MedImmune

A member of the AstraZeneca Group

When 2-become-1; Don't be a Stranger to the Challenges of Immunogenicity Testing in Dual Peptide Therapies

Nick White, Ph.D BMedSci

New analytical challenges for Immunogenicity testing

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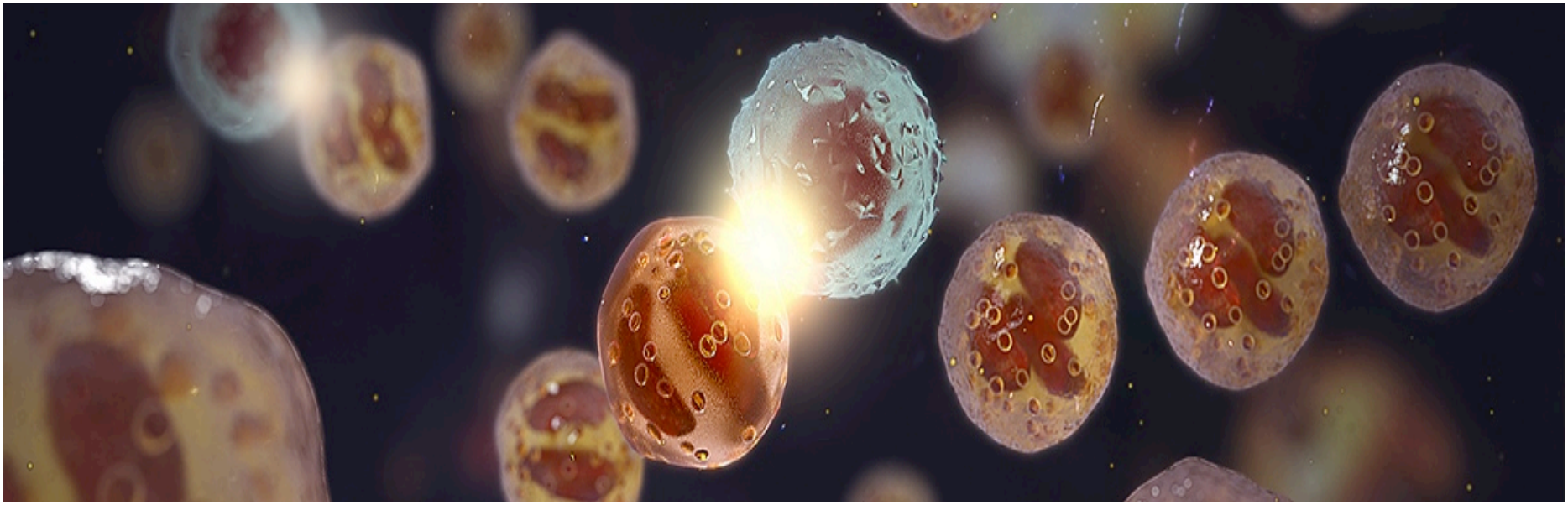

Content

- Marcromolecule/Biotherapeutic History
- Importance of Immunogenicity testing
- White papers and guidance documents
- Case studies
- Closing remarks

Macromolecules have Evolved Significantly over the Decades

- October 1982 – Human Insulin produced from recombinant bacterial expression systems licenced by the FDA
- 1986 – Muromonab-CD3, the first MAb to be approved for human use
- 1987 – Alteplase, first therapeutic enzyme approved
- 1989 to 1991 – EPO, G-CSF & GM-CSF approved
- 1997 – rituximab the first whole chimeric antibody & daclizimab the first humanised antibody approved
- 1998 – etanercept the first Fc-Fusion product was approved
- 2001 – Peg-G-CSF and Peg-GM-CSF approved
- 2002 – Humira the first human MAb generated via phage display approved
- 2005 – Exenatide the synthetic variant of endogenous peptide approved
- 2010 – Liraglutide approved by FDA
- 2014 – Dulaglutide approved by FDA

