Anti-Drug Antibody Testing In Toxicity Studies

Part 1: Scientific Background and Regulatory Expectation

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Outline

- The immune response to biological drugs
- Types and examples of anti-drug antibodies
- Evaluation of anti-drug antibodies in toxicity studies
- Updated regulatory recommendations

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The Immune Response

- The immune system responds to a foreign molecule or **antigen (Ag**), i.e. pathogen during an infection, to neutralize and eliminate it
  - Develops antigen-specific **antibody (Ab)**
- This response is retained as an immunological memory
  - Allows the adaptive immune response each time the Ag is encountered
- Ag-specific Ab production is a dynamic process
  - Single Exposure to Ag
    - Abs form in ~5-7 days: mostly IgM of low affinity/concentration
  - Multiple Exposures to Ag
    - Abs form in ~10-14 days: mostly IgG of high affinity/concentration

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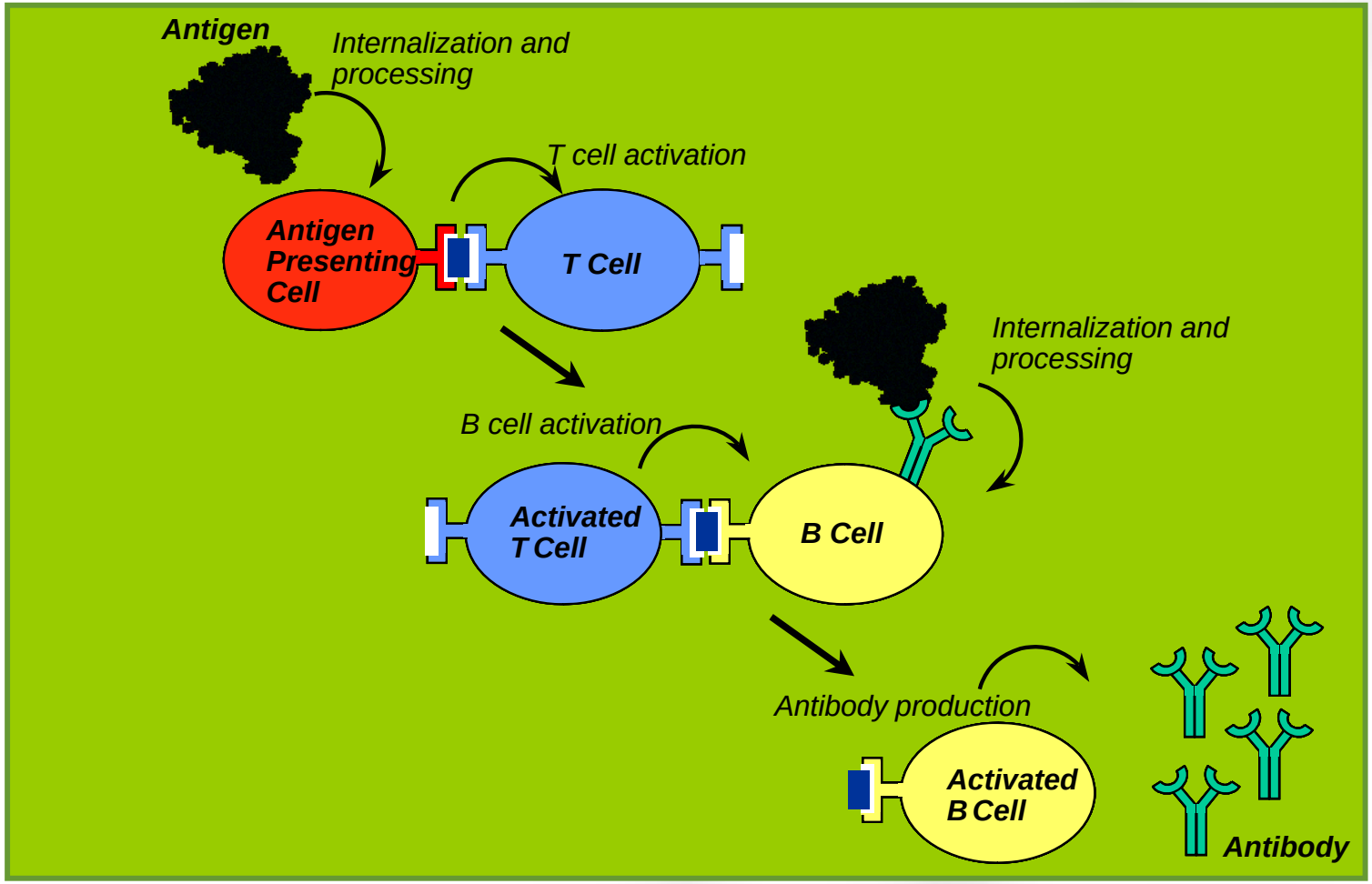
The Immune Response



