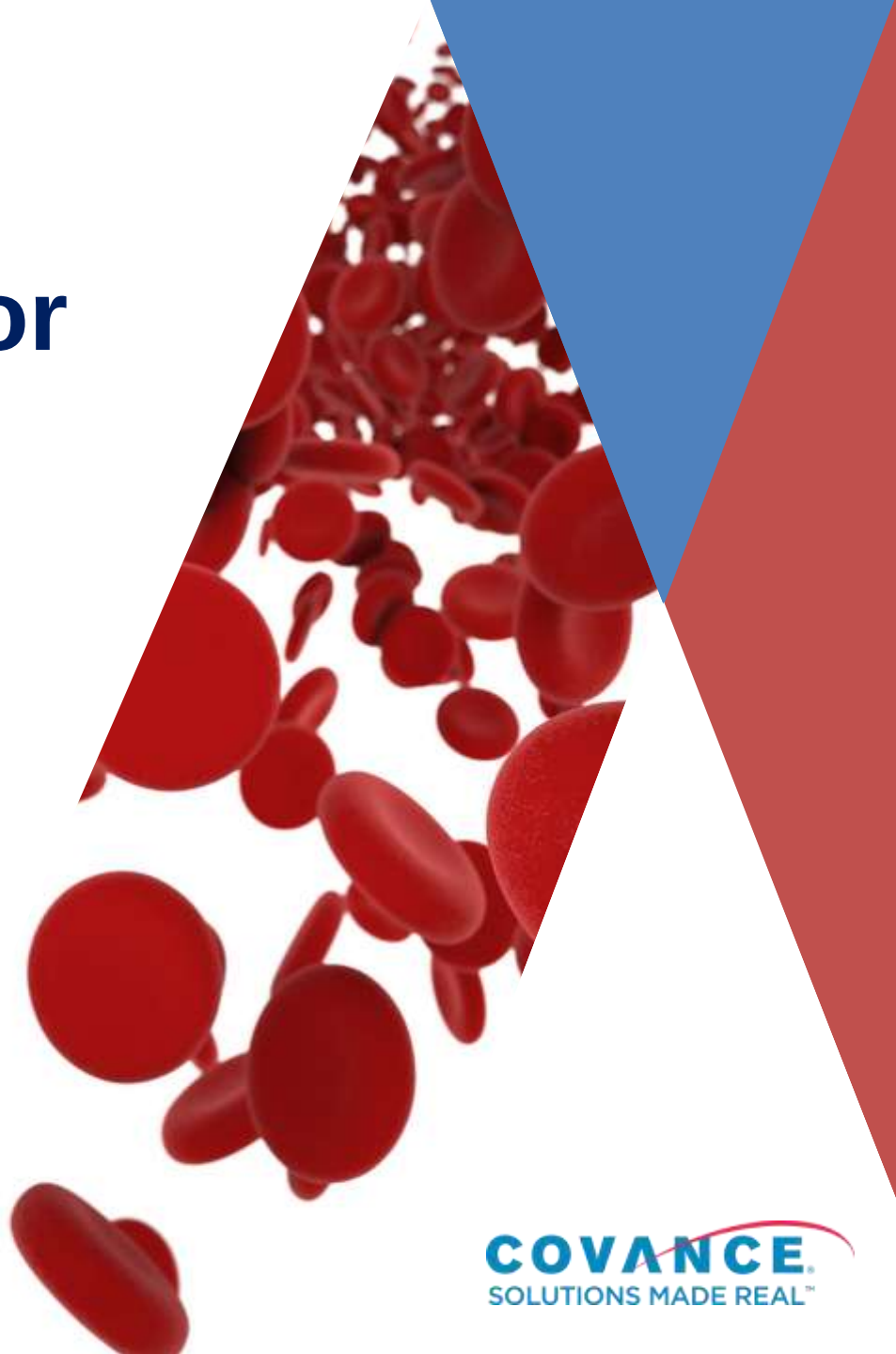
Paving the way for Non-Clinical Bioanalytical Partnerships