Dark Clouds Formed on the Horizon Were Largely Ignored

- Immunogenicity of protein therapeutics has been an issue throughout the history of biotechnology products
- All of a sudden this gained prominence in the early 2000's: **Erythropoietin (EPO)**
- Casadevall et al. NEJM (Feb 2002)
  - From 2000-2002 13 individuals were referred to their lab due to sudden resistance to the endogenous hormone and presented **transfusion dependent pure red-cell aplasia (PRCA)**
  - Hypothesis: Associated with a manufacturing change of the product distributed in Europe

Immunogenicity Assessments and Interpretation are Key Requirements in any Successful BLA or MAA

- Immunogenicity assessment is one of the regulatory requirements for a BLA or MAA
  - –Approval includes the review of immunogenicity studies
  - –Integrated Summary of Immunogenicity is a key document within the MAA and should be placed in chapter 2.7.2.4 of the CTD or Module 5
- WHO guidelines stipulate that immunogenicity assessment should be conducted in the relevant patient population
- EMA & FDA provided guidance documents, United States Pharmacopoiea and white papers published by industry all facilitate expectations in immunogenicity assessment

Regulatory Concerns of ADA to Biotherapeutics Products

Concern	Outcome
1). Safety	ADA causes hypersensitivity reactions ADA neutralise activity of an endogenous equivalent resulting in deficiency syndrome
2). PK	Altered PK by ADA causing a change in dose level Changes in clearance
3). Efficacy (PD)	Changes in drug effects Biotherapeutic no longer affects target
4). None!	Despite presence of ADA, there are no clinical effects

Information on Immunogenicity Testing is Published in the Label…

Immunogenicity

Patients in Studies RA-I, RA-II, and RA-III were tested at multiple time points for antibodies to adalimumab during the 6- to 12-month period. Approximately 5% (58 of 1062) of adult RA patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing *in vitro*. Patients treated with concomitant methotrexate (MTX) had a lower rate of antibody development than patients on HUMIRA monotherapy (1% versus 12%). No apparent correlation of antibody development to adverse reactions was observed. With monotherapy, patients receiving every other week dosing may develop antibodies more frequently than those receiving weekly dosing. In patients receiving the recommended dosage of 40 mg every other week as monotherapy, the ACR 20 response was lower among antibody-positive patients than among antibody-negative patients. The long-term immunogenicity of HUMIRA is unknown.

Immunogenicity

Consistent with the potentially immunogenic properties of protein and peptide pharmaceuticals, patients treated with Victoza may develop anti-liraglutide antibodies. Approximately 50-70% of Victoza-treated patients in the five clinical trials of 26 weeks duration or longer were tested for the presence of anti-liraglutide antibodies at the end of treatment. Low titers (concentrations not requiring dilution of serum) of anti-liraglutide antibodies were detected in 8.6% of these Victoza-treated patients. Sampling was not performed uniformly across all patients in the clinical trials, and this may have resulted in an underestimate of the actual percentage of patients who developed antibodies. Cross-reacting anti-liraglutide antibodies to native glucagon-like peptide-1 (GLP-1) occurred in 6.9% of the Victoza-treated patients in the 52-week monotherapy trial and in 4.8% of the Victoza-treated patients in the 26-week add-on combination therapy trials. These cross-reacting antibodies were not tested for neutralizing effect against native GLP-1, and thus the potential for clinically significant neutralization of native GLP-1 was not assessed. Antibodies that had a neutralizing effect on liraglutide in an *in vitro* assay occurred in 2.3% of the Victoza-treated patients in the 52-week monotherapy trial and in 1.0% of the Victoza-treated patients in the 26-week add-on combination therapy trials.

Among Victoza-treated patients who developed anti-liraglutide antibodies, the most common category of adverse events was that of infections, which occurred among 40% of these patients compared to 36%, 34% and 35% of antibody-negative Victoza-treated, placebo-treated and active-control-treated patients, respectively. The specific infections which occurred with greater frequency among Victoza-treated antibody-positive patients were primarily nonserious upper respiratory tract infections, which occurred among 11% of Victoza-treated antibody-positive patients; and among 7%, 7% and 5% of antibody-negative Victoza-treated, placebo-treated and active-control-treated patients, respectively. Among Victoza-treated antibody-negative patients, the most common category of adverse events was that of gastrointestinal events, which occurred in 43%, 18% and 19% of antibody-negative Victoza-treated, placebo-treated and active-control-treated patients, respectively. Antibody formation was not associated with reduced efficacy of Victoza when comparing mean HbA_{1c} of all antibody-positive and all antibody-negative patients. However, the 3 patients with the highest titers of anti-liraglutide antibodies had no reduction in HbA_{1c} with Victoza treatment.