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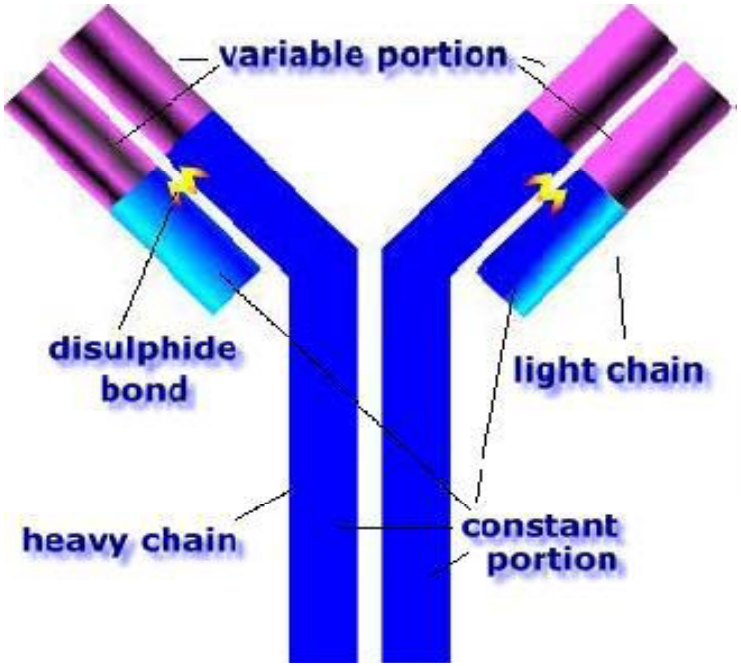
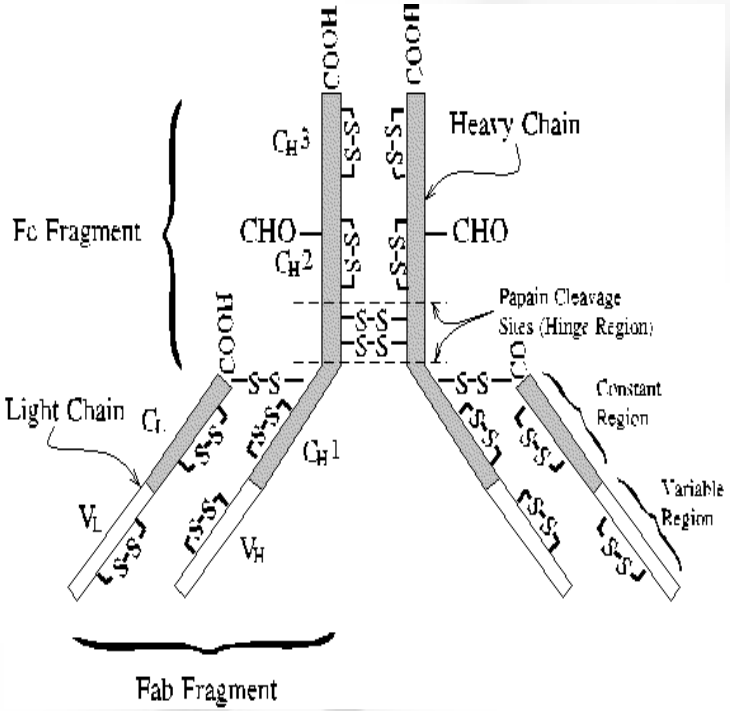
The Antibody Molecule





