Toxicokinetics for Pharmaceuticals and Biologics

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OBJECTIVES

- To provide some points to consider for regulatory affairs professionals regarding the use of toxicokinetic (TK) data, including those used for the overall determination of bioequivalence/biosimilarity
- To cover the “why” of toxicokinetics, not just the “how”

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Biotechnology-Derived Products (Biologics)

- Recombinant proteins
- Monoclonal antibodies
- Vaccines
- Gene transfer products
- Somatic cell therapy products
- Synthetic peptides
- Oligonucleotide-based technologies
- Biologics (blood and blood products, toxins, antisera, allergen extracts, *etc*.)

What is Toxicokinetics?

- **The use of pharmacokinetics to determine the relationship between the systemic exposure of a compound in animals and the toxicity profile**
- **Pharmacokinetics: "how a substance gets into the body and what happens to it in the body"**
  - Absorption
  - Distribution
  - Biotransformation (Metabolism)
  - Excretion

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What is Toxicokinetics? Cont.

- **Severity of toxicity – disposition and biological activity**
- **Disposition:**
  - Duration and substrate concentration at entry point
  - Rate/amount of drug absorbed
  - Body distribution and concentration at specific body sites
  - Biotransformation – nature of metabolites
  - Ability of molecule to interact with specific cell components
  - Amount/duration of storage of molecule in body tissues
  - Rate/site of excretion

Why Toxicokinetics?

- **Difficult to extrapolate the effects observed on a mg/kg (or mg/m^2) basis in the preclinical species to humans, without additional information on the ADME profile of the drug**

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Objectives of Toxicokinetic Endpoints in Nonclinical Studies

- Describe systemic exposure achieved in toxicity studies (*i.e.*, determine the TK profile)
- Relate exposure to findings observed in preclinical studies to evaluate toxicity
- Assist in determining whether findings in animals are relevant to humans prior to initiating clinical trials
- Provide justification for species, sex, dosing frequency, and study designs
- Good scientific approach

Basic Principles of Toxicokinetics

- Use of mathematical models to quantitate time course of drug absorption and disposition in animals
- PK studies – low pharmacological doses, linear kinetic process
- TK studies – high doses, susceptible to drug solubility problems, often nonlinear
- A model to estimate concentration and general parameters
- Data serve to bridge across species, *in vitro* vs. *in vivo*, preclinical to clinical, and link between physiology and genetics and disposition

What Do Toxicokinetic Data Tell Us?

Early Development (Discovery to Phase I/II)

- "understanding" of the drug, metabolic fate, *etc.*
- Starting dose in clinic primarily based on mg/kg basis (*i.e.*, NOAELs and appropriate safety factor)
- Dose proportionality
- Gender profile
- Correlate toxicity and systemic effects

Later Development (Phase II/III to Registration)

- Assume that there is a significant amount of clinical data
- Comparisons of toxicity profiles during this stage of clinical development are driven by an AUC-based rationale
- Primarily used for labeling purposes, *e.g.*, carcinogenicity studies, reproductive/toxicity studies

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When/Why Should You Carry Out Toxicokinetic Evaluations?

- Assist in interpretation of toxicity studies
- Aid in dose selection for next toxicity studies
- Understand exposure-response assessments
- Facilitate cross-species comparisons
- Determine whether additional toxicity studies are required
- Repeated-dose kinetic data
- Included in the design of distribution studies

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How Should Toxicokinetic Evaluations be Designed?

- Adhere to principles of GLP (if part of a GLP study)
- Assess exposure to parent and metabolites (when appropriate) in systemic compartments
- Use justified sampling time points (*i.e.,* sufficient numbers (~6 to 8) to estimate exposure)
- Use appropriate numbers of animals and dose groups
- Males and females (if both used in toxicity study)
- Not concerned with achieving high statistical precision
- Use specific, accurate, and precise bioanalytical methods
- Methods are validated and conform to GLP