In clinical trials of Victoza, events from a composite of adverse events potentially related to immunogenicity (e.g. urticaria, angioedema) occurred among 0.8% of Victoza-treated patients and among 0.4% of comparator-treated patients. Urticaria accounted for approximately one-half of the events in this composite for Victoza-treated patients. Patients who developed anti-liraglutide antibodies were not more likely to develop events from the immunogenicity events composite than were patients who did not develop anti-liraglutide antibodies.

6.3 Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The observed incidence of antibody (including neutralizing antibody) positivity in an assay is highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Rituxan with the incidence of antibodies to other products may be misleading.

Using an ELISA assay, anti-human anti-chimeric antibody (HACA) was detected in 4 of 356 (1.1%) patients with low-grade or follicular NHL receiving single-agent Rituxan. Three of the four patients had an objective clinical response.

A total of 273/2578 (11%) patients with RA tested positive for HACA at any time after receiving Rituxan. HACA positivity was not associated with increased infusion reactions or other adverse reactions. Upon further treatment, the proportions of patients with infusion reactions were similar between HACA positive and negative patients, and most reactions were mild to moderate. Four HACA positive patients had serious infusion reactions, and the temporal relationship between HACA positivity and infusion reaction was variable. The clinical relevance of HACA formation in Rituxan-treated patients is unclear.

Immunogenicity

Across four Phase 2 and five Phase 3 clinical studies, 64 (1.6%) TRULICITY-treated patients developed anti-drug antibodies (ADAs) to the active ingredient in TRULICITY (i.e., dulaglutide).

Of the 64 dulaglutide-treated patients that developed dulaglutide ADAs, 34 patients (0.9% of the overall population) had dulaglutide-neutralizing antibodies, and 36 patients (0.9% of the overall population) developed antibodies against native GLP-1.

The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, the incidence of antibodies to dulaglutide cannot be directly compared with the incidence of antibodies of other products.

Hypersensitivity

Systemic hypersensitivity adverse reactions sometimes severe (e.g., severe urticaria, systemic rash, facial edema, lip swelling) occurred in 0.5% of patients on TRULICITY in the four Phase 2 and five Phase 3 studies.

Injection-site Reactions

In the placebo-controlled studies, injection-site reactions (e.g., injection-site rash, erythema) were reported in 0.5% of TRULICITY-treated patients and in 0.0% of placebo-treated patients.

PR Interval Prolongation and Adverse Reactions of First Degree Atrioventricular (AV) Block

A mean increase from baseline in PR interval of 2-3 milliseconds was observed in TRULICITY-treated patients in contrast to a mean decrease of 0.9 milliseconds in placebo-treated patients. The adverse reaction of first degree AV block occurred more frequently in patients treated with TRULICITY than placebo (0.9%, 1.7% and 2.3% for placebo, TRULICITY 0.75 mg and TRULICITY 1.5 mg, respectively). On electrocardiograms, a PR interval increase to at least 220 milliseconds was observed in 0.7%, 2.5% and 3.2% of patients treated with placebo, TRULICITY 0.75 mg and TRULICITY 1.5 mg, respectively.

ADVERSE REACTIONS

Immunogenicity

As with all therapeutic proteins, there is the potential for immunogenicity. Neutralizing antibodies to erythropoietin, in association with PRCA or severe anemia (with or without other cytopenias), have been reported in patients receiving PROCRIT® (see WARNINGS: Pure Red Cell Aplasia) during post-marketing experience.

There has been no systematic assessment of immune responses, i.e., the incidence of either binding or neutralizing antibodies to PROCRIT®, in controlled clinical trials.

Where reported, the incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies across products within this class (erythropoietic proteins) may be misleading.

