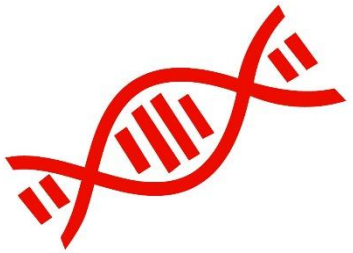


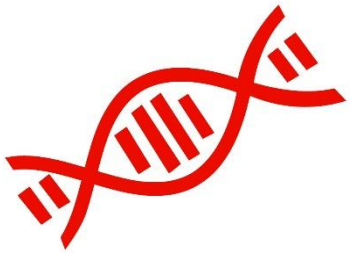
Meenu Wadhwa, PhD, UK

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

GaBI
Educational
Workshops

24 September 2019, Ankara HiltonSA, Turkey

2nd Turkish Interactive Workshop on
REGULATORY ASSESSMENT OF BIOSIMILARS



Immunogenicity assessment of biotherapeutics: the EU perspective

Meenu Wadhwa, PhD
24 September 2019

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

PPCI
Pro Pharma
Communications
International

NIBSC

Immunogenicity assessment of biotherapeutics: The EU Perspective

Meenu Wadhwa

Medicines & Healthcare products Regulatory Agency

Disclaimer

The views and opinions expressed in this presentation are entirely my own and should not be misconstrued as those representing any regulatory authority

Unwanted Immunogenicity

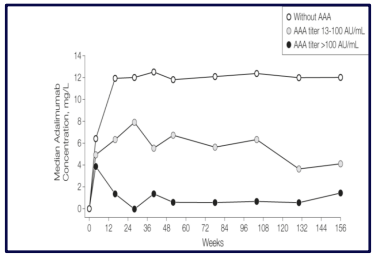
Product

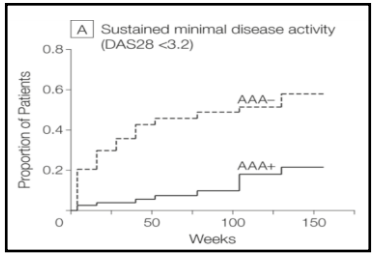
Efficacy

Antibodies

Safety

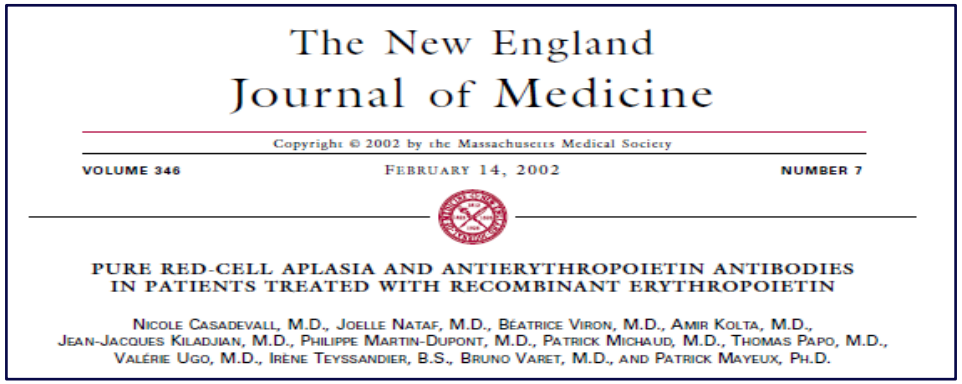
Infusion reactions, anaphylactic, hypersensitivity reactions, adverse effects





Adalimumab in RA patients

Bartelds et al:JAMA.2011;305(14):1460-68


The New England Journal of Medicine

Copyright © 2002 by the Massachusetts Medical Society

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 (not biosimilars)


original article

Biosimilar recombinant human erythropoietin induces the production of neutralizing antibodies

Clinical Impact

Efficacy – impaired clinical response

Safety – Infusion reactions, hypersensitivity reactions, serum sickness

– Cross-reactivity with an endogenous counterpart

Neurology. 2007 Oct 2;69(14):1391-403. Epub 2007 Aug 29.

The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL.

Calabresi PA[1], Giovannoni G, Confavreux C, Galetta SL, Havrdova E, Hutchinson M, Kappos L, Miller DH, O'Connor PW, Phillips JT, Polman CH, Radue EW, Rudick RA, Stuart WH, Lublin FD, Wajgt A, Weinstock-Guttman B, Wynn DR, Lynn F, Panzara MA; AFFIRM and SENTINEL Investigators.

CONCLUSIONS: The incidence of persistent antibody positivity associated with natalizumab is 6%. Reduced clinical efficacy is apparent in persistently positive patients. Patients with a suboptimal clinical response or persistent infusion-related adverse events should be considered for antibody testing.

Mult Scler. 2013 Apr;19(5):593-600. doi: 10.1177/1352458512460604. Epub 2012 Sep 19.

Clinical relevance of serum natalizumab concentration and anti-natalizumab antibodies in multiple sclerosis.

Vennegoor A[1], Rispens T, Strijbis EM, Seewann A, Uitdehaag BM, Balk LJ, Barkhof F, Polman CH, Wolbink G, Killestein J.

RESULTS: Antibodies were detected in 58% of the natalizumab-treated patients. All patients developed their antibodies before week 24. The large majority of these patients reverted to neutralizing antibody (NAb) negative status during follow-up. The presence of antibodies was inversely correlated with serum natalizumab concentration ($p<0.001$). Only high antibody titers are associated with very low or undetectable serum natalizumab concentration. Both high antibody titers and low serum natalizumab concentrations are associated with relapses and gadolinium-enhancing lesions on MRI.

Levels of Drug and Antidrug Antibodies Are Associated With Outcome of Interventions After Loss of Response to Infliximab or Adalimumab

Henit Yanai, Lev Lichtenstein, Amit Assa, Yoav Mazor, Batia Weiss, Arie Levine, Yulia Ron, Uri Kopylov, Yoram Bujanover, Yoram Rosenbach, Bella Ungar, Rami Eliakim, Yehuda Chowers, Raanan Shamir, Gerald Fraser, Iris Dotan and Shomron Ben-Horin

Clinical Gastroenterology and Hepatology, 2015-03-01, Volume 13, Issue 3, Pages 522-530.e2, Copyright © 2015 AGA Institute

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 J, Engdahl E, Lundkvist M, Brännström I, 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."

