

Patient

- Age
- Genetic background (HLA, MHC genotype)
- Immune status
- Disease state
- Prior sensitisation
- Pre-existing antibodies

Treatment

- Route of administration
- Dose and dose frequency
- Length of treatment
- Drug holidays, re-exposure
- Co-med therapy
- Storage

One of the most famous cases: erythropoietin (EPO)

- Recombinant human form of EPO
- EPO enhances red blood cell formation
- Original thought to be non-immunogenic
- Used successfully for chronic renal failure for 12 years as a replacement therapy
- Casadevall *et al.* NEJM (Feb 2002)
  - Sudden resistance to the drug
  - Pure red-cell aplasia (PRCA)
    - Causes a severe anaemia
    - Almost complete cessation of red blood cell production
  - EPO-induced PRCA caused by neutralising antibodies

Increases in EPO immunogenicity is likely multi-factorial

- Procrit: HSA formulation used within USA
- EPREX: HSA removed to comply with European regulations
  - Replaced with polysorbate 80 (Tween 80)
  - Most immunogenic formulation

- More cases seen when given by SC route compared to IV
- Patient population
  - More cases in CKD
  - None in cancer
- Multiple hypotheses
  - EPREX was a pre-filled syringe – possible leachates from the syringe plungers
  - Micelles forming repetitive epitopes
  - Handling and storage

Immunogenicity impacting launch or terminating a program

- **Bococizumab – untenable in a competitive landscape**
  - Ph2 trials AEs similar between ADA +ve and ADA –ve subjects
  - Ph3 trials
    - 48% incidence of ADA, 29% of patients developed neutralising antibodies
    - Higher rate of injection-site reactions than competitors
  - Drug development program terminated due to immunogenicity and variability in response to the study drug

- **Motavizumab – safety concerns**
  - Concerns over safety and allergic reactions
  - Not shown to be noninferior to parent drug palivizumab (Synagis)

- **Roctavian – delay to launch**
  - Formation of ADA required long-term safety and efficacy follow up (2 years)

Humira (adalimumab) still a best-selling biologic

- Fully human anti-TNFa monoclonal antibody
- Multiple indications with varying rates of immunogenicity
- After 52 weeks of treatment 225 (23/80) psoriatic arthritis patients had detectable ADA
- Humira concentrations were significantly lower at 28 and 52 weeks compared to patients without ADA
- Patients with detectable ADA had a poorer clinical outcome that ADA negative patients (disease activity score)
- Other therapies available which a patient could be switched to

Risk will depend on multiple factors

Risk of mounting an immune response due to ADA will vary with the product
Ideally evaluated early in drug development and documented

- **Clinical consequence**
  - Product
  - Patient population(s)
  - Risk-benefit
  - Pre-existing antibodies
- **Consequences for efficacy**
  - Neutralising antibodies
  - Cross-reactivity with endogenous counterparts (esp. non-redundant)
- **Consequences for safety**
  - Anaphylaxis
  - Cytokine Release Syndrome
  - Immune complexes
  - Cross-reactivity
- **Mitigation strategies**
  - Assays for ADA detection
  - Trial design and monitoring
  - Post-marketing strategies
  - Therapies for treatment

Risk = Severity x Probability (x Detectability)

SEVERITY	**LESS** Not endogenous Redundant activity Other therapies available End stage disease Reversible AE Non-replacement therapy	**MORE** Endogenous counterpart Unique activity Sole therapy Chronic disease Non-reversible AE Replacement therapy
PROBABILITY	**MORE** Autoimmune Chronic dosing Subcutaneous dosing route* Impure aggregates	**LESS** Immunosuppressed Single dose IV administration* Highly pure No aggregates