- **Immunoglobulin G (IgG)** is the most abundant Ab in the blood
  - Four subtypes with differing activities and concentrations
    - IgG1 (~9 mg/ml), IgG2 (~3 mg/ml), IgG3 (~1 mg/ml), IgG4 (~0.5 mg/ml)
    - Different activities correlate with the flexibility of their hinge region

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Immunogenicity of Biological Drugs

- All recombinant human or humanized proteins can potentially elicit the immune response and become immunogenic

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- Factors that contribute to immunogenicity
  - Characteristic of the molecule
  - Characteristic of the recipient (patient or animal)
  - Dosing regimen (dose level, frequency, route of administration)

- Stimulation of the innate immune system by the drug
  - May elicit „danger signals“
  - May induce cytokine release
  - May activate complement
  - Unknown factors...
- Induction of the adaptive immune response to the drug
  - Recognized as Ag and presented to T cells
  - **Development of antibodies against the drug: anti-drug antibodies (ADA)**

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Why Induction of ADA is a Problem?

- It concerns safety in patients
  - Potential ADA **impact on the health** of the patient
  - Clinically meaningful **antibody-induced alteration** of the response to the drug or its native counterpart
    - Rapid clearance and reduced exposure to the drug (Clearing ADA)
    - Prolonged exposure to the drug (Sustaining ADA)
    - Neutralization of the drug pharmacological activity (Neutralizing ADA)
    - Inhibition of production and/or activity of endogenous counterpart of the drug (Cross-reacting ADA)
    - Induction of allergic reaction (IgE response) to the drug
- May impact nonclinical risk assessment
  - Pharmacodynamic (PD) responses, toxicokinetics (TK), and/or toxicity profile in toxicology studies

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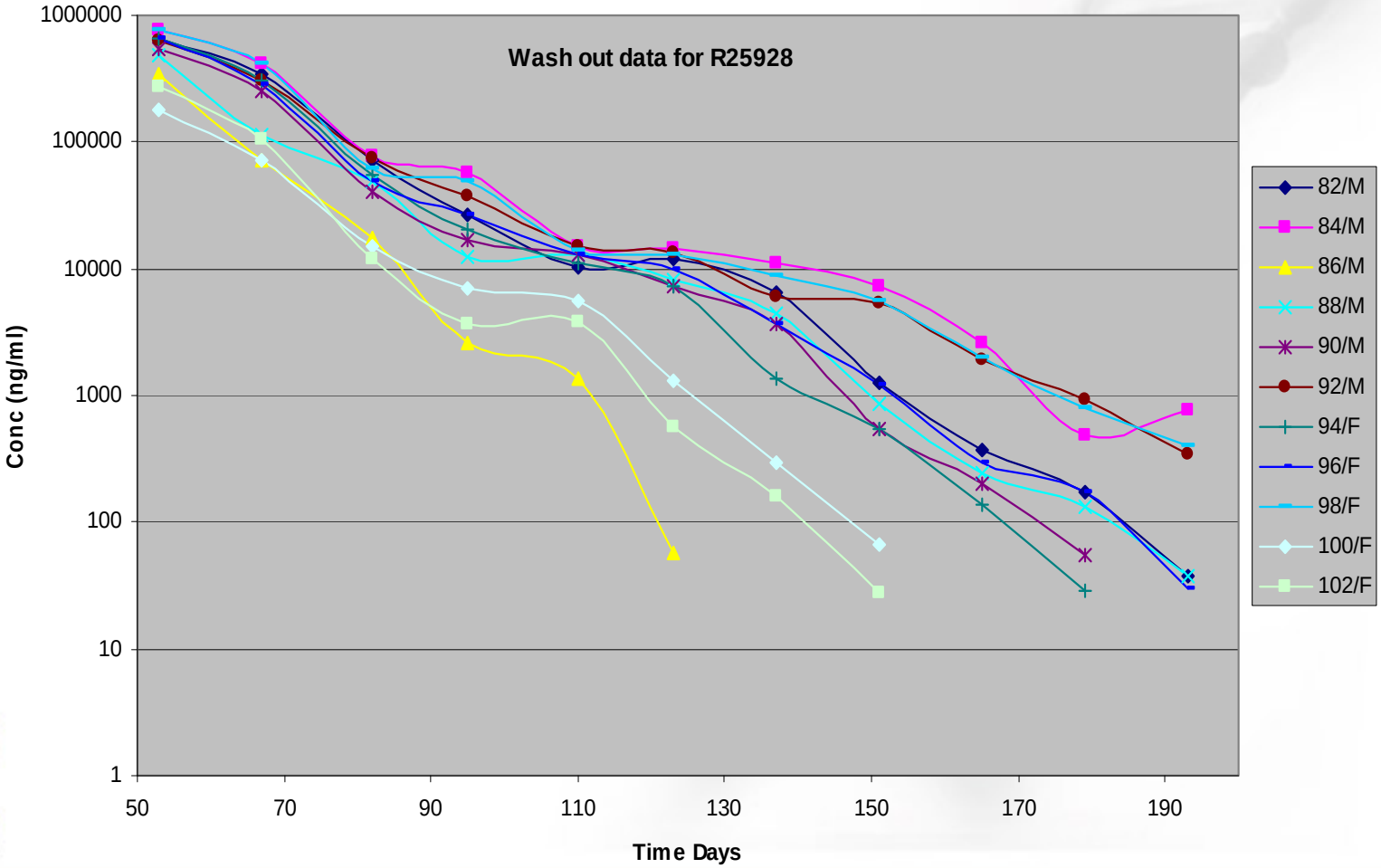
Example of Monoclonal Antibody Toxicokinetic Data

