Nonclinical Safety Evaluation of Biotechnology-Derived Therapeutics

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Presentation Overview

- History of biotechnology-derived products
- Fundamental differences between small and large molecule pharmaceuticals
- Challenges specific to biologics
  - Species specificity
  - Immunogenicity
- Alternative approaches/models
- Reproductive/developmental studies
- Studies in Nonclinical Programs
- Summary
Pharmaceutical Products

- Majority are small molecule (traditional) pharmaceuticals
  - Extensive experience and large historical database
    - Industry
    - Regulatory agencies (e.g., FDA, EMA)
  - Scientific and regulatory expectations for nonclinical toxicology studies are well defined
Pharmaceutical Products

- Biotechnology-derived products (Biologics)
  - Increased production over the last 25 years for use in various clinical indications
  - Relatively new (compared to small molecules)
  - Less experience (industry; regulatory agencies)
  - Smaller historical database (clinical and nonclinical)
Biologics: Definition

- Substances derived from living organisms
  - Humans
  - Animals
  - Plants
- Substances produced by biotechnology methods
The definition of biologics encompasses:

- Vaccines
- Blood and blood-derived products
- Anti-toxins
- Allergenics
- Cell, gene and tissue products
- Monoclonal antibodies
- Recombinant proteins
- Fusion proteins

Therapeutic proteins, biopharmaceuticals, biotechnology-derived products
Biopharmaceuticals

- Biologics (using recombinant DNA technology) initially developed in early 1980’s
  - Revolutionized the treatment of human disease by:
    - Mimicking or supplementing a human endogenous protein (growth hormone, erythropoietin)
    - Activating (agonistic) or blocking (antagonistic) a signaling pathway through specific receptor or ligand binding (monoclonal antibody, fusion protein)
Biopharmaceuticals: Examples

- **Humulin®** - first approved recombinant therapeutic protein was recombinant human insulin
  - Produced by genetically modified bacteria
  - Approved in 1982
  - Diabetes
- **Intron®** - interferon-α-2b
  - Approved in 1986
  - Hairy cell leukemia
- **Orthocline OKT-3® (muromonab)**
  - Anti-CD3 murine monoclonal antibody (IgG_{2a})
  - Approved in 1986
  - Renal transplant
Biopharmaceuticals: Examples

- **Enbrel® - Fc (IgG₁) fusion protein**
  - Approved in 1998
  - Specific to human tumor necrosis factor
  - Rheumatoid arthritis

- **Avastin® (Bevacizumab)**
  - Anti-vascular endothelial growth factor antibody (IgG1)
  - Approved in 2004
  - Colorectal cancer

- **Vectibix® (Panitumumab)**
  - Anti-epidermal growth factor receptor monoclonal antibody (IgG2)
  - Approved in 2006
  - Colorectal cancer
Biopharmaceuticals Vs. Small Molecules
Biopharmaceuticals Vs. Small Molecules

- Fundamental differences exist between small molecules and biopharmaceuticals
  - Differences influence the types of nonclinical toxicology studies to support the safety assessment
  - Guidance documents support different approach for the nonclinical toxicology studies (ICH S6(R1): Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals)
Biopharmaceuticals vs. Small Molecules

**Small Molecules**
- Manufactured by chemical processes

**Biopharmaceuticals**
- Derived from genetic manipulation of DNA
- Manufactured by living cells

**Low molecular weight**
- < 1000 daltons
- Potential for extensive distribution within the body

**High molecular weight**
- ≥ 1000 daltons
- High molecular weight proteins are largely confined initially to the vascular space
- More limited distribution within the body
Structure: Biopharmaceuticals Vs. Small Molecules

- $1^\circ$ structure
- Higher order structure
- Post translational modification
- Microheterogeneity

Statin
MW ~400 Da
Monoclonal Antibody MW ~ 150,000 Da
Consequence of Manufacturing from Living Cells– Comparability

- Complexity of protein structure results in microheterogeneity of final product
  - Glycosylation, deamidation, oxidation, disulfide bonds, free thiols, clipping, aggregates
- Manufacturing of biologics evolves with product development (i.e., frequent changes, scale-ups, etc)
- Since manufacturing changes may alter product, comparability assessments are frequently performed
- Comparability assessment ensures that the manufacturing changes have not affected the safety, identity, purity or efficacy, including immunogenicity, of the product
Consequence of Manufacturing from Living Cells– Comparability