Often the 'real impact' of ADA only becomes clear in a post-approval setting

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 on immunogenicity of therapeutic proteins

FDA

- **2014 –** Guidance for Industry. Immunogenicity Assessment for Therapeutic Protein Products

- **2019** – Guideline on Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection.

EMA

- **2017 –** Guideline on Immunogenicity assessment of therapeutic proteins → (EMEA/CHMP/BMWP/14327/2006 Rev 1)

- **2012** – Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use (EMA/CHMP/BMWP/86289/2010)

Biosimilars guidance from both agencies

Harmonised Approach to Immunogenicity Testing

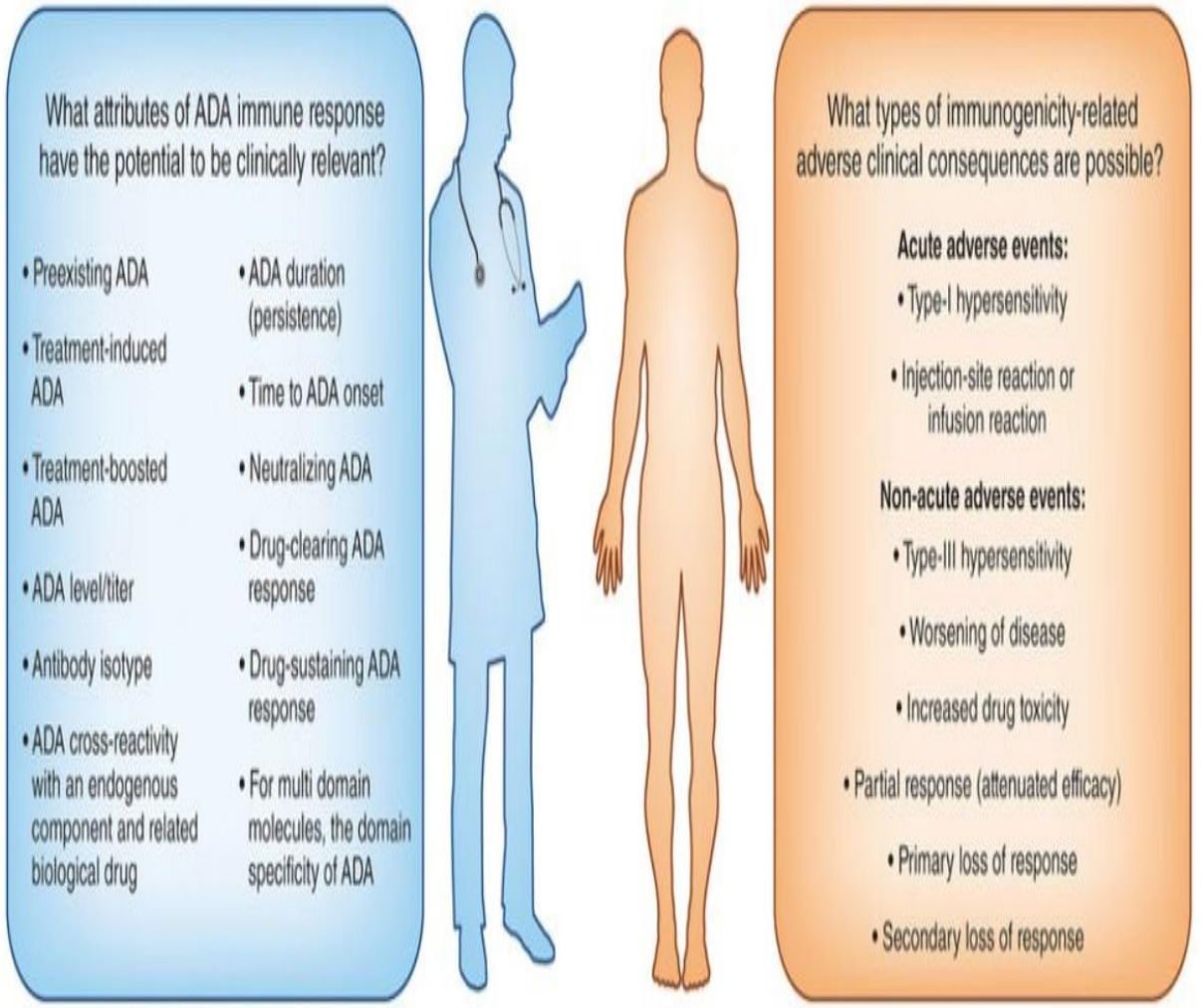
EMA

- General and pragmatic, adopting ‘industry practice’ where possible
- No specific guidance but ‘Guideline on bioanalytical method validation’ EMEA/ CHMP/EWP/192217/2009 Rev. 1 Corr. 2**

FDA

- Prescriptive but useful, aligned with industry where possible
- Specific guidance on ‘Assay development and validation’

Concepts and principles generally well-aligned and harmonized where possible
Deliver meaningful and clinically relevant immunogenicity results for patient safety and informed prescribing



Nat Biotechnol. 2015 Apr;33(4):334-6. doi: 10.1038/nbt.3181.
The quintessence of immunogenicity reporting for biotherapeutics.
Shankar G[1], Arkin S[2], Devanarayan V[3], Kromminga A[4], Quarmby V[5], Richards S[6], Subramanyam M[7], Swanson S[8]

EMA Immunogenicity Guideline (2017)

HOW?

‘Developing an integrated analysis strategy relevant for the intended treatment plan is critical for elucidating the clinical relevance of immunogenicity data’

Comprehensive Assessment

Risk-Based Approach: Analysis of risk factors

Testing: Well-Designed Studies, Sampling Strategy, Assays

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

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

- EGFR mAb – 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
- Cases clustered in different US states; IgE antibodies potentially due to tick bites etc

Product with same antigen as natural immunogen

Planning of Studies

- **Sampling strategy for ADA – frequency, timing and analysis dependent on risk assessment**
- Schedule adapted individually for each product and based on clinical trial design
  - Consider the PK of the product and assay capability (drug tolerance)
  - Characterise ADA - kinetics of induction, magnitude, transient/persistent antibodies
- Include **baseline and end of treatment sampling** (to allow conclusions e.g., persistent immune response or an immune response that was suppressed by the therapeutic)
- **ON-Drug**:
  - Early – 7 days, 3-4 weeks, monthly
  - Prior to re-randomisation
  - End of treatment
- **OFF-Drug – End of study**
  - Sufficient interval from last dose to accommodate drug tolerance of assays