Immunogenicity

Patients with RA, PsA, AS or PsO were tested at multiple time points for antibodies to etanercept. Antibodies to the TNF receptor portion or other protein components of the Enbrel drug product were detected at least once in sera of approximately 6% of adult patients with RA, PsA, AS or PsO. These antibodies were all non-neutralizing. Results from JIA patients were similar to those seen in adult RA patients treated with Enbrel.

In PsO studies that evaluated the exposure of etanercept for up to 120 weeks, the percentage of patients testing positive at the assessed time points of 24, 48, 72 and 96 weeks ranged from 3.6% - 8.7% and were all non-neutralizing. The percentage of patients testing positive increased with an increase in the duration of study; however, the clinical significance of this finding is unknown. No apparent correlation of antibody development to clinical response or adverse events was observed. The immunogenicity data of Enbrel beyond 120 weeks of exposure are unknown.

The data reflect the percentage of patients whose test results were considered positive for antibodies to etanercept in an ELISA assay, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed

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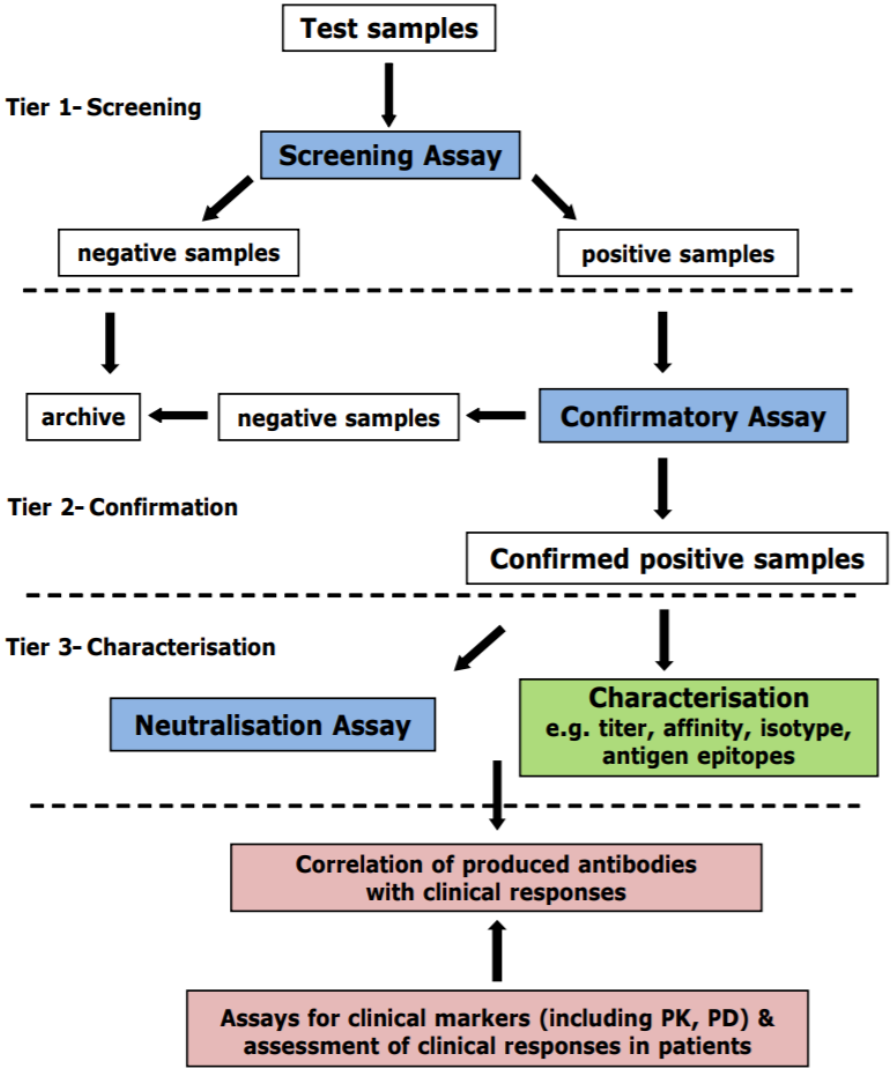
Enbrel® (etanercept) for Subcutaneous Injection

incidence of any antibody positivity in an assay is highly dependent on several factors, including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to etanercept with the incidence of antibodies to other products may be misleading.