Louise Angell



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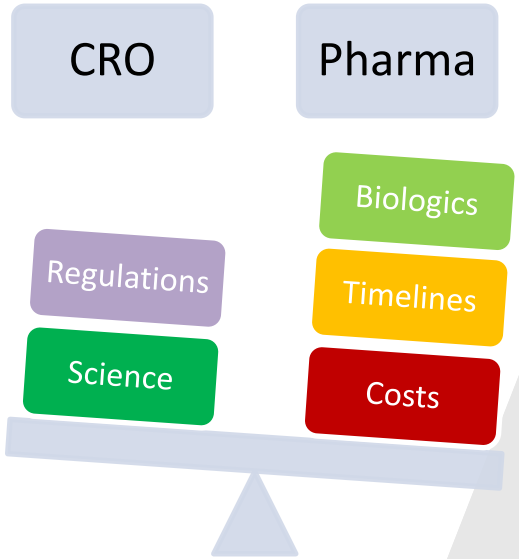
Content

- Overview of non-clinical immunogenicity testing for biologics
- Regulatory guidance
- Bioanalytical considerations
- Risk based approach



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Overview of Immunogenicity Testing

- All biologics (recombinant therapeutic proteins) will induce an immune response
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Unwanted ADA response has potential safety implications

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Overview of Immunogenicity Testing

- Test during non-clinical and clinical programs
  - Immunogenic response in non-clinical study is not predictive of an immune response in humans

- Novel Therapeutic Protein
  - **May assume** humanised protein to have less immunogenicity risk than prokaryotic engineered protein
  - Humanised protein will have greater homology to native sequence than non-humanised, however immunogenicity frequency is still variable
  - Understand BioCMC and *in vitro* data
  - Assess in repeat dose non-clinical studies, multiple species to enable *interpretation* of non-clinical data

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Regulatory Guidelines - Summary

- Regulatory agencies in the US and EU are consistent in their recommendation that immunogenicity be evaluated from a patient safety perspective due to non-predictability of data from non-clinical studies
- Immunogenicity testing is required for novel compounds
- Regulatory guidance for bioanalytical method validation is well defined for PK assays, with multiple white papers published for ADA assays, although ADA interpretation is evolving

- For Biosimilar products, there is no global guidance for immunogenicity testing, and EMA infer *in vivo* testing is not required due to lack of predictability
  - *in vivo* studies may be considered reasonable markers for potential immunogenicity assessment due to differences in product specification (quality and manufacturing)

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Regulatory Limitations

- Potential for differing interpretation of the guidelines by companies

- Biosimilar (ADA Assays)
  - Criteria for comparison of data?
  - It is not clear if one assay or two assay approach is optimal
  - Reference material?
  - One assay - additional assessments (cut point and drug tolerance?)

- Pre-existing antibodies
  - There is no regulatory guidance on how to deal with this

Bioanalytical Considerations

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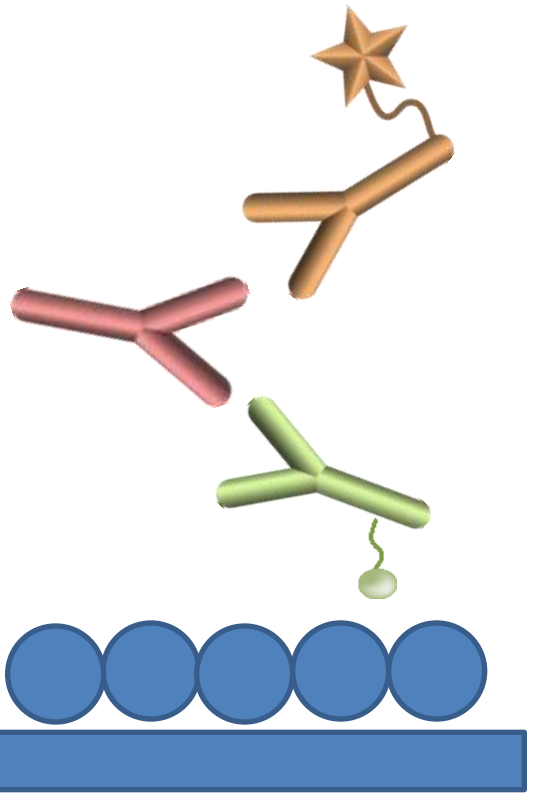
Overview of ADA assay