Plasma concentration of monoclonal antibody (mAb) after last dose



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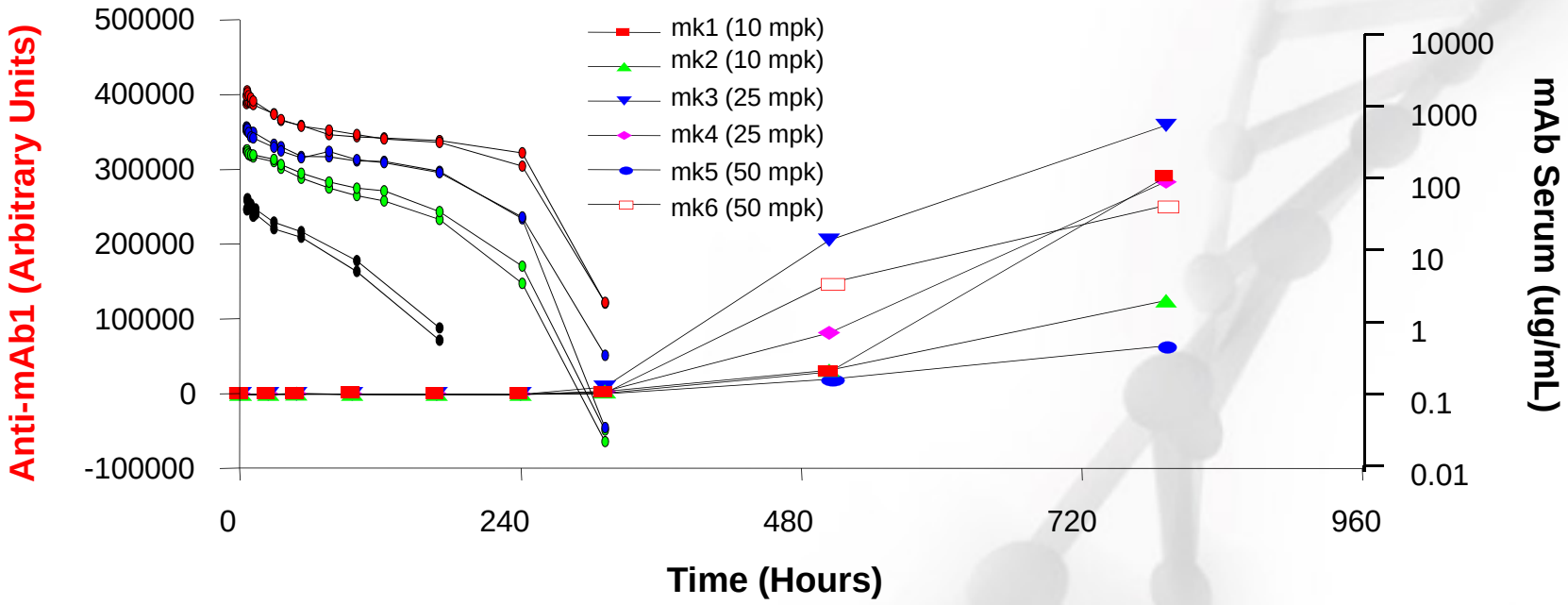
Immunogenicity Evaluation of mAb1