Additional Study Design Considerations

- **The extent of TK data required is often dependent on the toxicity profile**
- **If well-defined target organ toxicity, plasma TK used to obtain information to "interpret toxicity findings and determine the margin of safety"**
- **If poor correlation between systemic exposure and toxicity, consider target organ TK studies**
- **Other points to consider**
  - Route, age of animals, dosing frequency, satellite vs. main toxicity study animals
- **Goal: collect TK data in toxicity study using route and schedule ~ human use**

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Measuring Toxicokinetics: Factors to Consider

- Matrix: plasma (common) *vs.* whole-blood or serum (less common)
- <10% of circulating blood volume can be taken for analysis
- Exposure based on active entity (not salt)
- Racemate *vs.* enantiomer analyte
- Non-linear dose kinetics
- Parent (always) *vs.* metabolite (rarely) analysis
- Pro-drug: metabolite is the active entity
- Drug metabolized to pharmacological or toxicological relevant metabolites
- Extensive metabolism, systemic exposure based on major metabolites
- Human metabolite not identified in animal studies

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How are Drug Concentration Data Obtained and What Parameters are Determined?

- **Plasma/Whole Blood/Serum Levels Can Be Measured By:**
  - HPLC (UV, fluorescence)
  - HPLC-MS, HPLC-MS-MS
  - ELISA
  - Capillary electrophoresis (rarely, for proteins)
- **Parameters:**
  - C_{max}, AUC, T_{max}, $t_{1/2}$

How Should the Data be Reported?

- A stand-alone report that is included as an appendix in the toxicity study report
- A comprehensive description of the data generated

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Examples of How Toxicokinetic Profiles are Used to Assess and Interpret Toxicity

- Dose-dependent exposure
- Neutralizing antibodies
- Dosing regimen effects (daily *vs.* cumulative actions of drug)
- Metabolites
- Pro-drug

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Preclinical Studies that Typically Include Toxicokinetics

- General toxicity (acute, repeated-dose)
- Carcinogenicity
- *In vivo* genotoxicity assay
- Tissue distribution studies
- Development and reproductive toxicity

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Acute Toxicity Studies

- In rodents, blood samples may be collected and stored for possible TK analysis; however, it is more common for exposure data to be obtained in pilot PK studies (which can also be used to test different formulations, dosing regimens, *etc*.)
- In nonrodent MTD studies, it is more likely that TK will be a component of the study design (*e.g.*, to confirm exposure is adequate if an emetic effect observed)
- Justify selection of high-dose for subsequent repeated-dose toxicity studies (*i.e.*, if plateau in exposure is observed)

Repeated-Dose Toxicity Studies

- Assess whether dose or duration (Day 1 *vs.* last day of dosing) have any effects on systemic exposure
- Determine whether there is an induction/inhibition of systemic clearance
- Evaluate whether systemic exposure data support toxicity profile (if not, may need to obtain target organ exposure data)

Toxicokinetic-Based Approaches to Selecting High-Dose for Rodent Carcinogenicity Study

- **Assumption for use of TK-based approaches:**
  - compound is non-genotoxic (based on standard battery) and displays a low degree of toxicity
- **MTD often used to determine the high-dose for a carcinogenicity study, based on the results of a 3-month range-finding toxicity study**
- **It is recognized that there is a threshold for non-genotoxic carcinogens**

Toxicokinetic-Based Approaches to Selecting High-Dose for Rodent Carcinogenicity Study – Cont.

- **Option #1 : 25-fold multiple of human systemic exposure**
  - Metabolism (qualitative): rodents ~ humans
  - Adjust for plasma protein binding (especially if >80%, significantly greater in animals than in humans)
  - Systemic exposure based on parent drug; parent + metabolite; or solely metabolites
  - Human systemic exposure used for calculation is referred to as the Maximum Recommend Human Dose (MRHD)
  - High dose = 25 X exposure (AUC) at the MRHD

Toxicokinetic-Based Approaches to Selecting High-Dose for Rodent Carcinogenicity Study – Cont.