anti-drug antibody (ADA)

labelled drug

biotin-labeled drug

Streptavidin coated plate

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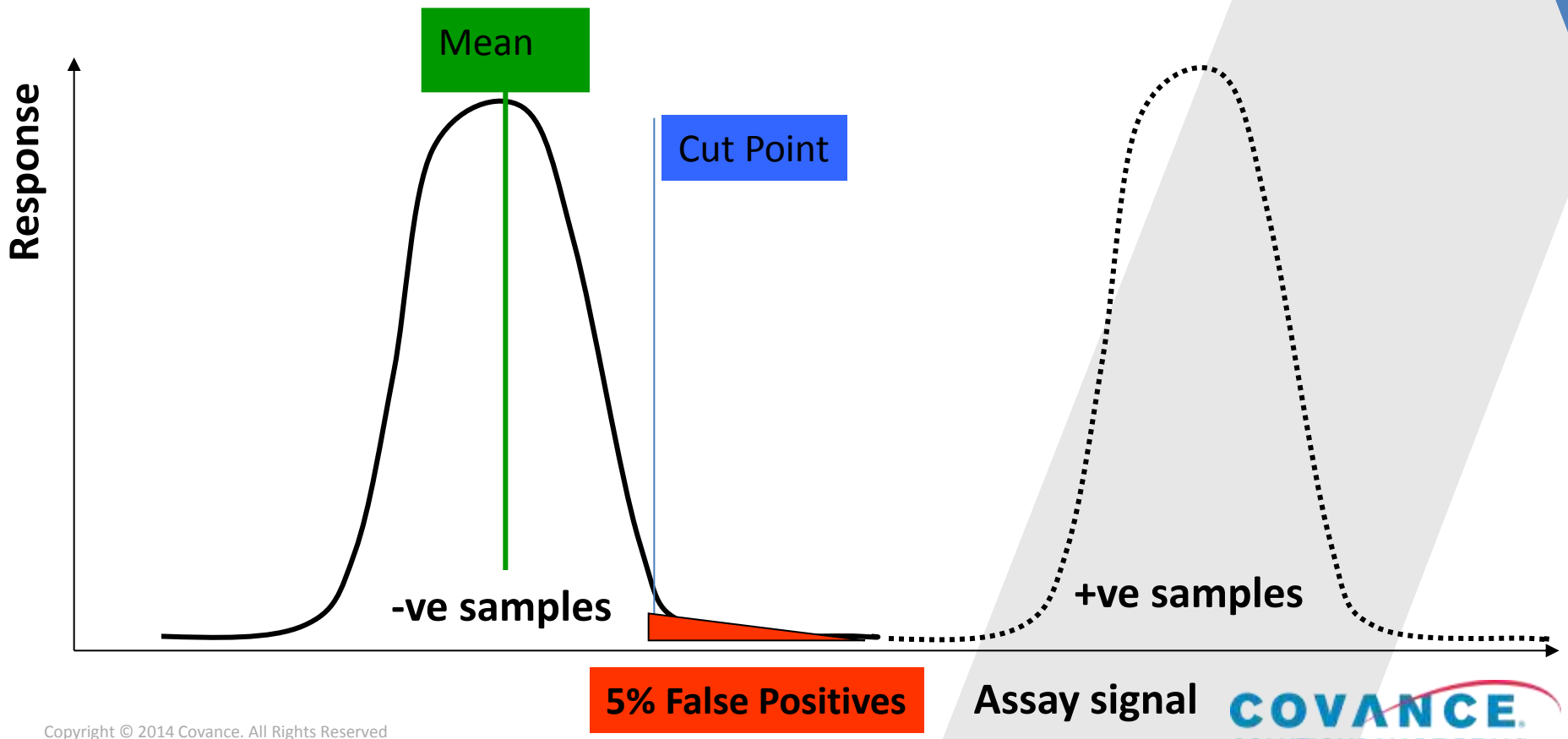
Overview of ADA assay

