HESI IMMUNO-SAFETY TECHNICAL COMMITTEE

On-demand Training Course

Nonclinical Development of Biologics

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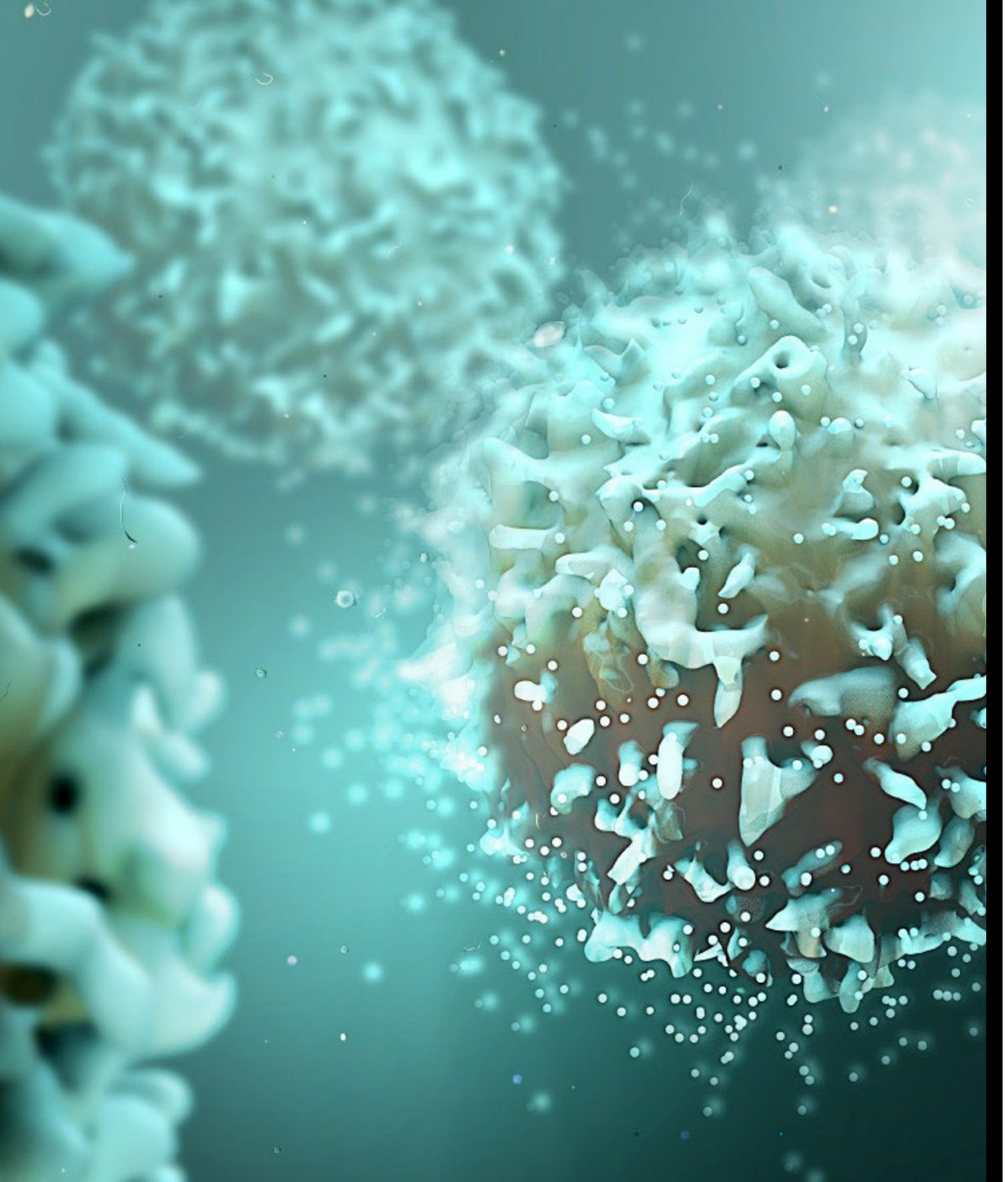
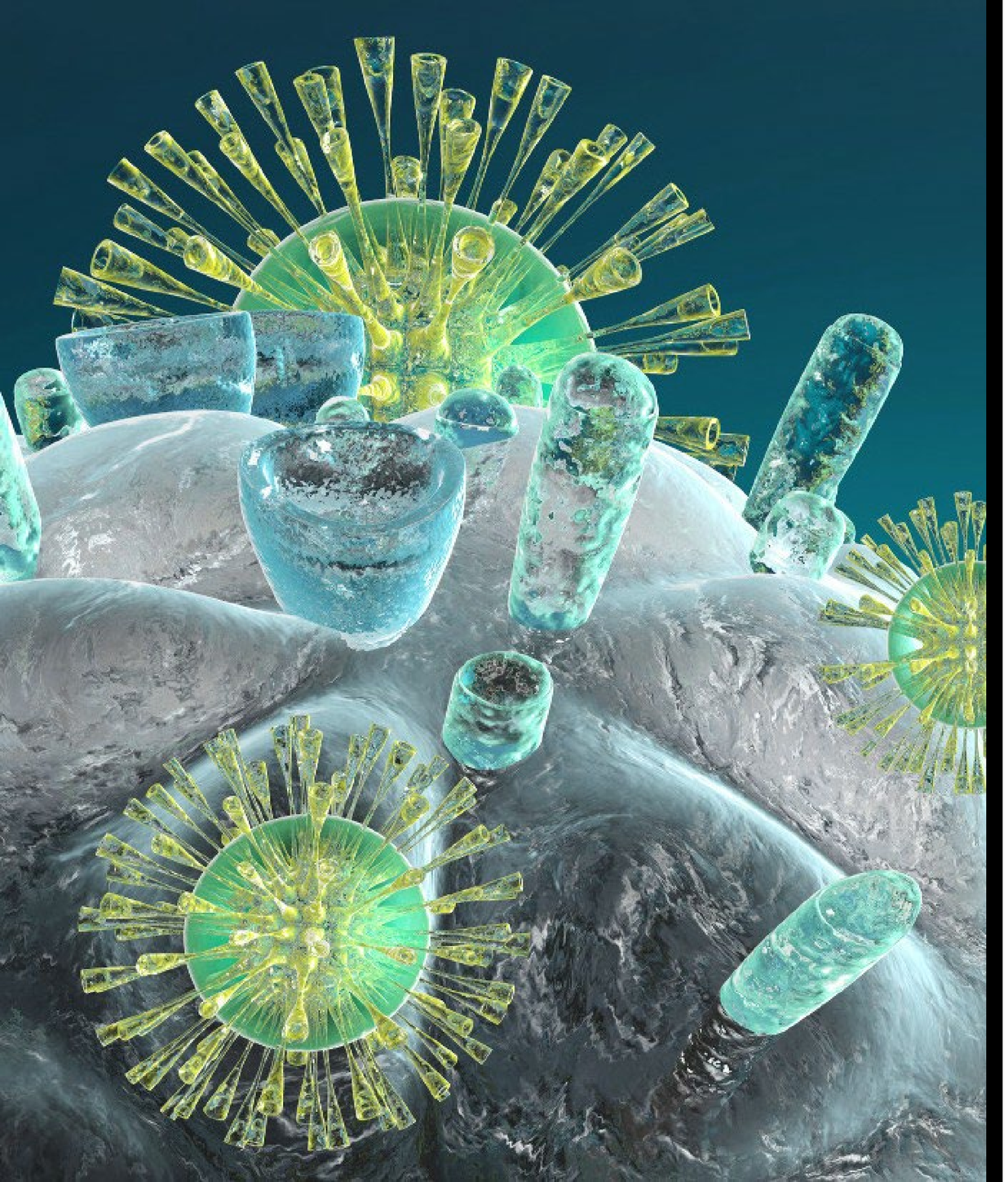