- Analytical and functional assays performed (quality attributes/ CMC issues)
- If product comparability cannot be established with quality assessments, preclinical studies may be required
  - PK assessments
  - PD assessments
  - Toxicology studies
- Product used in nonclinical studies not required to be “identical” to that going into the clinic, but does have to be “comparable” in order to extrapolate the preclinical safety data to the clinical scenario
Biopharmaceuticals Vs. Small Molecules

- **Small Molecules**
  - Metabolized by Liver
  - Biotransformation studies
    - Metabolites identified
    - Potential for active or toxic metabolites

- **Biopharmaceuticals**
  - Catabolized by body (proteolytic degradation)
    - Amino acids
    - Small peptides
  - Active or toxic breakdown protein products generally not a concern
  - Biotransformation studies generally not performed

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**Figure No. 1: DRUG METABOLISM PATHWAYS**

- Oxidation (Cytochrome P450's)
- Conjugation (Glucuronidation etc.)
- Metabolite
- Polar Species
- Renal Elimination (Urine)
- Stable Adducts
- Non-polar Species
- Biliary Elimination (Stool)
Biopharmaceuticals Vs. Small Molecules

- **Small Molecules**
  - Drug-drug interactions potential concern
  - Half-life relatively short
    - Minutes
    - Hours
    - Few days
  - Impurities generally consist of:
    - Organic residuals
    - Solvents
  - Toxicity studies for impurities may be necessary

- **Biopharmaceuticals**
  - Drug-drug interactions are less of a concern
  - Half-life can be long
    - mAb-Days to weeks
  - Impurities generally consist of host cell molecules:
    - DNA
    - Protein
  - Toxicity studies for impurities usually not needed
Biopharmaceuticals Vs. Small Molecules

- **Small Molecules**
  - Less target specificity *(compared to biologics)*
  - Toxicities often non-specific/not related to the intended target
    - “Off-target toxicity”

- **Biopharmaceuticals**
  - High target specificity
  - Toxicity often related to intended target/pharmacological action of the drug
    - “On-target toxicity”
    - Exaggerated pharmacology
Biopharmaceuticals Vs. Small Molecules

- **Small Molecules**
  - Immunogenicity generally not a concern
  - Species limitations generally not a concern
    - 2 species usually used for toxicology studies
      - 1 rodent; 1 non-rodent

- **Biopharmaceuticals**
  - Immunogenicity is often a challenge
  - Species limitations may be a challenge
    - Only 1 relevant animal species may be available
Properties of Biologics that Can Influence Nonclinical Toxicology Programs

- High degree of target and species specificity
- Immunogenicity
Species Specificity
Properties of Biologics: Species Specificity

- Most biopharmaceuticals are:
  - Human proteins that are highly targeted to a human receptor
  - Antibodies specific for a human protein or receptor

- Due to high specificity to the human target, many biopharmaceuticals do not recognize the target in all animal species commonly used in nonclinical toxicology studies.
  - Mouse, rat, rabbit, dog, minipig, monkey
Species Specificity

- Toxicology studies for biopharmaceuticals should be conducted in a pharmacologically relevant species (ICH S6(R1)).

- Relevant species is one in which the biopharmaceutical is pharmacologically active due to the expression of the intended target (e.g., receptor or epitope).

- Toxicology studies in non-relevant animal species (one that does not express the target) can be misleading and are discouraged (ICH S6(R1)).
  - Unreliable safety data
  - Not predictive of potential human toxicity
Species Specificity

- How to demonstrate relevant species:
  - Sequence identity of target
  - Target expression and distribution
  - Binding assays
    - Biacore, flow cytometry, ELISA
  - Functional bioassays
    - Cell proliferation, signal transduction modulation, enzyme changes
  - In vivo pharmacology
Species Specificity

- There are cases when only one relevant animal species can be identified
  - Most often nonhuman primates (e.g., cynomolgus monkeys)
  - One species is acceptable with sufficient justification
Species Specificity – Nonhuman Primates