- At early developmental 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

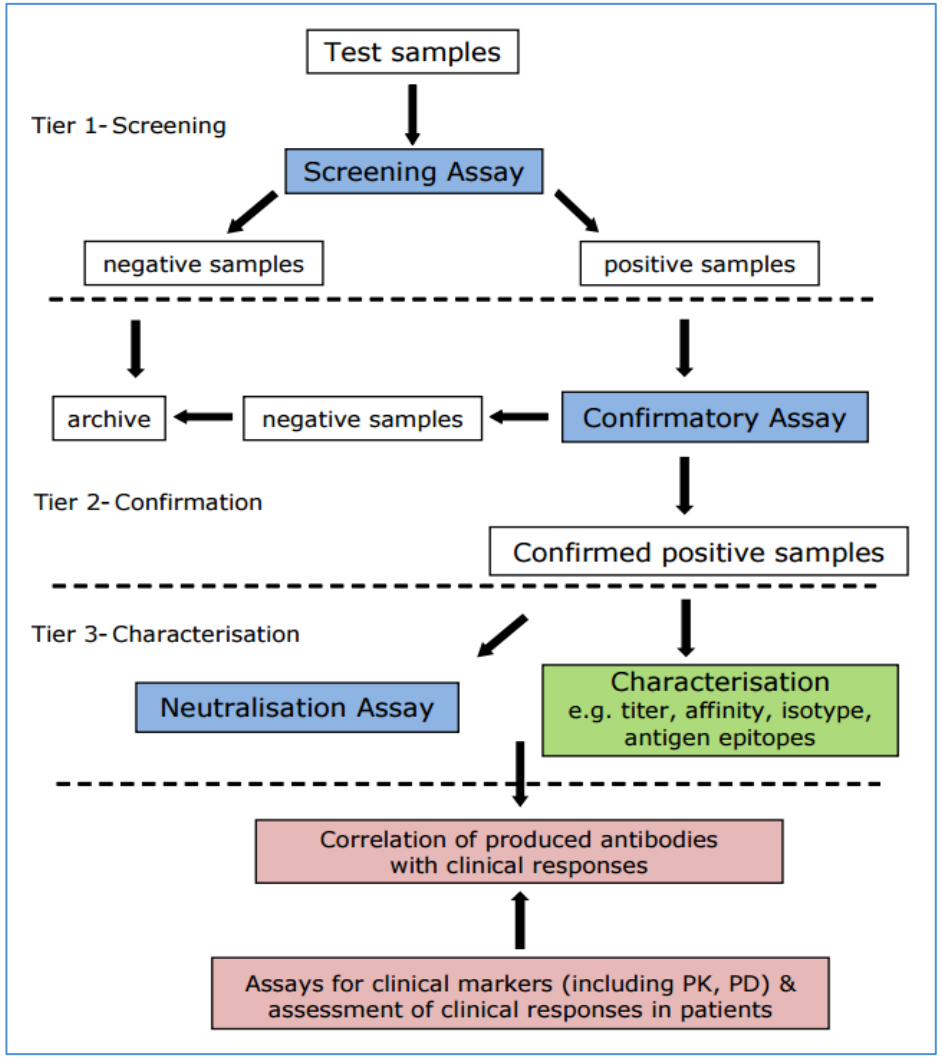
EMA Immunogenicity Guideline (2017)

Integrated planning, analysis and assessment

- Multi-tiered approach
- Assays, positive controls
- Data on immunogenicity and analysis

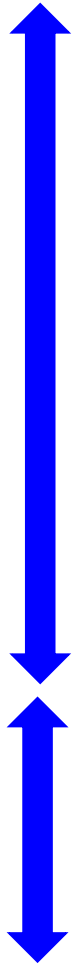
  ADA profile (incidence, titres, neutralisation, onset, persistence)
- Data on PK, PD
- Integrated analysis of clinical impact
- Conclusion on the risk of immunogenicity – indication-specific, risk managing and mitigating measures, pharmacovigilance etc

Integrated Summary of immunogenicity



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



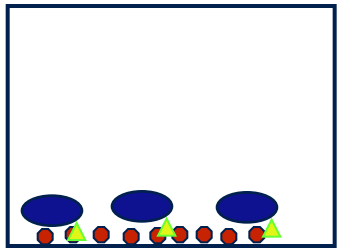
Screening

Neutralization

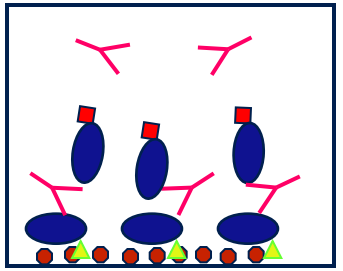
Bridging ELISA Formats

- Popular – ease of use, throughput
- Dual arm binding
- No requirement of secondary antibody

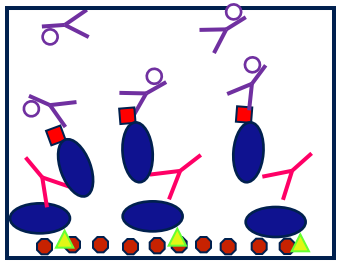
- Requires labelled therapeutic - Labelling may alter epitopes.
- May fail to detect rapidly dissociating antibodies.
- Affected by therapeutic/target interference, matrix components e.g. rheumatoid factors
- Lacks sensitivity toward IgG4



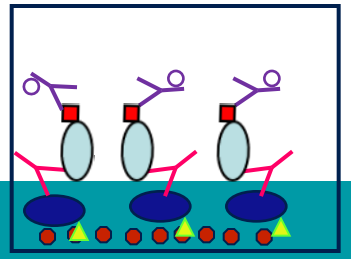
Streptavidin (●) plates
coat biotinylated antigen



add sample / control Ab
& DIG - antigen



add anti-DIG Ab
AP conjugate



Add substrate
& measure OD

Bridging Assay - Electrochemiluminescence (ECL)