A Brief History of the Whitepapers & Regulations

- Findlay *et al*., J. Pharm. Biomed. Anal. 21 (2000) 1249–1273; first proposed Bioanalytical Methods to detect antibodies to marcromolecules
- A.R. Mire-Sluis *et al*., JIM 289 (2004) 1–16; Standardised recommendations for the design & optimisation of ADA immunoassays
- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins; EMA Draft 2006, Effective 2008
- G. Shankar *et al*., Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281 formed the basis of most ADA method validation designs
- Assay Development for Immunogenicity Testing of Therapeutic Proteins; FDA 2009
- Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products; FDA 2016
- Guideline on Immunogenicity assessment of therapeutic protein; EMA Draft 2015, Effective 2017
- Ishii-Watabe, Shibata, Nishimura *et al.* Bioanalysis (2018) 10(2), 95–10 Immunogenicity of therapeutic protein products: current considerations for anti-drug antibody assay in Japan

Clinical Strategy for Immunogenicity Determination: 'Tiered Approach'



Guideline on Immunogenicity assessment of therapeutic proteins
EMEA/CHMP/BMWP/42832/2005 Rev1

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Antibody Peptide Fusion

Method Development and Validation Considerations

- Molecule Conjugation for critical reagent utilisation
  - Labelling reagents may affect small molecular peptides
- Positive Control
  - Fully characterised; ensure various epitopes on the molecule to identify ADA to all molecular portions
- Sample Pre-treatment
  - Acid dissociation may affect the tertiary structure of the peptide portion
- Target inference
  - Agonistic peptides maybe bound to circulating receptor
  - Bound antibody may sterically hinder ADA detection

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Antibody Peptide Fusion

Method Development and Validation Considerations

- Screening tier should detect ADA to antibody, linker and peptide
  - An appropriate SCPF (Screening Cut Point Factor) should be established
- Pre-existing antibodies
  - Peptide was based on current IP
- Confirmatory tier should confirm response is specific for drug
  - An appropriate CCPF (Confirmatory Cut Point Factor) should be established for each drug moiety

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Antibody Peptide Fusion

3rd Tier Characterisation Considerations

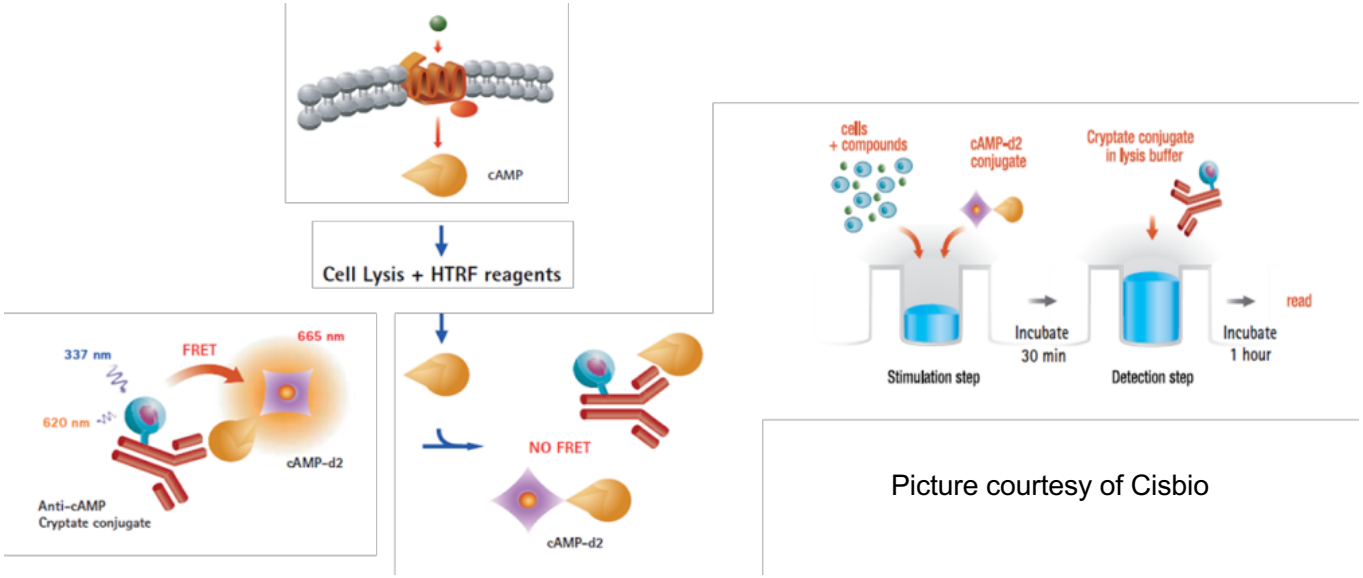
- Regulators may request full characterisation ADA response
  - Is the ADA response Antibody, linker or peptide mediated?
  - MAb panel was created via hybridoma and epitopes characterised to define specificities
  - Specificity (confirmation) assays against different moieties were developed