NHP Toxicology study with mAb1: 10, 25, 50 mg/kg SC



mAb1 was highly immunogenic in toxicology studies

- Detected 'clearing' ADA at 2 weeks after 1st dose
- Pretreatment with high dose (200 mg/kg) overcame the clearing effect
- Associated with toxicity in a repeat dose study

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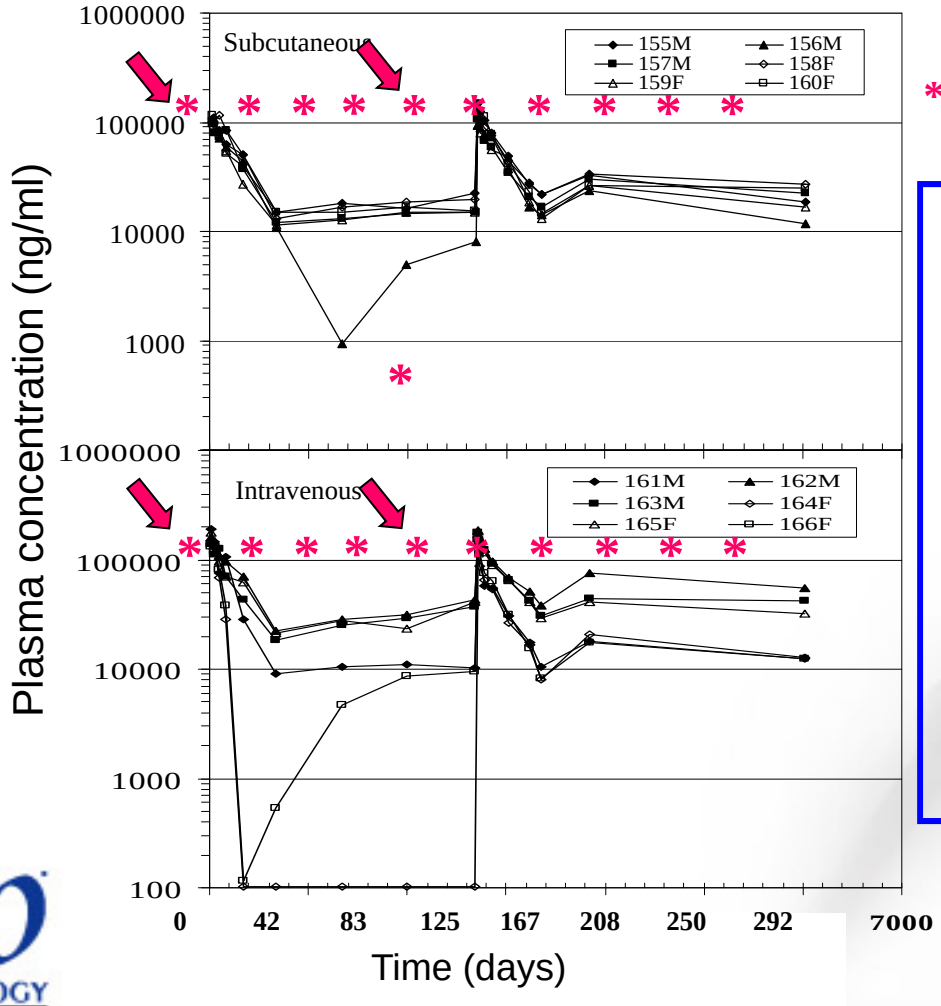
Immunogenicity Evaluation of mAb2