- **Option #2 : Saturation of Absorption**
  - Systemic exposure reaches a plateau for compounds that are poorly absorbed or have a receptor-mediated mechanism (note: as absorption occurs by passive diffusion for most drugs, it is not a saturable process)
  - Investigated by testing a wide range of doses (up to 1,500 mg/kg or MFD)
  - Plateau if ≥ 20% increase in systemic exposure at next highest dose
  - In reality, should look at several different dose levels and may even need statistics to interpret the plateau
  - High dose: lowest dose which displays maximum systemic exposure
  - Important to demonstrate that limitations in systemic exposure not due to increased metabolism (*i.e.,* need to look for possible metabolites)

Comments Regarding Toxicokinetic Evaluations in Carcinogenicity Studies

- Ensure steady state equilibrium of ADME is consistent with TK profile in 3-month range-finding studies (typically achieved after about 6 half-lives)
- Do not need to calculate/determine AUC
- Typically 1-3 time points is adequate, which include T_{max} (peak) and T_{min} (trough)
- Monitor for first 6 months (*e.g.*, rats: 3 month and 6 months; mice: 1 month, 6 months)

In vivo Genotoxicity Studies

- **If result of *in vivo* assay is negative, need to demonstrate that it is not due to a lack of systemic exposure or exposure in target tissue**
  - Plasma levels
  - Bone marrow levels
  - Autoradiography of bone marrow
  - “Effects” on bone marrow (toxicity)

Tissue Distribution Studies

- **Tissue distribution data are valuable for interpreting target organ toxicity**
- **How would these data support target organ toxicity?**
  - Target tissue half-life > dosing interval by 2-fold
  - Half-life of parent drug/metabolite is significantly greater following repeated-dose *vs.* single dose
  - Unanticipated target organ toxicity (based on histopathology in short-term studies)
- **Duration:**
  - Only long enough to monitor the drug at steady state in target organs/tissues (~1-3 weeks)

Development and Reproductive Toxicity Studies

- Data from non-pregnant animals is useful to set dose levels
- Dependent on the extent of observed toxicity in range-finding studies (if low toxicity, may be justified to include exposure data)
- An expectation, but no requirement per se, to obtain TK data, with most companies getting this information at the start and end of gestation in teratology studies
- May need to use another species if placental transfer is not adequate

Metabolites

- Comments applicable to pharmaceuticals not biologics
- Quantitative differences are common between animals and humans
- Assumption that preclinical species used in toxicity studies have a qualitatively similar metabolic profile as humans (*e.g.,* based on results from liver slices, hepatocytes, hepatic microsomes)
- Qualitative differences are uncommon, but some reactions are limited to primates
- If you are not able to demonstrate exposure to human-specific metabolites, separate safety data may be required (*e.g.,* limited *in vitro* genotoxicity, subchronic toxicity studies in single species (rodent) with duration dependent on proposed clinical use (2 weeks-13 weeks), teratology in single species)

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Quantitation of Metabolites

- **Pro-drug converted to bio-active metabolite**
- **Highly potent metabolite**
- **Metabolites constitute predominant circulating drug related moieties**
- **Note: A "major metabolite" accounts for a significant proportion of the AUC of total drug-related entities (*i.e.*, >25% of total systemic exposure)**
  - If no impact on safety, then this metabolite is not considered important *per se*

Active Metabolites

Metabolic Activity:

- **Same as parent**
- **Different than parent**
- **A mixture of both processes**

Is an active metabolite important?

- **Determined based on:**
  - relevant systemic exposure of parent drug and metabolite (*e.g.*, AUCs)
  - Relevant potencies of parent and metabolite against desired pharmacological target and/or toxicological target.

Active Metabolites – Cont.