Learning Objectives

- To be able to identify the common themes for developing nonclinical programs across different biologic product types
- To understand at a high-level, types of nonclinical studies required for different biologic product types
- To discern the clinical relevance of immunogenicity and immune reactions for different biologic modalities



Outline of Presentation

- Definition of Biologic Products
- Regulatory Authority of Biologic Products at FDA
- Nonclinical Development
  - Common elements for all biologics
  - General program for therapeutic proteins, vaccines and cell and gene therapies
- Immunogenicity and Immunotoxicity

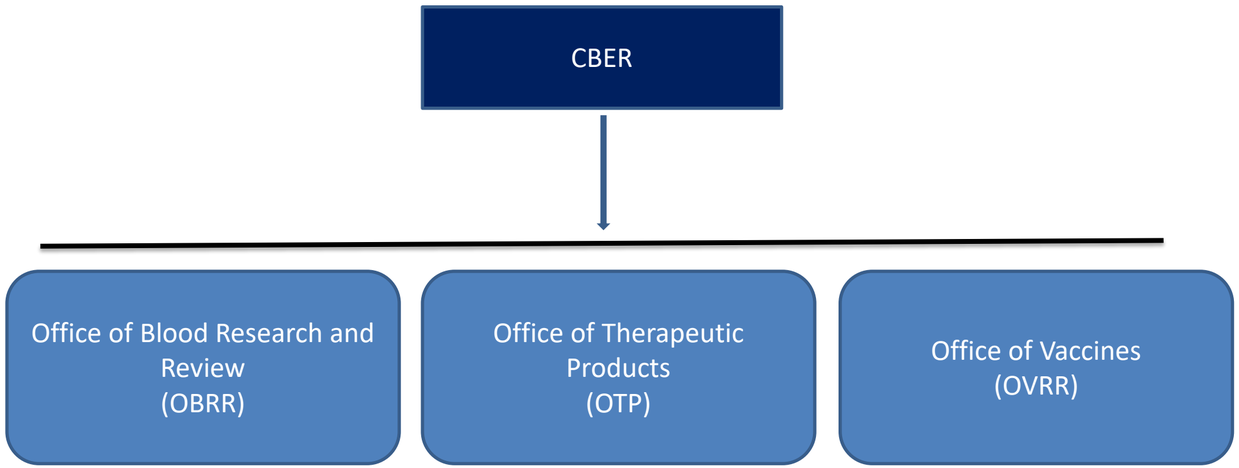
Biologics- Definition

- Biological Product- Section 351 of the Public Health Service (PHS) act (amended in 2020 [21 CFR part 600.3(h)]):
  - Virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound) applicable to the prevention, treatment, or cure of a disease or condition of human beings.

- Types of products
  - Blood-derived
  - Vaccines
  - Allergenic
  - Immunoglobulins (monoclonal and polyclonal antibodies)
  - Proteins
  - Cell and gene therapies
  - Live microorganisms

Center for Biologic Evaluation and Research (CBER)

- CBER regulates all biologics except therapeutic proteins (other than coagulation factors)