- For Regulatory interaction, sequence homology was a key consideration…
  - How similar was the peptide to endogenous counterpart?
  - Would ADA affect normal function of this?
  - Does the agency agree with the Sponsors position that cross-reactive ADA is not monitored?

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Antibody Peptide Fusion

3rd Tier Characterisation Considerations

- Neutralising Assays (in place for Phase 3)
  - Traditionally bioassays are developed to be indicative and reflective of the affect of NAb on a molecules mechanism of action
  - These molecules contain 2 distinct mechanisms of action, requiring 2 distinct assays, potentially with 2 reporters



Picture courtesy of Cisbio

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Dual Agonist

Method Development and Validation Considerations

- Size and molecular structure may affect conjugation; many conjugation protocols are optimised for peptides <40KDa
  - Remember; No-weigh biotin – 556Da, Sulfo-Tag – 1141Da
  - Significant potential these may block epitopes in small molecules
- Investigate conjugation chemistry; Free amines tend to be at peptide *n*-terminus. May affect PC binding…
- Buffer selection; molecules properties should be taken into consideration
  - i.e. mechanisms to increase $T_{1/2}$

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Dual Agonist

Method Development and Validation Considerations

- Sequence homology; interrogate the peptide structure against the endogenous counterparts of the molecule
  - Specifically called out in FDA guidance Section III & IV
- Develop/validate endogenous cross-reactivity assays
  - Panel of MAbs were assessed to develop reasonably sensitive method to establish specificity of ADA against endogenous molecules
- Assay the impact of consequences of antibodies to endogenous molecule 'normal' function
- Consider 'matrix' interference
  - Endogenous molecules may adversely effect the assay, binding PC

Dual Action Molecules Require a More Detailed Immunogenicity Investigation: Dual Agonist

3rd tier characterisation considerations

- FDA (or EMA) may ask the question or require that:
  - Cross-reactivity characterisation of confirmed ADA be performed
  - Samples were collected to ensure the full range of ADA can be detected (IgM & IgG at minimum)
  - The assay (screening and confirmatory) was suitable sensitive? Able to detect <100 ng/mL ADA in expected levels of drug (i.e. C_{max} or C_{trough})
- Ensured NAb assay was in place that was appropriate to the mechanisms of action for the drug
  - Peptides are agonists so required a bioassay
  - Be prepared to report NAb titre too, based on risk of the molecule!
- Characterise ADA positive samples against drug lot used

REMEMBER

STORE SUFFICIENT CLINICAL IMMUNOGENICITY SAMPLES UNDER APPROPRIATE STORAGE CONDITIONS TO ALLOW FOR ADDITIONAL TESTING IF REQUIRED

Closing Remarks

- Biotherapeutics molecules have a progressed significantly over the past few decades, becoming evermore complicated and evermore important in pharmaceutical company's portfolios
- The past 2 decades has seen regulatory spotlights focus on immunogenicity testing with the introduction of agency guidance documents and their subsequent revisions
- Little regulation exists for these novel modalities; however, thought about the molecule structure and relationships with the endogenous moieties and regulatory interaction can lead to complex characterisation in addition to the traditional tiered approach

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