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

Transfer to SA-coated plates

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

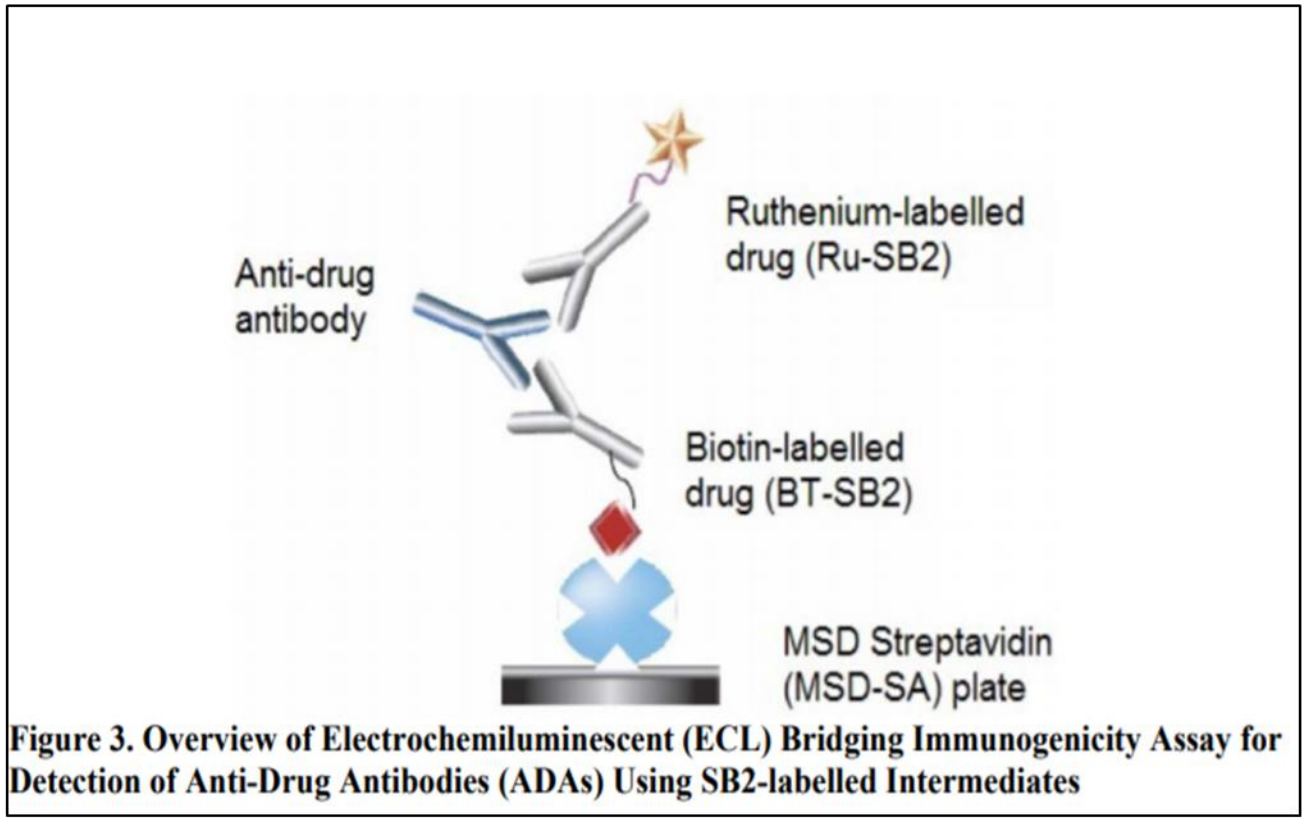


Figure 3. Overview of Electrochemiluminescent (ECL) Bridging Immunogenicity Assay for Detection of Anti-Drug Antibodies (ADAs) Using SB2-labelled Intermediates

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

Challenging to detect rapidly dissociating abs, IgG4

https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/UCM557890.pdf

Screening Assays

- Several platforms for detection of antigen-antibody binding
  - Relative merits and weaknesses need to be considered
- Selected assay is sensitive, specific and not confounded by:
- Matrix effects – any interfering factors - false positive/negative
  - Soluble target, disease specific e.g., rheumatoid factors, others
- Residual therapeutic/immune complexes – false negative
  - mAbs persist or given chronically at high doses so high levels of drug and/or immune complexes expected. 'Drug tolerant' assay
- If corrective measures required - must be validated for effectiveness & adopted on a case-by-case basis based on their suitability and need

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

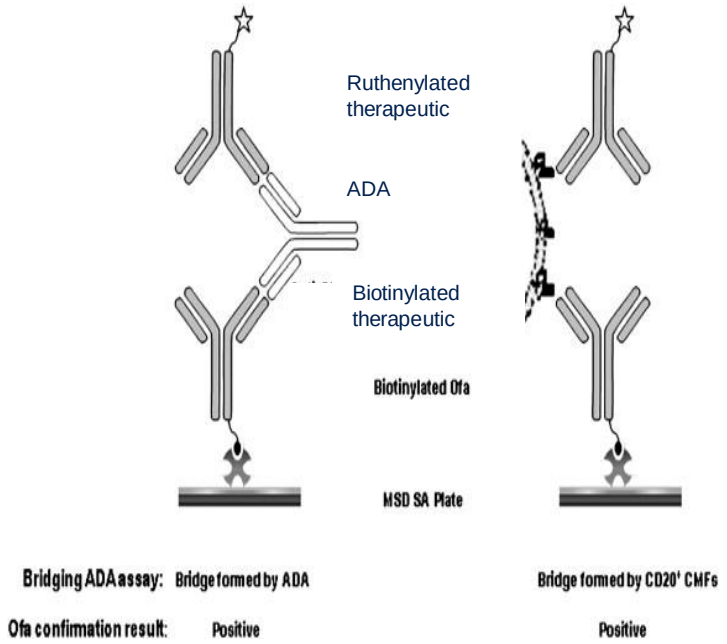
Mitigation: Deplete target - dissociate & affinity capture with Ab
Block drug target interaction - sol receptor , another Ab