- Qualitative screening assay, confirmation, titre quantification



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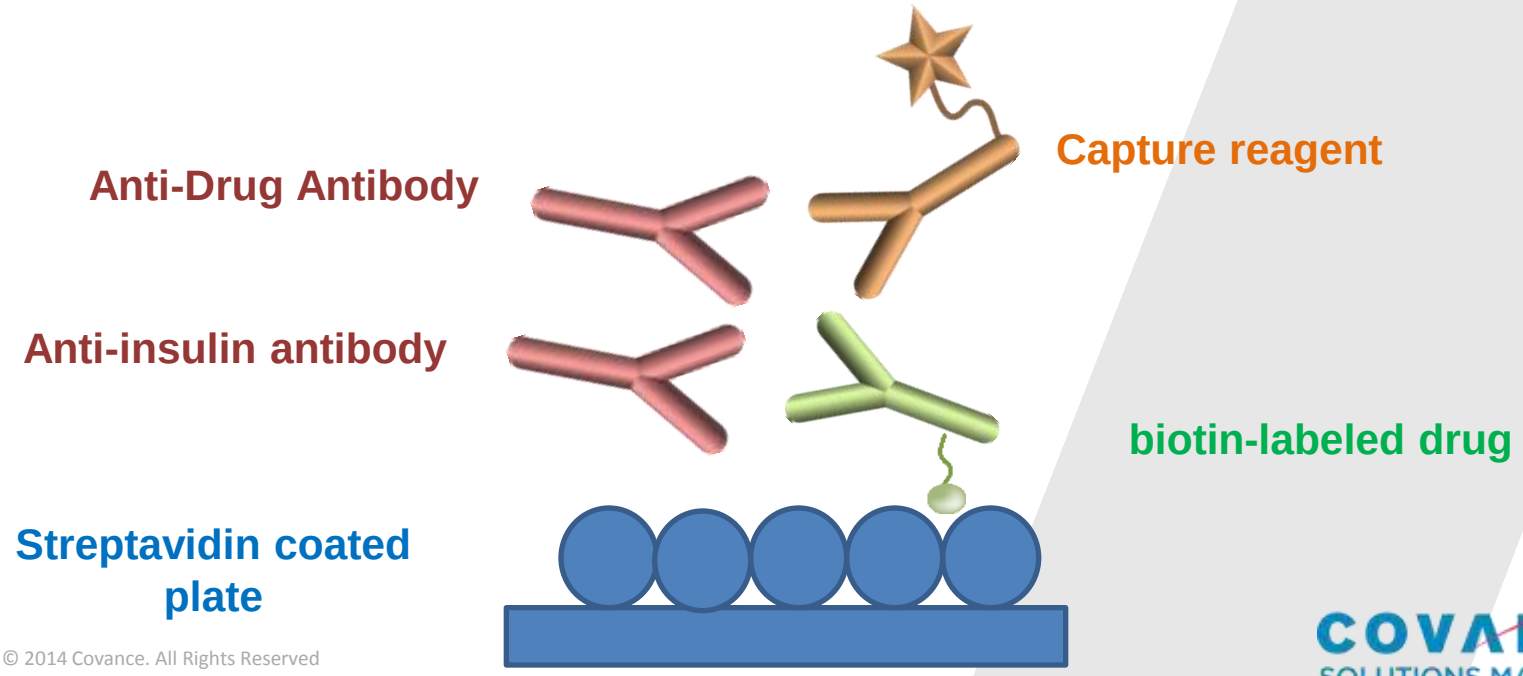
Insulin BioSimilar Case Study

- EMA Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant insulin and insulin analogues (draft April 2014)
  - Risk based approach for toxicology studies

- Presence of pre-existing antibodies against insulin is well documented



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Insulin Biosimilar Case Study