NHP Toxicology Study with mAb2: 10 Doses at 10 mg/kg SC or IV



* Day of dosing

mAb2 was minimally immunogenic in toxicology studies

- Effect on TK after single dose
- Continued dosing overcame any antibody effect

mAb2 was not immunogenic in clinical studies

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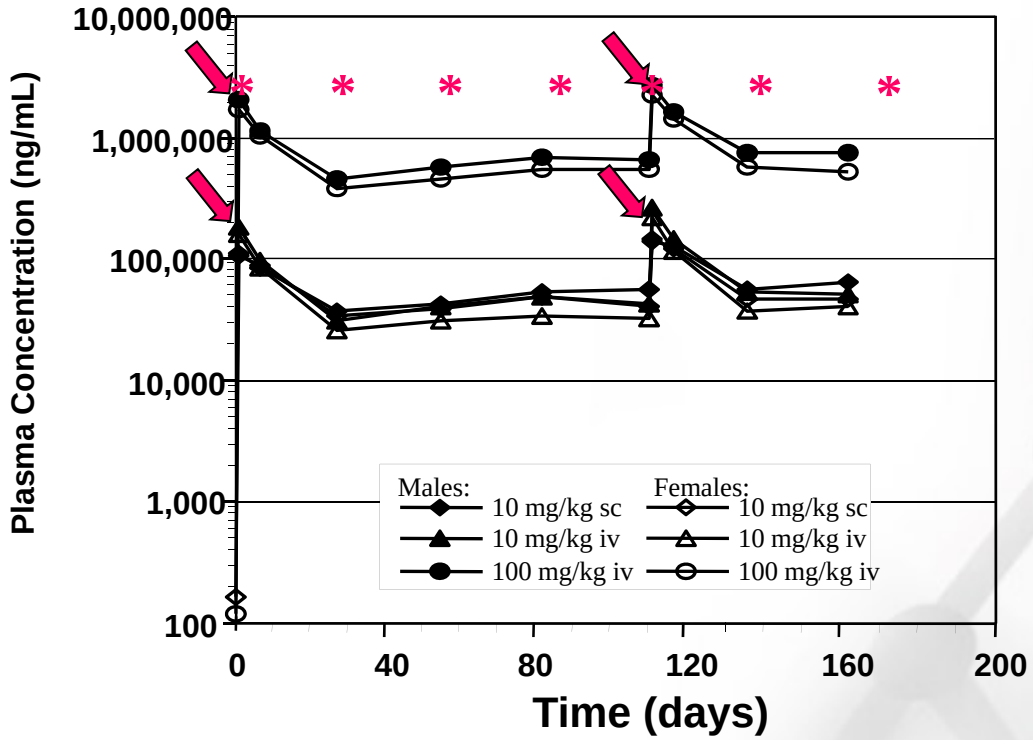
Immunogenicity Evaluation of mAb3

NHP Toxicology Study with mAb3: 7 Doses at 10 mg/kg SC or IV and 100 mg/kg IV



* Day of dosing

mAb2 was not immunogenic in toxicology and clinical studies

Immunogenicity Evaluation of mAb4

Animal	AUC(0-168) ug*hr/mL	ADA Titer	Heart Inflam	Eye Inflam	Stifle Inflam
M	144000	11.1	-	-	-
M	353000	Neg	minimal	-	minimal
M	212000	Neg	moderate	minimal	slight
M	237000	Neg	slight	minimal	slight
M	110000	8.5	minimal	minimal	--
F	124000	6.6	moderate	slight	moderate
F	ND	Neg	slight	moderate	moderate
F	180000	Neg	-	-	minimal
F	176000	Neg	-	-	-
F	29.6	24.8	-	-	-

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mAb4 was moderately immunogenic in toxicology studies