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

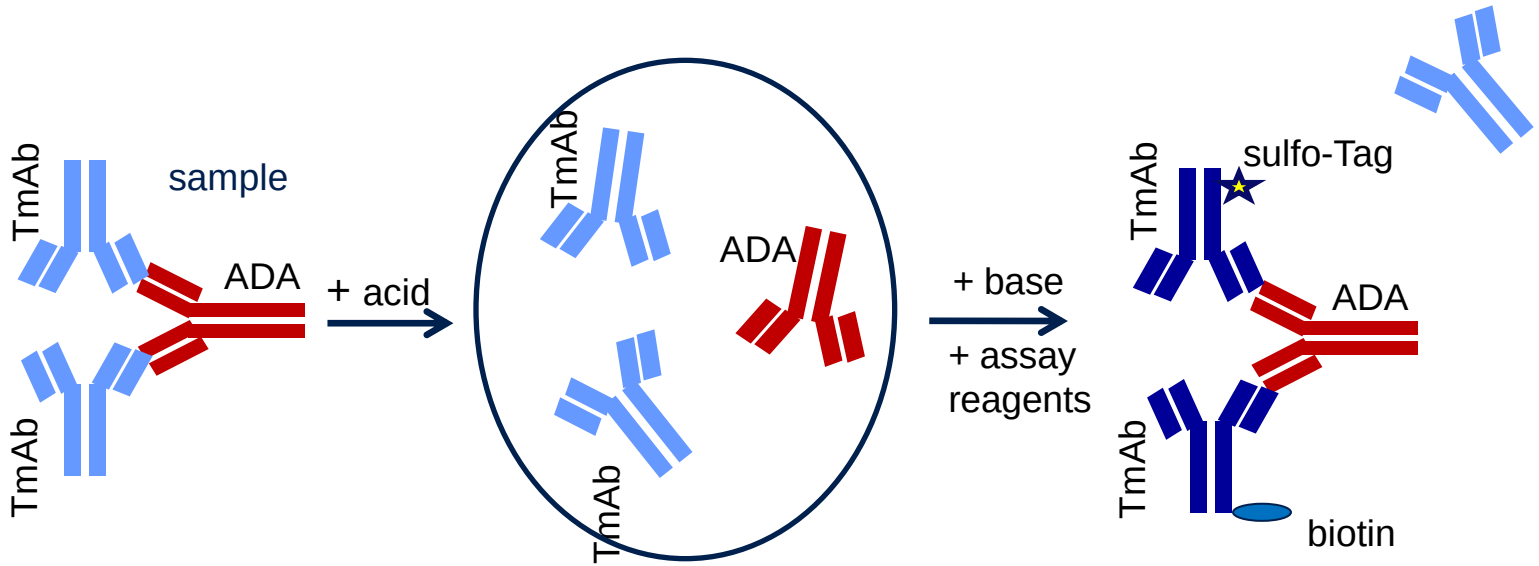


Adapted from Chen K. et *al*, 2013,JIM 394:22-31

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):



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

EMA Immunogenicity Guideline (2017)

Drug Tolerance

The Applicant has to demonstrate that the drug tolerance of the assay exceeds the levels of the therapeutic protein in the samples for ADA testing. Due to technical limitations it may not be always possible to develop fully tolerant assays. If this occurs, the best possible assay should be employed and the approach taken should be properly justified.

Neutralizing Antibody Assays

- Neutralizing capacity of positives needs to be evaluated …..since this often correlates with diminished efficacy. Deviation possible if strong justification for a waiver e.g., experience (GH, Insulin)

- For most products, 2 assay types largely dictated by mechanism of action

Cell-based bioassay	OR	Competitive ligand binding assay (CLBA)
Examples - IFN-beta, Rituximab		Example - Etanercept

- Cell-based : Better insight on functional effects, favored by regulators
  Complex assay design, Validation can be difficult
- CLBA : Rapid, Simple assay design, no cells, relatively easy to use

Neutralizing Antibody Assays

- Experience from TNF mAbs

- Evidence from public domain (benefit to biosimilars)

The immunogenic part of infliximab is the F(ab′)2, but measuring antibodies to the intact infliximab molecule is more clinically useful

Shomron Ben-Horin, Miri Yavzori, Lior Katz, Uri Kopylov, Orit Picard, Ella Fudim, Daniel Coscas, Simon Bar-Meir, Itamar Goldstein, Yehuda Chowers

Ann Rheum Dis. 2013 Jan;72(1):104-9. doi: 10.1136/annrheumdis-2012-201445. Epub 2012 Jul 3.

Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation.

van Schouwenburg PA[1], van de Stadt LA, de Jong RN, van Buren EE, Kruithof S, de Groot E, Hart M, van Ham SM, Rispens T, Aarden L, Wolbink GJ, Wouters D.

Ann Rheum Dis. 2015 Jan;74(1):311-4. doi: 10.1136/annrheumdis-2014-206237. Epub 2014 Oct 23.

The antibody response against human and chimeric anti-TNF therapeutic antibodies primarily targets the TNF binding region.

van Schie KA[1], Hart MH[1], de Groot ER[1], Kruithof S[1], Aarden LA[1], Wolbink GJ[2], Rispens T[1].

Assay choice: Cell-based - product MOA

Cell assays difficult, highly susceptible to therapeutic

If sufficient sensitivity, precision, robustness not achieved

Engage with regulators; Strong justification and data (transparency) – alternative approach may be acceptable

Testing is challenging

- No perfect screening assay.
- 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’.

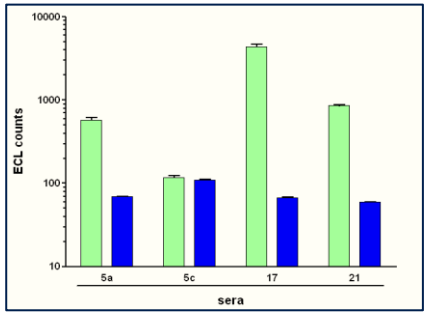
    Healthy sera, diseased /baseline sera, irrelevant antibody
- Clear criteria for discriminating +ves from –ves
- Regulatory obligation to validate assays

Target : Measure Polyclonal response

Immunogenicity Assays: Reporting Data

- **Screening cut point (SCP)** - assay threshold at/above which samples are defined as +ve
  - Detect all (potentially) clinically relevant ADA
  - Define statistically using healthy (or diseased) controls (~5% false-positives), check suitability of SCP with pre-dose clinical samples; justify outliers, how pre-existing antibodies handled, show derivation of SCP
- **Confirmatory cut-point (CCP)** - level of signal inhibition at/above which a sample is judged to have specific antibody
  - Derived by testing drug-naïve samples with and without therapeutic

    (for eliminating false positive samples post-screen)



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

- **Titre determination** - maximal dilution giving a signal above SCP
  - Should be informative as it can be linked to ADA of clinical impact. Need to be explicit as to how this is defined and calculated.