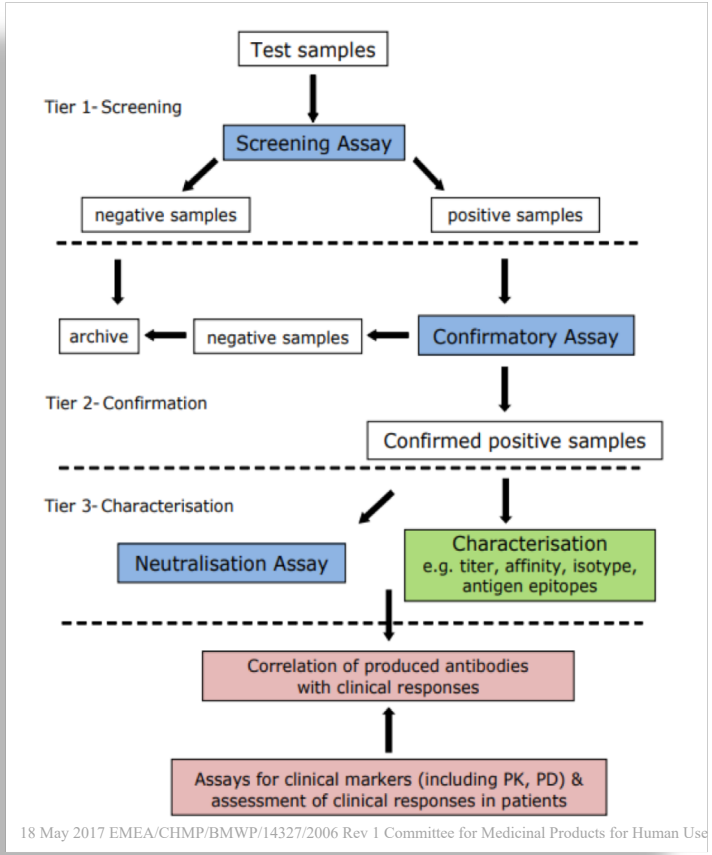
* Not always the case

Prediction methods exist but are not 100% accurate

- Immunogenicity is not translatable from animals to humans (ICH S6(R1))
  - Human or humanised drugs will be seen as foreign and cause an immune response

- **In silico tools**
  - Identification of T cell and B cell epitopes in a protein sequence
  - Databases for screening epitopes
  - May provide opportunity for candidate molecule selection or molecule engineering
- **In vitro screening of T cell epitopes**
  - MHC binding of a peptide is necessary for recognition by a T cell
  - MHC-peptide binding assays
- **In vitro prediction of antigen presentation**
  - Dendritic cells from healthy volunteers
  - Isolate and sequence peptides presented by HLA
- **In vitro prediction of T cell activation**
  - Dendritic cells and assess proliferation using fluorescent dye
- **In vitro cytokine release assays for likelihood of infusion reactions**

Clinical testing follows a tiered approach



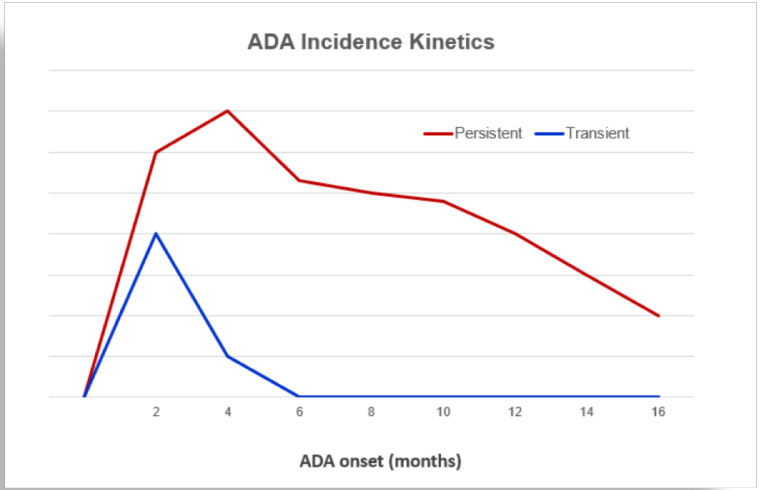
18 May 2017 EMEA/CHMP/BMWP/14327/2006 Rev 1 Committee for Medicinal Products for Human Use (CHMP)

Strategy modified based on risk and mode of action of the biotherapeutic

ADA responses following treatment

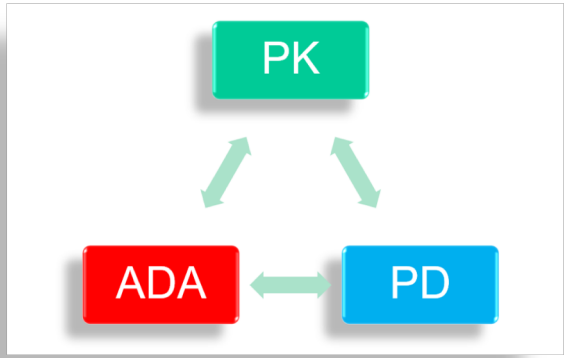
- ADA response by treatment or placebo
- Number and percentage of subjects ADA +ve
- Kinetics – time to onset, persistent or transient
- Magnitude of the response: titre
- Neutralisation (if tested)
- Treatment-induced ADA
- Treatment-boosted ADA
- Treatment-emergent
- Pre-existing
- Specificity for multi-domain molecules