- Cut po itive" samples
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Scientists came together to discuss *potential* issues

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  - Pre-incubate cut point sample with insulin to remove pre-existing anti-insulin Ab whilst capturing biological matrix variation
  - Skip confirmation step
  - Titre pre and post dose for comparison

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Non-Clinical Study Design

- Some Clients have well defined BioCMC package and good prediction of immunogenicity risk

- Other Clients have limited experience with developing biologics
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Enhanced relationships help to guide projects

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Non-Clinical Study Design

- Learnings for non-clinical sample collection and testing
  - Importance of pre-dose samples
  - Align ADA with TK sampling
  - Collect enough sample to allow tier testing (screen, confirmation, titer, nAb)
  - Design to account for sample volume limitations (rodents)
  - Choice of bioanalytical assay
  - Drug interference (extend wash-out period so drug is BLQ)

Bioanalytical Considerations

ADA	In-Lab Analytical Time

In 2014, ADA transfer studies lasted an average 6 week in-life period

- **Not just analytical time, time is required for data review, Client discussions, Client internal debate**
- **What are Client (submission) timelines**
- **Additional time for positive control generation**

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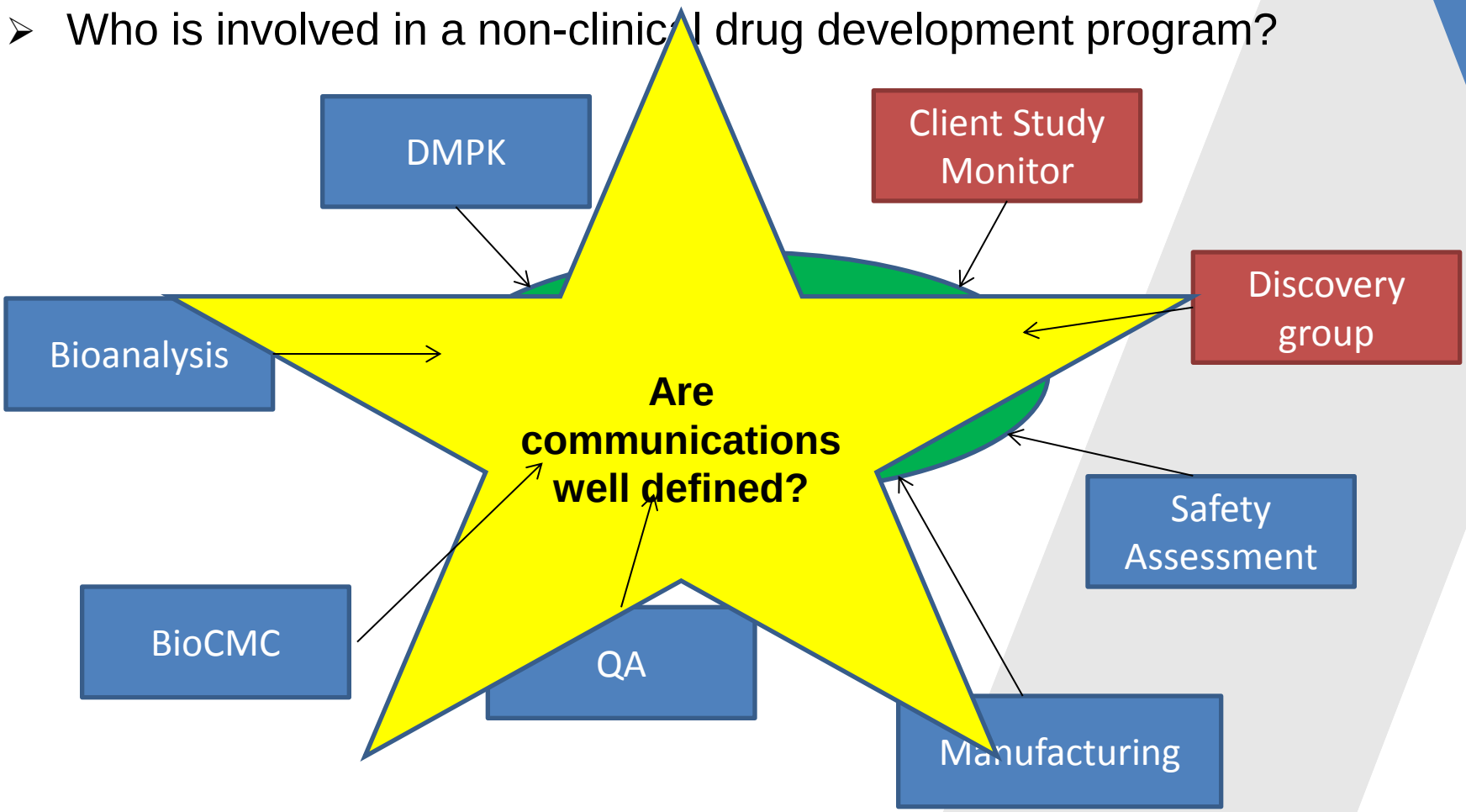
Improved Communication