Immunogenicity Results

- Explicit indication of samples tested, positive/negative/equivocal at timepoints
- Screen positive ADA samples and subjects by treatment group
  - Baseline, ON-Drug, End of study & OFF-drug samples: treatment emergent, boosted & total
- Same applies for confirmed positive ADA samples
- Sequential Data for transient/persistent ADA/post-treatment
- Assay titres over time

- Presentation of data with summary Tables and Figures

- For PK/PD impact stratified groups based on ADA
  - Visualise individual profiles in subjects with and without ADA when there is a high incidence of ADA formation
  - **A subgroup analysis** of ADA negative and ADA positive subjects comparing PK parameters between treatment groups if feasible

- Impact on efficacy/safety etc?

Example: Benralizumab (Fasenra)

- Humanised, afucosylated mAb - IL-5Rα subunit on basophils, eosinophils and induces their apoptosis in the presence of NK cells via enhanced ADCC
- Indication - add-on maintenance therapy for severe asthmatic adults (eosinophilic phenotype)
- Phase III – 2 dosing frequencies; 30 mg sc every 4 weeks vs every 4 weeks for the 1st three doses, then every 8 weeks thereafter
  - 3-tiered testing – screening, confirmatory and titre, NAb assay
  - ADA +ve - Baseline 2%, post-treatment 7-14% study based (boost & new btw 8-16 wks); median titres peaked ~400; very high titres >25,600 in 0.5% patients
  - 68-80% were NAbs and persistent; high median titres (ADA and nAb titres)
  - ADA incidence slightly higher and increased NAbs with low freq vs 4-week regimen
  - ADAs impacted trough levels ↓ and eosinophils ↑ to pre-treatment levels (rare)
  - No clear effect of ADAs on efficacy/safety incl hypersensitivity reactions.

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/004433/WC500245333.pdf

Example: Benralizumab (Fasenra)

- Further data on the long-term impact of persistent neutralising ADAs will be provided from the extension trials (2 studies) as part of phamacovigilance & RMP - Q4 2018 & Q4 2019

- SmPC ➡

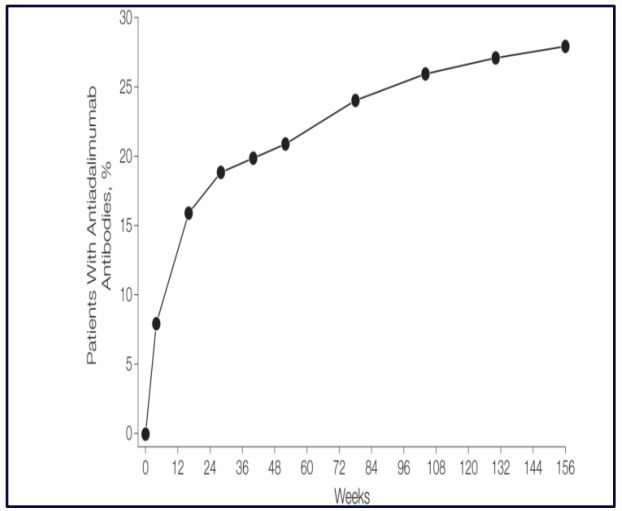
Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Loss of/reduction in long-term efficacy due to persistent neutralising anti-drug antibodies	SmPC section 5.1 (Pharmacodynamic properties) states: Immunogenicity Overall, treatment -emergent anti- drug antibody response developed in 107 out of 809 (13%) patients treated with Fasenra at the recommended dosing regimen during the 48 to 56 week treatment period of the exacerbation trials. Most antibodies were neutralising and persistent. Anti -benralizumab antibodies were associated with increased clearance of benralizumab and increased blood eosinophil levels in patients with high anti-drug antibody titres compared to antibody negative patients; in rare cases, blood eosinophil levels returned to baseline levels. Based on current patient follow-up, no evidence of an association of anti- drug antibodies with efficacy or safety was observed	None

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Public_assessment_report/human/004433/WC500245333.pdf