- Parent is a pro-drug
- Metabolite is next-generation drug
- Potency
- Selectivity
- Bioavailability
- Safety profile
- Half-life
- Distribution between plasma and tissues

Case Studies

- Toxicity studies to support a novel drug combination
- Proposed label change based on new reproductive toxicity data
- Modification of clinical development program
- Selection of doses for carcinogenicity study

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Case Study No. 1

- Combination of a marketed drug + Drug X
- FDA suggested that the Sponsor conduct a 1 mo. rat study
- Mortality (>90%) at 2 weeks at dose levels <u>not</u> associated with mortality for single use of each agent
- <u>Why?</u>
- 2-fold ↑ AUC for Drug X at Day 1, with no change in C_{max} and ↑ t1/2 (thus, would yield higher steady-state concentrations after repeated doses)
- Information used for establishing clinical monitoring procedures and adjusting starting doses in clinical trials

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Case Study No. 2

- **Clinical routes of administration:**
  - oral and IV (IV dose is 15-fold greater than oral on mg/kg)
- **Sponsor generated new reproductive toxicity data (oral teratology studies in rats and rabbits) and requested that FDA concur with the proposed labeling change**
- **Findings: no toxicity in F_0 generation**
- **Why?**
- **Exposure data:**
  - **high** oral bioavailability in **humans**
  - very **low** oral bioavailability in **rats**
  - exposure at highest oral dose level tested in rats = human clinical exposure following oral administration, but 50-fold lower than human systemic exposure following IV administration
- **Overall, data did not support a change in labeling**

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Case Study No. 3

- **Drug Y being developed primarily for use in women**
- **No gender-differences in toxicity profile or total systemic exposure**
- **Rat metabolism study**
  - Males: parent drug < 10% total exposure
  - Females: parent drug > 50% total exposure
  - Similar results by IV route
- **Male dogs also display rapid metabolism of parent drug (female dogs not tested)**
- **Phase I studies in healthy male volunteers and extensive PK studies in males carried out**
- **Kinetics in females first investigated in Phase II and ~6-fold greater systemic exposure than males**
- **Clinical development program would have proceeded much differently if additional preclinical studies carried out up front**

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Case Study No. 4

- 13-week dietary admix dose range-finding study in rats (male, female) submitted in support of proposed dose levels for carcinogenicity study
- High-dose based on MTD, but FDA aware of a separate oral gavage study in same strain of rats that displayed dose limiting toxicities at dose levels 3-fold greater than the highest level used in the dietary administration study
- TK data for dietary admix and gavage administration yielded equivalent toxicity at ~ equivalent systemic exposure levels (thus, if no TK data were available, high-dose would have had to rely on gavage data)
- FDA concurred with Sponsor's proposed dose levels

Toxicokinetics for Biologics

- General principles discussed to this point apply for both pharmaceuticals and biologics (biopharmaceuticals)
- Single- and repeated-dose toxicokinetics and tissue distribution studies are useful, mass balance studies are not
- Species differences can have a significant impact on dose-response relationships, data extrapolation, and risk assessment
- Use clinically relevant routes and regimens
- Measure systemic exposure