- NHP often the only pharmacologically relevant animal species for toxicology studies
  - Old world NHPs
    - Cynomolgus monkey
      - Most common NHP used (larger historical database)
    - Rhesus monkey
  - New world NHPs (marmosets)
    - Limited historical database for toxicity studies
    - Very sensitive to disruptions in environment
    - More often used for PK studies
  - Great apes
    - Chimpanzees are NEVER appropriate for toxicology!
      - Limited data obtained (*endangered species*)
      - Small animal number and large variability
      - Difficult data interpretation
      - Experimentally naïve animals not available
Challenges Using Nonhuman Primates

- Various challenges using NHP as the animal model in toxicology studies
  - Limited availability \textit{(compared to rodents and canines)}
    - Contact CROs early to place study
  - Limited number of animals/group \textit{(compared to rodents/canines)}
    - Can make data interpretation difficult
  - Cost is high \textit{(compared to rodents/canines)}
    - particularly sexually mature animals!
  - Background and/or sub-clinical disease \textit{(viruses, bacterial, parasites)}
    - Hepatitis, malaria, shigella
    - Impact data interpretation
    - Particularly if the molecule is an immunosuppressive
      - Are observed changes due to pre-existing infection or molecule?
Challenges Using Nonhuman Primates Cont.

- NHPs of different origins
  - Indonesian vs. Chinese origin
- NHPs are heterogenous
  - Compared to inbred mice or rats
  - Increases variability in data
Species Specificity

- There are cases when NO relevant species can be identified
  - Specific for human or human and chimpanzee
  - Alternative approaches can be used to identify potential safety risks
- Alternative approaches:
  - Surrogate/analogous proteins
  - Transgenic animals
  - Knockout animals
  - Animal models of disease
Immunogenicity
Immunogenicity

- Immunogenicity = immune response against the product, resulting in production of anti-product antibodies.
- Biopharmaceuticals are often immunogenic in animals.
  - Abbreviations that you may see:
    - ADA = anti-drug antibodies
    - RAHA = rat anti-human antibodies
    - PAHA = primate anti-human antibodies
    - MAHA = monkey anti-human antibodies
Immunogenicity

- Types of anti-product antibodies
  - Binding
    - Minimal to no impact on product exposure
  - Clearing
    - Increase clearance of product which decrease systemic exposure of product
    - Can alter distribution of product to tissues
  - Sustaining (rare)
    - Decrease clearance of product which prolong half-life/exposure of product
    - Potentially lead to or increase toxicity
Immunogenicity

- Types of anti-product antibodies
  - Neutralizing
    - Interfere with product binding to target
    - Neutralize pharmacological activity of product
  - Cross-reactive with endogenous proteins
    - Neutralize the pharmacological activity of the product and the corresponding endogenous protein
    - Concern is for neutralization of activity of nonredundant system
Immunogenicity

- Anti-product antibodies can affect the outcome of a toxicology study
  - No effect
  - Decrease exposure by increasing clearance of the product or neutralizing the product’s activity
  - Sustain exposure by decreasing clearance (rare)
  - Cause adverse effects not directly related to the product (anaphylaxis, immune complex deposition)
  - Cross-react with the endogenous protein
Immunogenicity

- Included as an endpoint in biopharmaceutical toxicology studies
  - Aid in the interpretation of the study
- Primary concern is for the development of clearing and/or neutralizing anti-product antibodies
  - Lower exposure of the target organs to biopharmaceutical
  - Generation of misleading toxicity data
Immunogenicity

- Mere presence of anti-product antibodies is not a reason to terminate a study (does not invalidate the study)
  - Sufficient number of animals may be exposure to active product
  - Studies terminated when complete loss of exposure of activity of product and/or severe adverse reactions preclude additional dosing
Immunogenicity

- Various factors can influence the immunogenic potential of biopharmaceuticals
  - Route of administration
    - Inhalation > SC > IP > IM > IV > topical*
  - Duration of dosing
    - Typically repeated dosing is more immunogenic than a single dose
  - Product
    - Protein structure
    - Manufacturing processes (glycosylated vs. nonglycosylated)
    - Impurities (DNA, host cell proteins)
    - Aggregates (high order)