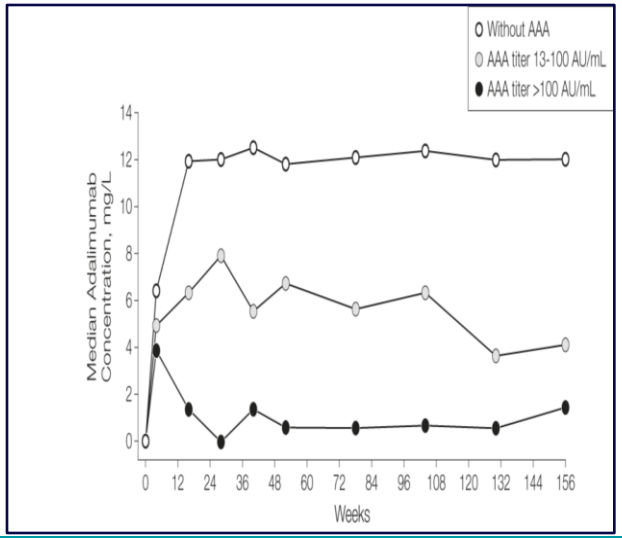
Antibodies and clinical impact

RA patients treated with Adalimumab over 3 years

A



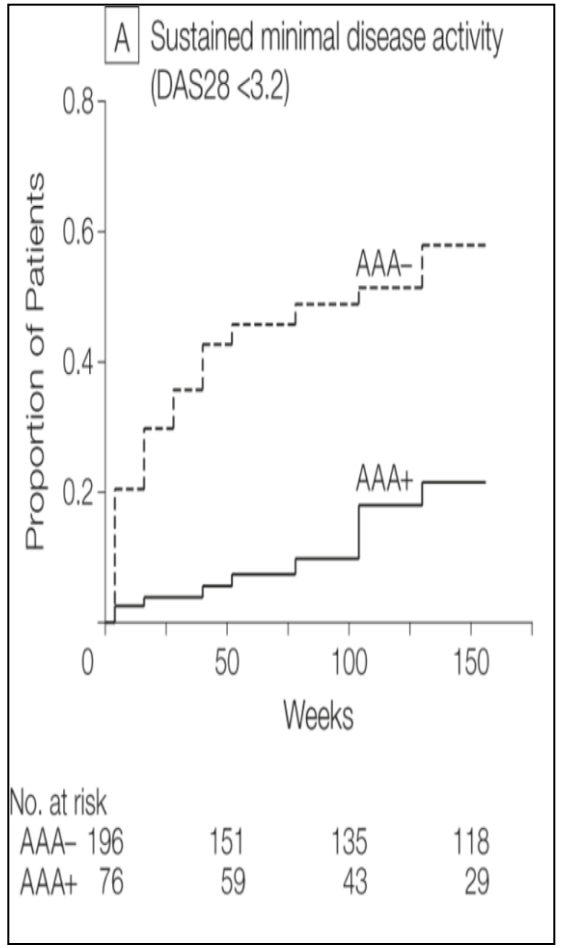
B



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

- Head-to-Head studies
  - Sensitive, homogeneous and clinically relevant patient population (ideally naïve). Extrapolation perspective
  - Suitable design, size – allows conclusion on ADA and clinical impact
  - Same assay platform, sampling points (baseline, sequential, post-termination) based on product PK, sampling when therapeutic levels low (wash-out period)

- Sampling for ADA (& for drug) in pivotal PK, PD, safety & efficacy studies
- Study duration – product based; in chronic treatment (1 year normally)
- Consider risk (previous experience, any potentially immunogenic structures, patient population)

Comparative Immunogenicity : Biosimilars

State-of-art assays

- **Options:**
  - A. 2 assays using administered therapeutic product (true immunogenicity) with similar sensitivity & specificity and no bias in recognition
  - B. Single assay using ‘biosimilar’ for both arms (relative). Should detect antibodies to all epitopes of the biosimilar followed by a confirmatory step using both products *

  **Minimises Variability: risk of under-estimating RMP immunogenicity (acceptable)*

Expectation

Antigenic equivalence shown and assay suitable (antibody control/s)
Clinical sample data showing concordance (excess drug – equivalence)

Comparative Immunogenicity

Approved biosimilars of Humira

Approach for ADA assay

Biosimilar	Manufacturer	Screening Assay	Neutralizing Antibody Assay
Imraldi	Samsung Bioepis	ECL (1-assay)	Competitive ligand binding
Cyltezo[1]	Boehringer Ingelheim	ECL (1-assay)	Cell-based[2]
Amgevita/Solymbic	Amgen	ECL (1-assay)	Cell-based[3] and ligand-binding
Hyrimoz	Sandoz	ECL (1-assay)	Competitive ligand binding
Hulio	Mylan	ECL (1-assay)	Competitive ligand binding[3]
Idacio	Fresenius Kabi Deutschland GmbH	ECL (1-assay)	Competitive ligand binding

Information taken from EPARs of the different products from EMA website
Product withdrawn in Europe in 2019, [2] ADCC assay; [3]cell-based initially and superseded with ligand binding assay due to poor drug tolerance.

Comparative Immunogenicity

Expectation :

Antigenic equivalence using antibody controls

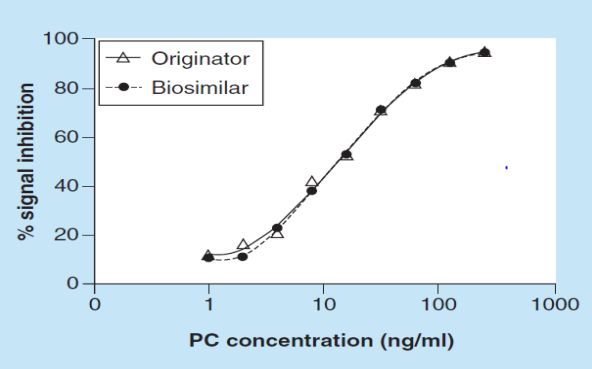


Figure 4. Relative binding curves with titrated positive control (0–250 ng/ml) versus spiked confirmatory concentration of biosimilar/originator (50 µg/ml)

Anti-adalimumab antibody assay methodology

Anti-Drug-Antibodies (ADAs) were measured by a bridging ligand-binding electro-chemiluminescent (ECL) assayin both Humira- and SB5-treated patients. (For validation of these methods, see Analytical methods in the Discussion on Clinical pharmacology section of this AR and Clinical AR.)

Neutralising antibodies

Neutralising antibodies (Nabs) were measured. The neutralising activity was assessed by inhibition of TNF-α binding to immobilised SB5 by circulating ADAs.

Antigenicity of SB5 and Humira

The Applicant claims that adalimumab of EU-Humira and SB5 are antigenically equivalent by showing a similar inhibition of the signal by a monoclonal human anti-adalimumab antibody. In addition, the Applicant has used dilution curves of polyclonal rabbit anti-SB5 and rabbit anti-adalimumab (from EU Humira) to demonstrate similar reactivity in the ECL assay.

Ryding et al (2017) Bioanalysis 9(18), 1395-1406

Comparative Immunogenicity: Biosimilars

- Data assessed in context of totality of evidence
- Similar ADA incidence, titres, neutralisation, kinetics of development
- Identify root cause of any differences e.g. impurities, aggregates etc
  - Excess immunogenicity not compatible with biosimilarity BUT
  - Lower immunogenicity does not preclude biosimilarity: *Justification required*
- **Expectation** - Clinical consequences / impact no worse than RMP
  - Compare clinical impact of ADA on PK,PD, efficacy, safety etc
- Post-approval surveillance of immunogenicity
  - Key requirement for biosimilars: monitoring any immune-mediated adverse effects
- Special studies in high risk situations
  - Where serious but rare effects (anaphylaxis) known with reference product

Some Examples

Remsima vs Remicade

- Initial testing with a single antigen (reference product) → no difference seen
- Assay using biosimilar as antigen developed → no difference, including ADA titers
- Cross testing of sera with both assays
  → good concordance → evidence for similar immunogenicity
- **Indications studied**

	Remsima	Remicade (reference)
Ankylosing Spondylitis	37.5%	36.1%
Rheumatoid arthritis	55.6%	54.3%

- Similar impact on clinical efficacy and safety

APPROVED - EMA

Etanercept Biosimilar: Benepali (SB4) vs Enbrel

Phase 3 in RA patients (+ MTX): ECL assay with SB4 as target (One assay approach)

	ADA Incidence (any positive)				
Phase III timepoint	**SB4**		**EU Enbrel®**		
	N=299	**(%)**	**N =297**	**(%)**	p
Week 24	2	**0.7**	39*	**13.2**	< 0.001
Week 52	3	**1.0**	39*	**13.2**	

Sampling: Baseline, Weeks 2, 4, 8, 12, 16 & 24; 1 patient NAb+ve *

Rationale for putative lower immunogenicity?