- No association between the observed toxicity and ADA responses

To date, mAb4 has not been immunogenic in clinical studies

Immunogenicity Testing in Toxicology Studies is Resource Intensive

- Method development of drug specific ADA assays
  - Generation of positive controls
    - Typically immunization (multiple injections) of rabbits with the drug
    - Purification of anti-drug antiserum to obtain ADA standard
    - Labelling the drug and/or ADA control with chromogenic enzyme or other ‘tag’ for the detection
  - Validation for the use in tox species matrix (serum/plasma)
  - Low throughput assays
  - May need multiple methods for ADA characterization
- Need to balance the use of high resources with the purpose of anti-drug antibody animal data

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Purpose of Nonclinical Anti-Drug Antibody Testing

- Addressed in BioSafe Sponsored White Paper

  R.A. Ponce, L. Abad, L. Amaravadi, T. Gelzleichter, E. Gore, J. Green, S.Gupta, D. Herzyk, C. Hurst, I. Ivens, T. Kawabata, C. Maier, B. Mounho, B. Rup, G. Shankar, H. Smith, P. Thomas, D. Wierda. Immunogenicity of biologically-derived therapeutics: Assessment and interpretation of nonclinical safety studies. *Regulatory Toxicology and Pharmacology,* 54: 164-182 (2009)

  – To establish best practices for the use of immunogenicity data
  – To understand this information in the context of human health risk assessment

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Conclusions and Recommendations

(Ponce et al.)

- Case-by-case immunogenicity assessment
  - Information required to interpret TK, PD and toxicology data
- Study designs should be flexible to enable minimization of immunogenicity when problematic
- Immunogenicity testing is not necessary absent changes in study parameters
- Develop decision strategy for measuring / characterization of ADA
  - Relate decision process to our ability to interpret tox studies

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Addendum to ICH S6 (R1)

- Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals (ICH S6)
- At Step 4 of the ICH Process
  - 5 topics
    - ✓Species Selection
    - ✓Study design
    - ✓Immunogenicity
    - ✓Reproductive/developmental toxicity
    - ✓Carcinogenicity

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ICH S6 (R1) Immunogenicity Topic

- **Revised recommendations**
  - Immunogenicity assessments are conducted to assist in the interpretation of the study results and design of subsequent studies
  - Measurement of ADA in nonclinical studies should be evaluated when
    - Evidence of altered PD activity
    - Unexpected changes in PK/TK in the absence of a PD marker
    - Evidence of immune-mediated reactions (immune complex-related, vasculitis, anaphylaxis, etc.)
  - It is useful **to obtain appropriate samples** during the course of the study**,** which can subsequently **be analyzed if needed** to aid in interpretation of the study results
  - **Characterization, specifically of neutralizing potential, is generally not warranted**, particularly if adequate exposure and pharmacological effect can be demonstrated by a PD marker of activity in the *in vivo* toxicology studies

Summary

- The immune response to biological drugs is a modality-dependent phenomenon
- Anti-drug antibodies are frequently observed in animal studies
- The need, scope and extent of immunogenicity testing in toxicology studies should be established based on the study findings

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Additional Information
(not covered in the presentation)

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Translation of Nonclinical ADA Data

(Ponce et al.)

- All biopharmaceuticals are potentially immunogenic
- Formation of ADA in animals does not impede drug development
  - Lack of appropriate immunogenicity assays and characterization can invite regulatory action
  - Decision tree for characterization of ADA based on scientific rationale
- General lack of correlation between nonclinical studies and clinical experience with regard to the incidence of ADA
- Perception that nonclinical immunogenicity data informs potential clinical safety liabilities