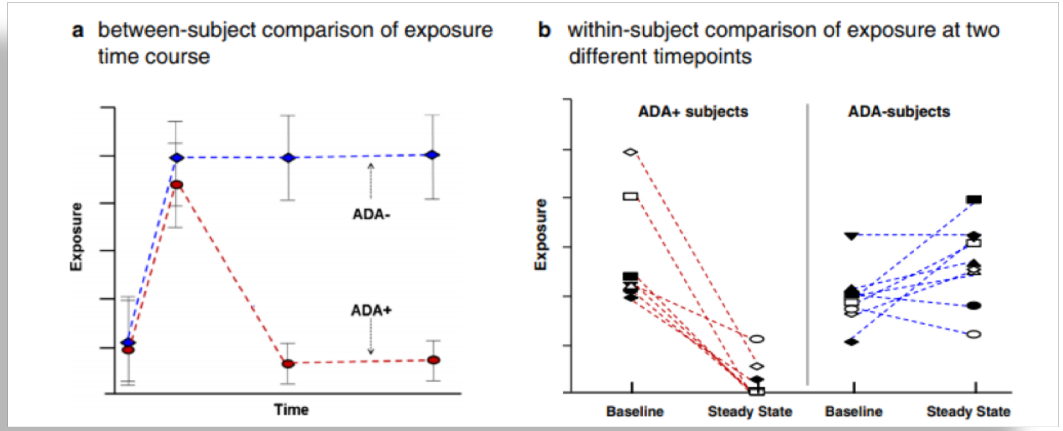
Shankar *et al. AAPS Journal*, Vol. 16, No. 4, July 2014 for further details



Correlation of the data

- Immunogenicity can impact exposure (PK) or pharmacodynamic markers (PD), safety and efficacy





Wang *et al. AAPS Journal*, Vol. 18, No. 2, March 2016

Immunogenicity assays are not PK assays

	LBA PK Assay	LBA* Immunogenicity Assay
Assay type	Quantitative	Qualitative or semi-quantitative Use titre for magnitude
Reference standard	Dosed molecule Well-characterised	Surrogate positive controls Negative control
Standard curve	Uses reference standard Used in sample testing Back calculated concentrations	Positive or negative based on a cut point Titration
Sensitivity	Lowest concentration with acceptable accuracy and precision	Varies on the positive control used Based on the cut point (equal to or just above)
Accuracy and Precision	Both determined	Only precision
Results	Mass units	Use of mass units discouraged Reporting either positive/negative or titre

*LC-MS or other technologies may also be used and have different assay considerations

Immunogenicity assays may take different formats and have a different context of use

Binding ADA assays

- Direct format
- Bridging format
- Multiple technologies
- Screening, confirmatory and titre

Neutralising ADA assays

- Cell-based
- Competitive ligand binding
- Will depend on mode of action of the drug

Seeing more complexity

Further characterisation – specificity, isotyping
New modalities – C&GT products, oligos, mRNA, CRISPR etc.
Vector, lipid nanoparticles, transgene, gene product, cellular responses etc.
Use of other technologies such as flow and ELISPOT

Summary

- Immunogenicity can cause a **wanted (vaccine) or unwanted immune response (biotherapeutics)**
- Immunogenicity assessment is **required for market approval**
- **Reported in the product label**
- **Many factors can affect immunogenicity** such as molecule attributes, formulation, patient factors and treatment regimen
- **Can result in alterations in drug exposure and efficacy** and may result in serious **clinical consequences**
- Immunogenicity should follow a **risk based approach**
- Clinical immunogenicity assessment should be an **integrated approach evaluating PK PD, efficacy and safety**
- **Increasing complexity with new modality drugs**

Acknowledgements

- Training Day team
- EBF SC

Contact Information

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European Bioanalysis Forum vzw

www.e-b-f.eu