- SB4 - lower aggregate content and HCP
- Insufficient to explain difference in immunogenicity
- ADAs appeared early (between weeks 2 - 8) and most disappeared after week 12

Reviewed validation of ADA assay

- Assay drug tolerance close to mean trough concentrations which differed at weeks 4, 8
- Sufficient to cause bias in ADA testing?
- Re-evaluated – Ignoring week 4, 8 samples

Table 31. Incidence of Overall ADA by Treatment Group by Ignoring Samples Taken at Weeks 4 and 8 (Safety Set, Study SB4-G31-RA)

Overall ADA Status	SB4 n/n' (%)	EU Enbrel n/n' (%)	*p*-value
24-week Overall ADA Incidence	0/299 (0.0)	2/296 (0.7)	0.2471
52-week Overall ADA Incidence	1/299 (0.3)	2/296 (0.7)	0.6225

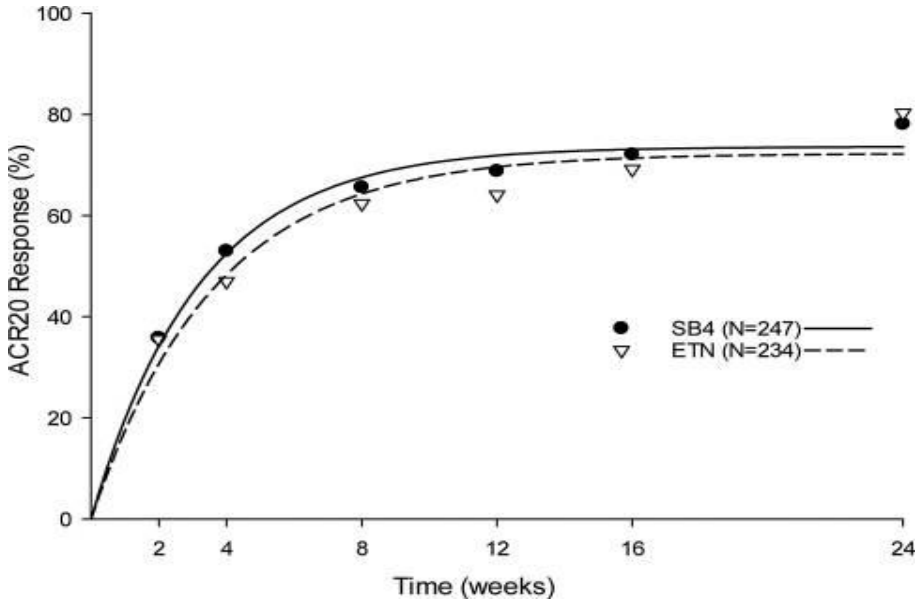
Conclusion:
SB4 no less immunogenic than Enbrel

Emery P et al Ann Rheum Dis. 2017; 76(1):51-57.

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004007/WC500200380.pdf;

Benepali (SB4) versus Enbrel

Week 24 - ACR20 response rate in the per-protocol set was 78.1% for SB4 and 80.3% for ETN



No impact on PK and safety.....
No difference in efficacy noted..

APPROVED - EMA

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004007/WC500200380.pdf; Emery P et al Ann Rheum Dis. 2017; 76(1):51-57.

Adalimumab ABP 501 vs Humira

Table 29. Summary of Binding and Neutralizing ADAs Following Repeat Dosing in Study 262 and Study 263

	Rheumatoid Arthritis Study 262		Plaque Psoriasis Study 263				
			Through Week 16		Week 16 to EOS		
	ABP 501 40 mg (n=264)	US-ADA 40 mg (n=262)	ABP 501 40 mg (n=174)	US-ADA 40 mg (n=173)	ABP 501/ ABP 501 40 mg (n=152)	EU-ADA/ EU-ADA 40 mg (n=79)	EU-ADA/ ABP 501 40 mg (n=77)
Binding ADA-positive, n (%)	101 (38)	100 (38)	96 ( )	110 ( )	104 ( )	59 ( )	56 ( )
Neutralizing ADA-positive, n (%)	24 ( )	29 (11)	17 ( )	24 ( )	21 ( )	16 ( )	19 ( )

Source: FDA analysis of data from Amgen 351(k) BLA submission
US-ADA: US-licensed Humira; EU-ADA: EU-approved Humira; EOS: end of study

APPROVED

https://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/arthritisadvisorycommittee/ucm510293.pdf

Clinical Impact of ADA

ADA incidence and impact: Similar for the reference and biosimilar ABP501

For both products:

- ADA-positive patients had a lower exposure (troughs)
- ADA-positive patients had inferior efficacy
- Hypersensitivity/injection-site reactions were similar regardless of ADA status
- NAbs did not have a statistically significant differential impact on efficacy between the two products

Conclusion : Both products (reference and ABP501) analytically and clinically similar in terms of efficacy, safety and immunogenicity

APPROVED

Markus R et al (2019) A Review of the Totality of Evidence Supporting the Development of the First Adalimumab Biosimilar ABP 501. Adv Ther. 36(8):1833-1850. doi: 10.1007/s12325-019-00979-6.

What is required?

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

Methods

- Antibody incidence and 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

Antibodies for host cell proteins if appropriate.

Data

Conclusion

Immunogenicity is an issue for all biologicals (incl biosimilars)…….

An immunogenicity testing approach based on

- scientific knowledge and risk considerations with sufficient data which
- informs the prescriber of product immunogenicity and potential outcomes for clinical decision-making

Image : From Wikimedia Commons, the free media repository

Validation Aspects and Terminology

- **EMA Guideline on bioanalytical method validation' EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2****
- **Shankar et al (2008)** Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal, 48, 1267-81
- **Gupta et al (2011)** Recommendations for the validation of cell-based assays used for detection of neutralizing antibody immune responses elicited against biological therapeutics. J Pharm Biomed Anal, 55, 878-88
- **Shankar et al (2014)** Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology & tactical recommendations. AAPS J 16(4):658-73
- **Devanarayan V (2017)** Recommendations for Systematic Statistical Computation of Immunogenicity Cut Points. AAPS J 19(5):1487–1498

Thank You!