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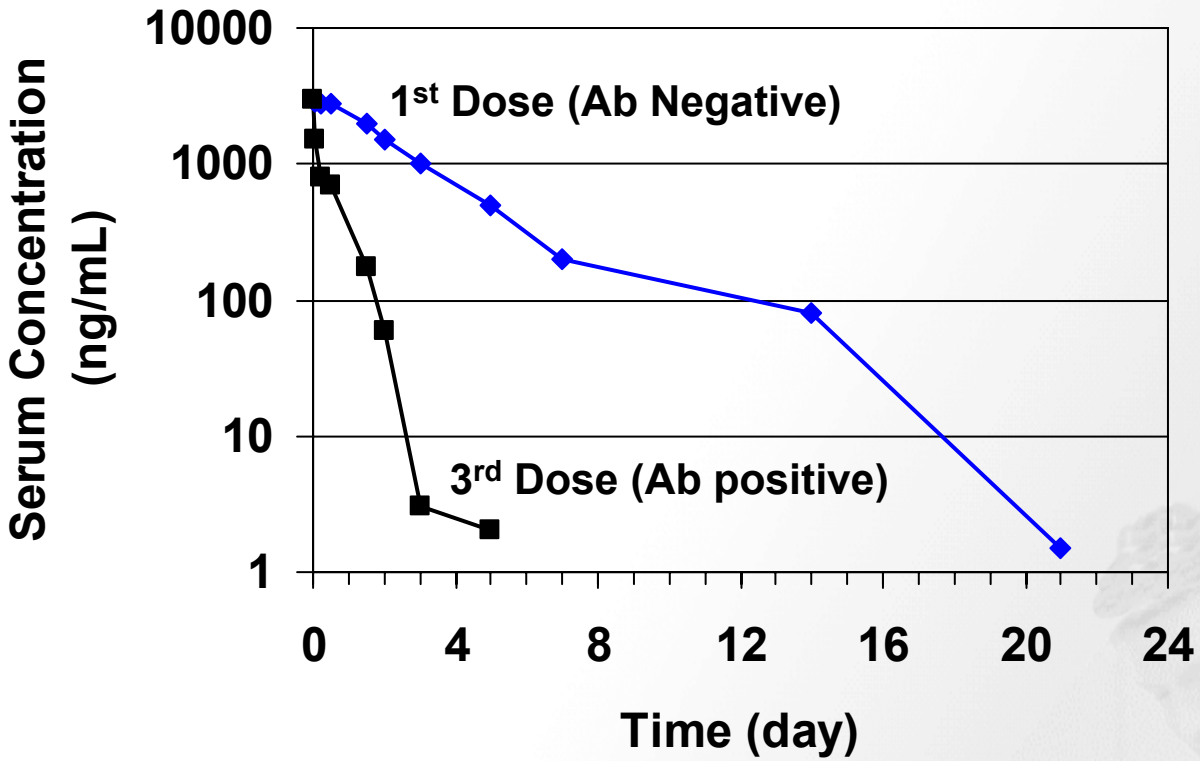
Immunogenicity

- **Assumed that most biologics for humans will be immunogenic in animals**
- **Antibodies generated must be measured and characterized to determine the potential effects on pharmacokinetics/toxicokinetics**
- **Clinically relevant anti-drug antibodies**
  - Clearing antibodies
  - Sustaining antibodies
  - Neutralizing antibodies
  - Antibodies that cross-react with endogenous proteins

Clearing Antibodies



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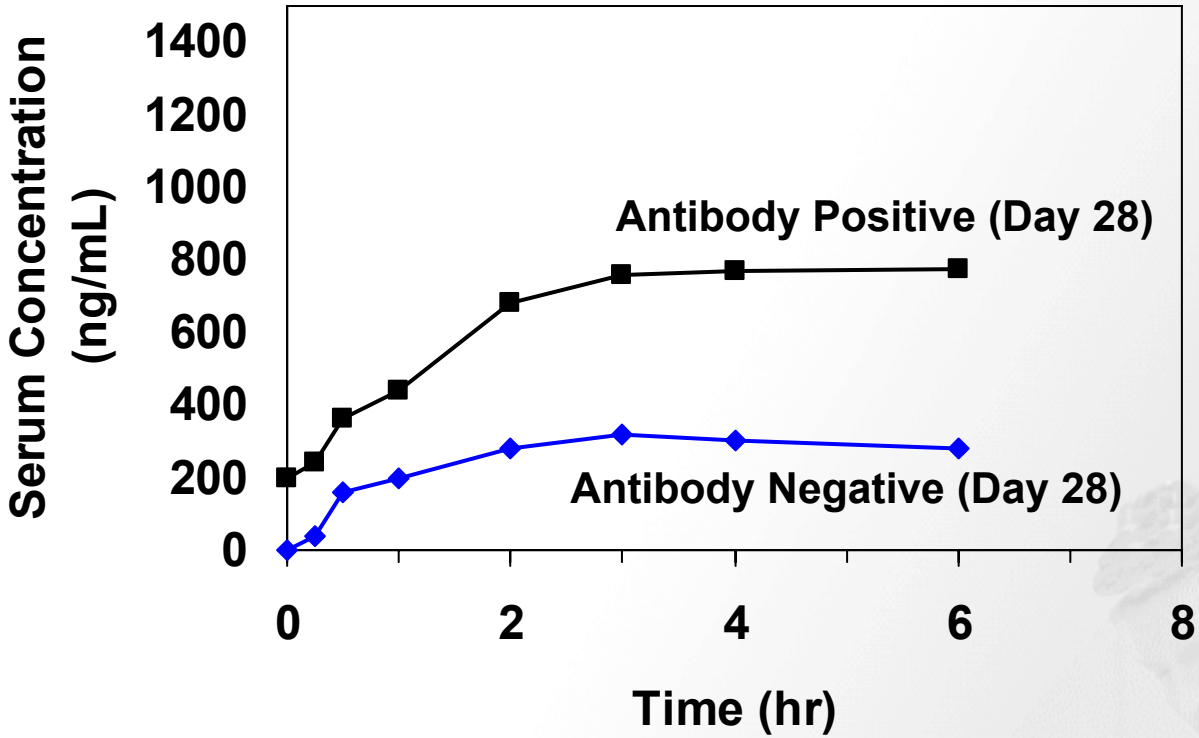
Sustaining Antibodies