- Who is involved in a non-clinical drug development program?



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Risk Based Approach for ADA Testing

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Interpretation of Immunogenicity

Immunogenicity *must be* considered with other endpoints

- **CRO challenge to generate data**
- **Pharma challenge to consider all data and make immunogenicity assessment**
- **Pharma could increase efficiency of CRO by ensuring data are shared**

ADA + PK + PD + AE = Immunogenicity Assessment

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Risk Based Approach for ADA Testing

- Regulatory agencies consistently recommend that immunogenicity be evaluated from a patient safety perspective

- As such, companies should utilize a risk-based approach in evaluating potential immunogenicity of their drug products

- Immunogenicity is difficult to predict
  - Potential immunogenicity of the protein
  - Biological function of the protein
  - Endogenous counterparts
  - Route of administration
  - Dose and frequency of administration
  - Health status of subjects

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Bioanalytical Cost Limitations

- Is ADA testing required?
- Is a fully validated method required?

T

- **Use risk based approach to understand BioCMC and *in vitro* data**
- **Plan *in vivo* studies and consider commitment to ADA assay**

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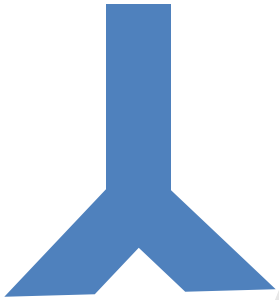
Summary

- Regulatory agencies in the US and EU are consistent in their recommendation that immunogenicity be evaluated from a patient safety perspective, however this is open to interpretation

- CROs can be well placed to advise Clients of potential bioanalytical limitations
  - Pre-existing antibodies
  - Non-clinical study design
  - Consideration to bioanalytical timelines

- Increased communications between Pharma and CRO can benefit

- Use risk based approach to consider extent of bioanalytical testing required



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Thank You

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Additional info for reference as required

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Regulatory Guidelines?

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> "Many biotechnology-derived pharmaceuticals intended for human are immunogenic in animals. Therefore, measurement of antibodies associated with administration of these types of products should be performed when conducting repeated dose toxicity studies in order to aid in the interpretation of these studies. Antibody responses should be characterised (e.g. titer, number of responding animals, neutralising or non-neutralising), and their appearance should be correlated with any pharmacological and/or toxicological changes. ……"

US

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> "Animal immunogenicity assessments generally do not predict potential immunogenic responses to protein products in humans. However, when differences in manufacturing (e.g., impurities or excipients) between the proposed product and the reference product may result in differences in immunogenicity, measurement of anti-protein antibody responses in animals may provide useful information relevant to patient safety."

Regulatory Guidelines?