Immunogenicity

- Anti-product antibodies may make long term toxicity studies and reproductive and developmental studies difficult.
- Immunogenicity in animals is not necessarily predictive of human response (ICH/S6(R1)).
Immuneogenicity Case Study
Immunogenicity

- Study Design:
  - Human monoclonal antibody
  - Administered to cynomolgus monkeys
    - Intravenous injection
    - Once weekly for 4 weeks
- Development of clearing ADAs resulted in:
  - Increase drug clearance
  - Decreased exposure over time
Clearing Antibody: Impact on Exposure

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**Antibody negative**

**Antibody positive**
Alternative Approaches and Models
Alternative Approaches/Models

- In some cases, alternative approaches for the nonclinical toxicology studies for biologics are necessary
  - Product pharmacologically active in humans only
  - Anti-product antibodies imposed limitations on the ability to conduct comprehensive nonclinical safety studies
    - Clearing and/or neutralizing antibodies
      - Unable to maintain exposure/pharmacological activity throughout study
  - ICH S6(R1) – allows for flexibility so alternative approaches can potentially be used for the nonclinical safety assessment
Alternative Approaches/Models

- Homologous (Surrogate/Analogous) proteins
- Transgenic animals
- Knockout animals
- Animal models of disease
Homologous Protein

- Homologous proteins recognize the ortholog of the original human target
  - Animal models
    - Mouse (most common)
    - Rat
    - Cynomolgus monkey

- Advantage
  - Good model for molecules that cross-react only with human target
    - Most commonly used alternative method
Homologous (Surrogate) Protein

- ICH S6(R1)- homologous proteins should resemble the clinical candidate as much as possible with regard to:
  - Production process
  - Range of impurity and/or contaminant profile

- Necessary to characterize the homologous protein to ensure it is a suitable surrogate for the clinical candidate
Homologous (Surrogate) Protein

- Demonstrate surrogate has pharmacological mechanism similar to clinical candidate
  - Binding studies
  - Assays/biomarker of pharmacological activity
    - Downmodulation/upmodulation of receptor/target
    - ↑ or ↓ expression of cytokine(s)
    - ↑ or ↓ proliferation of certain cell populations

- Necessary to characterize PK and ADA responses of surrogate in animal species used in toxicology studies
Homologous (Surrogate) Protein

- Necessary to determine the dose of the homologous protein in your animal species that is pharmacologically equivalent to the human dose with the clinical candidate
  - Based on pharmacology and PK data
  - Data used to determine doses used for toxicology studies with the homologous protein
Homologous (Surrogate) Protein

- Challenges using an homologous protein:
  - It will never be your clinical candidate!
    - Differences from clinical candidate
      - Manufacturing process
      - Different impurity profile
      - PK and ADA profile
        - Example: lower exposure with surrogate due to high rate of ADA responses
      - Pharmacological activity
        - Potency equal to clinical candidate?
        - Mechanism of action similar?
Homologous (surrogate) Protein

- Challenges using an homologous protein:
  - No established criteria or regulatory guidance for pharmacology or toxicology studies
    - How rigorous should the assessment of the pharmacological/toxicological activities of the homologue be?
      - Aggregates
      - Host-cell proteins
    - How rigorous should assessment of the homologous material be?
  - Company/regulatory concerns
    - Suitability of homologue to characterize safety of clinical candidate when compared to human data
Homologous (Surrogate) Protein

- Challenges using an homologous protein:
  - Resource intensive!
  - Development & manufacturing resources needed (essentially developing a 2nd molecule!!)
    - Generation of material
    - Characterization of material
      - Impurities
      - Aggregates
      - Formulation excipients differ from clinical candidate
    - Studies to demonstrate comparability to the clinical candidate
    - Assay development for PK and ADA
Alternative Approaches/Models

- Transgenic animals (mice)
  - Animal model expressing human target
    - Often used in pharmacology studies
  - Advantage
    - Allows for evaluation of clinical candidate (unlike surrogate)
  - Limitations
    - Expensive to develop and maintain colony
    - Lack of historical data; data interpretation can be difficult
    - Limited lifespan; limited number of animals
    - Spontaneous pathologies can be associated with having the gene expressed
    - May be difficult to produce mice with consistent expression and tissue distribution of the human target
Alternative Approaches/Models