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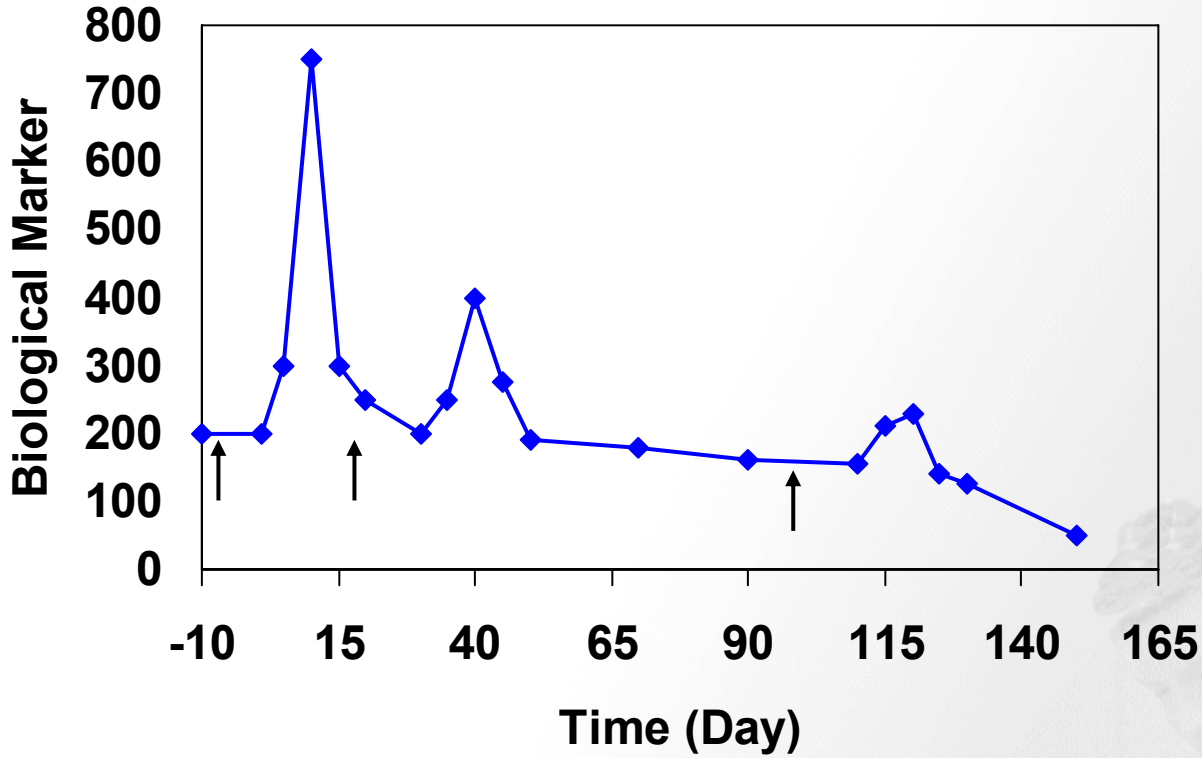
Neutralizing Antibodies



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Conclusion

- **Toxicokinetic data are an important component of the design and interpretation of toxicity studies for pharmaceuticals and biologics**

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THANK YOU!

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