EU

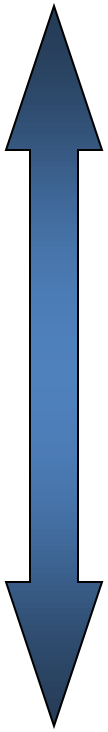
"……..the predictivity of non-clinical studies for evaluation of immunogenicity is considered low. Non-clinical studies aiming at predicting immunogenicity in humans are normally not required. However, ongoing consideration should be given to the use of emerging technologies …which might be used as tools. Measurement of antibodies in non-clinical studies are however requested as part of repeated dose toxicity studies, in order to aid in the interpretation of these studies …….. the comparison of the antibody response to the reference product in an animal model may be part of the comparability exercise both for similar biological medicinal products ……. and for changes in manufacturing……

"Due to the different production processes used by the biosimilar and reference product manufacturers, qualitative differences of process related impurities will occur…….. Qualitative or quantitative difference(s) of product-related variants ……may affect biological functions of the mAb and are expected to be evaluated by appropriate *in vitro* assays. These quality differences may have an effect on immunogenic potential and potential to cause hypersensitivity. It is acknowledged that these effects are difficult to predict from animal studies and should be further assessed in clinical studies. Immunogenicity assessment in animals is generally not predictive for immunogenicity in humans, but may be needed for interpretation of *in vivo* studies in animals. Blood samples should be taken and stored for future evaluations if then needed."

"Non-clinical toxicity as determined in at least one repeat dose toxicity study, including toxicokinetic measurements. ……"

Immunogenic Classes of Therapeutic Proteins

Strong



Weak

Class	Description	Human Protein Homology	Immunogenicity Frequency	Examples
A	Prokaryotic	Low	High	Staphylokinase
B	Mammalian	Low	High	OKT-3
C	Novel Construct	Medium	Variable	High: Denileukin Low: human growth hormone
D	Chimeric Human	High	Variable	H: chMuL6 L: rituximab
E	Humanized	High	Variable	L: Campath-1 H: Human anti-CD3
F	Human	Identical	Variable	H: GM-CSF L: Human insulin

Bugleski and Treacy, Cur Opinion Mol Ther 6: 10-16, 2004

Animal Data Not Predictive

Protein Therapeutic	Preclinical Immunogenicity?	Clinical Immunogenicity?
Streptokinase and Staphlyokinase	High	High
Keyhole Limpet Hemocyanin	High in rodents	High
Human interferon α- 2a	High in rodents	Low
Human Growth Hormone	High in rodents	Low
Human Interferon-λ	High in Cynomolgus monkeys	Low
Human Interleukin-3	High in Rhesus monkeys	Low

Bugleski and Treacy, Cur Opinion Mol Ther 6: 10-16, 2004

Estimated Risk and Study Design

Risk Level	Drug Characteristics	Examples	Consequences
Low	• *Not structurally identical to endogenous protein* •• Non agonistic	• Enzymes • Antibody drugs	• Infusion site reactions • Loss of efficacy • Mild allergic reactions
Medium	•• Partially or completely identical to endogenous protein •• Endogenous counterpart is either missing or redundant or •• Not structurally identical to endogenous protein/agonistic	• Replacement therapy, such as Factor VIII • Antibody drugs	• Same as Low Risk • Overstimulation of endogenous mechanism • Immune complex formation
High	•• Partially or completely identical to endogenous protein •• Endogenous counterpart is not redundant	• Erythropoeitin • GM-CSF	• Same as Medium Risk • Neutralization of endogenous counterpart

Estimated Risk and Study Design

Bioanalytical Testing Strategy		
Risk Level	**Sampling Frequency**	**Assessment ADAs**
Low	More frequently earlier in development, less frequently in Phase III (baseline, end of study and possibly follow-up)	Screen / Confirm Titer assessment Further characterization may be helpful Nab, Mapping ADA
Medium	More frequently earlier in development, less frequently in Phase III (baseline, end of study and possibly follow-up)	Screen / Confirm Titer assessment Further characterization may be helpful Nab, Mapping ADA
High	More frequently throughout all phases of clinical trails. Consider real-time testing of ADA and Nab	Screen / Confirm / Titer / NAb Further characterization of ADA by mapping, isotyping etc. Sequential patient dosing rather than cohort model for first-in-human studies

Rosenberg and Worobec; Biopharm. Int. 17, 17, 18 (2004)