- Knockout models
  - Evaluate the pathologies associated with not having the target expressed
  - Limitations
    - Expensive to develop and maintain colony
    - Lack of historical data
    - Limited lifespan
    - Limited animal availability
    - Other spontaneous pathologies associated with not having gene expressed
      - Develop immediately or over animal’s life span
    - Does not mimic therapeutic setting
Alternative Approaches/Models

- Animal models of disease
  - Allows for evaluation of general toxicity and undesirable promotion of disease progression during treatment with drug
  - Limitations
    - Lack of historical data
    - Limited lifespan or number of animals
    - Confounding effects of disease
      - May interfere with interpretation of the toxicological data
    - GLP compliance may be difficult due to the complexity of these studies
      - Often available in research facilities that do not have GLP capabilities
Reproduction and Development Toxicity Studies
Reproduction/Development Studies

- ICHS6(R1)- Studies performed according to ICHS5(R2); however, study design and dosing schedule may be modified based on species specificity, the nature of the product and mechanism of action, immunogenicity and/or PK behavior and embryo-fetal exposure

- Studies should only use pharmacologically relevant species, ideally with clinical product
Reproduction/Development Studies

- Studies should be performed in rat and rabbit, if pharmacologically relevant species.
- When no relevant species, alternative models may be explored.
- If weight of evidence (e.g., mechanism of action, phenotypic data from genetically modified animals, class effects, etc) suggests adverse effect on fertility or pregnancy outcome may not need to perform studies.
Reproduction/Development Studies

- If NHP only relevant species:
  - Only developmental studies are necessary
    - May use alternative model with scientific justification
    - Study may be conducted during Phase 3 and submitted at time of licensure, as long as appropriate clinical precautions are taken
  - ICHS6(R1) provides a design consideration for combined embryo-fetal and pre/post-natal development (PPND) study (referred to as ePPND)
Reproduction/Development Studies

- Effects on fertility can be assessed in repeat-dose toxicity studies of at least 3 months
  - Sexually mature NHPs
  - Evaluation of reproductive tract (organ weights and histopathological evaluation)
  - If concern, menstrual cyclicity, sperm count, sperm morphology/motility, and reproductive hormone levels can be evaluated
- Not recommended to develop homologous protein or transgenic model to conduct mating studies in rodents
- Studies are used for hazard identification
Nonclinical Studies to Support the Safety of Biopharmaceuticals
Biopharmaceutical Nonclinical Programs

- Pharmacodynamic studies
  - Mechanism of action, proof of concept
- Safety Pharmacology Studies
  - Many times can be incorporated into the general toxicology studies (ICH(S)7a)
  - hERG assay not performed
- Pharmaco-/toxicokinetics
  - Absorption studies
  - Tissue distribution studies not typically performed due to limitations with data interpretation
  - Metabolism/ excretion studies not typically performed (catabolism)
Biopharmaceutical Nonclinical Programs

- General toxicology studies
  - Program designed based on properties of the biopharmaceutical
- Human Tissue Cross-reactivity study (generally mAbs only)
- Local tolerance studies
  - Incorporated into the general toxicology studies
- Genotoxicity assay
  - Not typically performed, inability of product to enter living cells
Biopharmaceutical Nonclinical Programs

- Reproductive and developmental toxicology studies
  - Study designs dependent upon relevant species, immunogenicity, mechanism of action, embryo-fetal exposure
  - If NHP the only relevant species- studies are more complicated
    - ICHS6(R1) provides design considerations

- Carcinogenicity studies
  - Weight of evidence approach
    - Published data, class effects, product biology and mechanism of action, etc
  - Studies may not be required and/or may not be feasible
Summary

- There are fundamental differences between biopharmaceuticals and small molecules.
- Properties of biologics can influence the approach and design for the nonclinical toxicology studies.
- Critical to conduct the appropriate study based on the properties of the product:
  - pharmacologically relevant species
  - Immunogenicity profile
Summary

- Consider using alternative models for the program when appropriate
  - Ex: mAb that cross-reacts only with human target
    - Develop surrogate antibody rather than conducting toxicity studies in non-relevant animal species
  - There is “no one program fits all” approach for biopharmaceuticals
Summary