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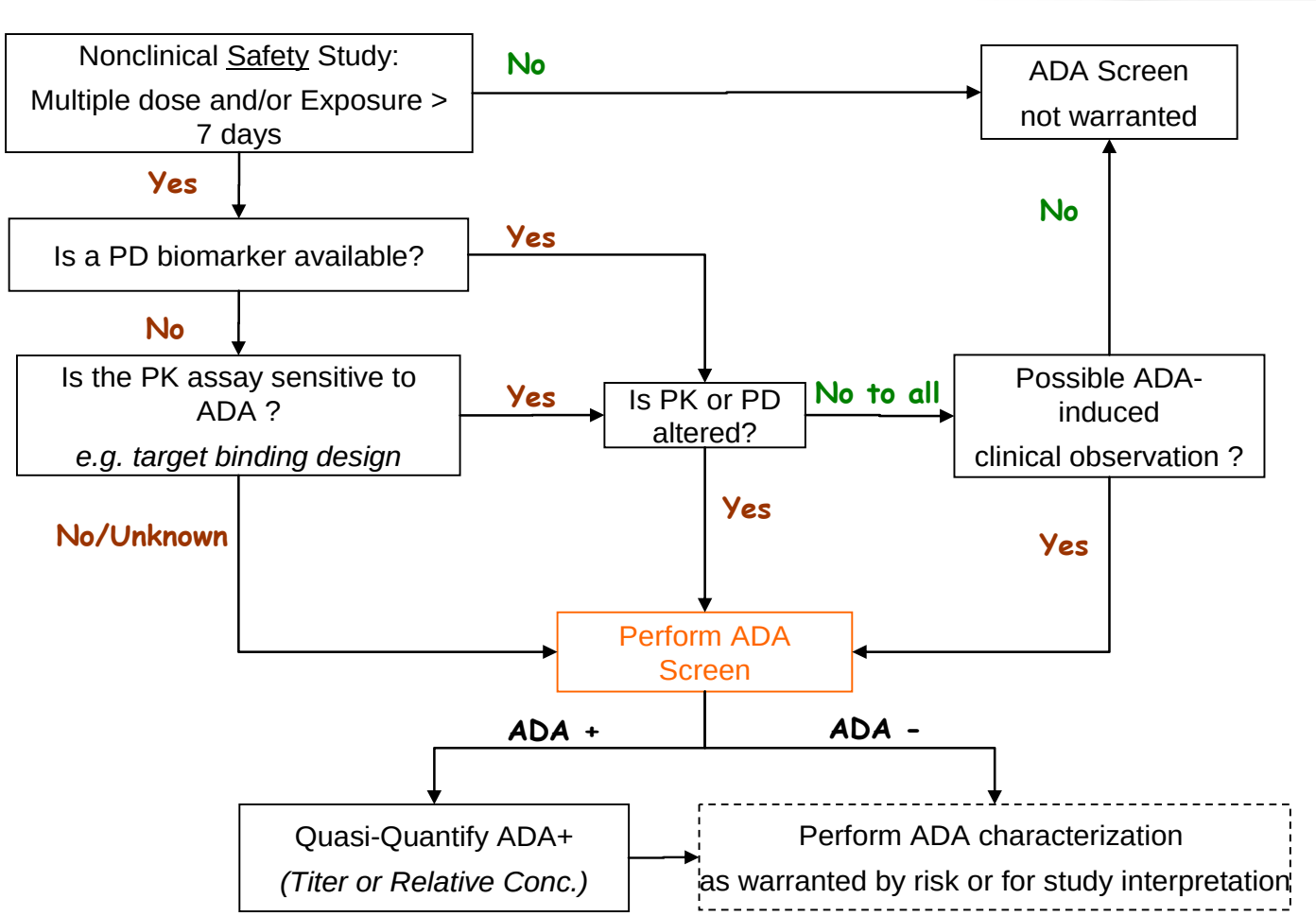
Decision Tree

(Ponce et al.)



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Causes of Immunogenicity of Biologic Products

- AA sequence differences between therapeutic protein and endogenous protein
- Glycosylation differences
- Structural alterations
  - Aggregation
  - Oxidation
  - Deamidation and degradation
  - Conformational changes
- Storage conditions
- Production/purification
- Formulation
- Route, dose and frequency of administration
- Immune status of patient / animal
- Genetic background

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Immunoassay Platforms for Detecting Antibodies

- ELISA
  - Bridging format
  - Direct format
  - Indirect format
- Radioimmune precipitation
- Surface plasmon resonance
- Electrochemiluminescence

- Early immune response
  - Typically IgM, low affinity and concentration
  - Difficult to detect
- Increasing immune response with repeated doses
  - T-cell help is needed for class switching and affinity maturation is required for a robust immune response
- High affinity mature IgG antibodies are more likely to neutralize effects of therapeutic proteins
  - Likely to be produced at a higher concentration

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