- Approach for the nonclinical safety assessment strategy for a biopharmaceutical should be based on:
  - Scientific rationale
    - Relevant species, mechanism of action, study feasibility, other product properties
  - Indication/patient population
    - Ex: Oncology vs psoriasis
    - Ex: Women of child-bearing potential vs. post-menopausal women
  - Relevance of the studies for determining and understanding human risk
References

- Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (CBER, 1997)
- Raptiva®, FDA Review, Application Number 125075/0, approval 10/27/03
- Clarke, J. et al (2003). The Toxicologist #361 pg 74
- www.bio.org
- www.fda.gov
Extra Slides
Homologous Antibody: Case Example
Case Example

- Efalizumab (Raptiva®)
  - Humanized IgG1 monoclonal antibody to human CD11a
    - Cross-reacts only with human and chimpanzee CD11a
  - Indication: moderate-severe plaque psoriasis
    - Men & women of childbearing age
    - Administered chronically
  - Chronic and reproductive toxicity studies necessary

- Developed surrogate antibody
  - Chimeric rat/mouse anti-mouse CD11a antibody
    - muM17
    - Toxicology studies to support registration were conducted in CD-1 mice

Raptiva, FDA Review, Application Number 125075/0, approval 10/27/03
Case Example Cont.

- Evaluation of muM17 as suitable surrogate antibody for efalizumab
  - Binding affinity to CD11a
    - efalizumab to human CD11a ~ 3.0 nM
    - muM17 to murine CD11a ~ 2.7 nM
  - Similar tissue binding properties
    - Efalizumab: human leukocytes in blood and spleen
    - muM17: mouse leukocytes in blood and spleen
  - Characterize PK/antibody responses of muM17 in mice
  - Demonstrate pharmacological activity of muM17 in mice similar to efalizumab in humans
    - decrease CD11a expression on T lymphocytes

Raptiva, FDA Review, Application Number 125075/0, approval 10/27/03
Case Example Cont.

- Determined dose of muM17 in mice that was pharmacologically equivalent to the efalizumab human dose
  - Based on CD11a downmodulation on T lymphocytes
    - Very sensitive biomarker on pharmacodynamic activity for efalizumab in humans and muM17 in mice
  - 3 mg/kg/week identified as minimal muM17 dose that maintained maximal CD11a downmodulation
    - Approximately equivalent to efalizumab human dose of 1 mg/kg/week
    - muM17 doses for toxicology studies
      - 3, 10, and 30 mg/kg (10-fold safety factor based on dose)
Case Example Cont.

- Toxicology program with muM17
  - Repeated-dose toxicity (up to 6 months duration)
  - Reproductive toxicity
    - Fertility
    - Embryo/fetal development
    - Peri/postnatal
- Immunotoxicology
  - Adult mice
  - Offspring born to dams administered muM17 during gestation and lactation
Transgenic Animal Model: Case Example
Transgenic Animal Model: Case Study

- **Kelixinab**
  - Human-cynomolgus monkey chimeric mAb directed against human CD4
  - Cross-reactivity limited to human CD4
  - Indication
    - Rheumatoid arthritis
    - Males and females of childbearing age
    - Chronic treatment

- **Developed human CD4 transgenic mouse**
  - Model (HuCD4/Tg mice)
    - Murine CD4 knockout, human CD4 knock-in

Case Study Cont.

- Necessary to characterize HuCD4/Tg mice to ensure suitable animal model for toxicology studies
  - Compare distribution of cells expressing human CD4 transgene in comparison to murine CD4
  - Demonstrate pharmacological activity of keliximab in HuCD4/Tg mice
    - Depletion of HuCD4+ lymphocytes
  - Determine suitability of mice for chronic studies with regard to background pathologies and survival
  - Ensure immune system of mice was competent
    - Immunotoxicology studies
  - Developing/maintaining animal model resource intense!
Case Study Cont.

- Toxicology studies using HuCD4/Tg mice
  - Short term and chronic (6-month) toxicity studies
  - Male/female fertility
  - Embryo-fetal development
  - Immune function in F1 mice
    - Host resistance to infection
    - Immune function
      - Host resistance to infection
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https://www.surveymonkey.com/s/VNZC9MQ