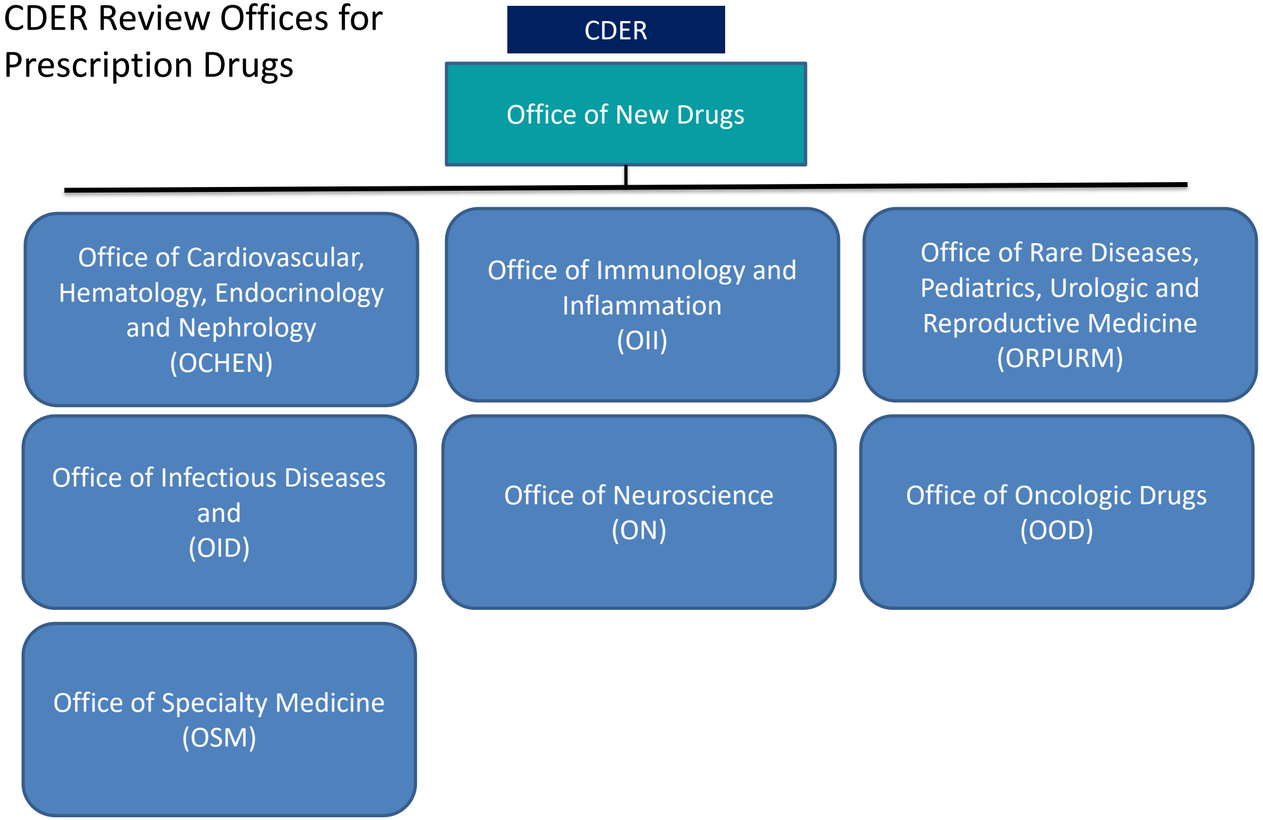


Types of Products CBER Regulates

- Cell and Gene Therapies
- Xenotransplantation products
- Therapeutic vaccines and other antigen-specific active immunotherapies
- Blood
- Tissues
- Allergenics
- Prophylactic vaccines
- Blood components
- Polyclonal antibody products against a single target

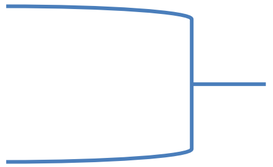
Center for Drug Evaluation and Research (CDER)

CDER Review Offices for Prescription Drugs



Types of Products CDER Regulates

- Synthetic small molecules
- Synthetic oligonucleotides (DNA and RNA)
- Recombinant and isolated proteins
- Monoclonal antibodies and derivatives



Therapeutic proteins, Biologics, Biopharmaceuticals, Biotechnology-derived products

Proteins

- Polymer containing > 40 alpha amino acids (associated with each other in a manner that occurs in nature), produced by recombinant DNA techniques in cells in vitro (ex: human, animal, insect and, bacteria) or in transgenic animals and plants or are purified endogenous proteins.
- Polymer 41-99 amino acids manufactured by chemical synthesis
- Proteins (> 40 amino acid) are regulated under 351 PHS Act and licensed as a Biologics License Application (BLA)
- Peptides (≤ 40 amino acids) are regulated under the FD&C Act as a New Drug Application (NDA), unless peptide meets definition of a biological product (e.g., peptide vaccine)

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Common Elements of Biologic Nonclinical Development

Common Elements Of Biologic Nonclinical Development

- Toxicities are typically associated with the pharmacology of a biologic
- Studies should be conducted in pharmacologically/biologically relevant species
  - Therapeutic protein: A species in which the product is pharmacologically active due to the expression of the intended target (e.g., receptor or epitope)
  - Vaccine: A species in which the desired immune response is produced
  - Cell Therapies: A species in which the product has pharmacological activity
  - Gene Therapies: A species in which the vector has appropriate tissue tropism and the transgene is pharmacologically active
- Non relevant species can result in safety data that is misleading

Common Elements Of Biologic Nonclinical Development

- Biologic products are specific to their targets, which can limit use in other species
- Due to species specificity, alternative models may be needed to evaluate the pharmacology and/or safety of a biologic product
  - Homologous (surrogate/analogous) proteins/products
    - Homologous proteins recognize the ortholog of the intended human target
    - E.g., mouse GMCSF, mAb to a mouse epitope, species-specific cell type, species specific transgene in gene therapy
  - Transgenics models
    - Animal model expressing human target
  - Knock out models
    - Evaluate the pathologies associated with not having the target expressed
    - Replacement of deficient genes

Common Elements Of Biologic Nonclinical Development

- Due to species specificity alternative models may be needed to evaluate the pharmacology and/or safety of a biologic product (cont.)
  - Disease models
    - Allows for evaluation of general toxicity and undesirable promotion of disease progression during treatment with product
    - Commonly used for cell and gene therapies
    - Rarely used for therapeutic proteins for safety assessments
  - In vitro primary cultures/organoids
    - Allows for evaluation of direct effects of product
    - E.g., CAR-T products, other genetically modified immune cells, tumor oncolytics

Common Elements Of Biologic Nonclinical Development

- Immunogenicity/immune response against product
  - Majority of biologics: Immune response against the product, resulting in production of unwanted anti-product antibodies
    - Can dramatically alter nonclinical development plans for a biologic
  - Vaccines: Part of the mode of action
  - Host immune rejection of a cell therapy

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Therapeutic Protein Development

General Information for Therapeutic Proteins

- Primary Guidances
  - ICHS6(R1): Preclinical safety evaluation of biotechnology-derived pharmaceuticals (2011)
  - Nonclinical evaluation of the immunotoxic potential of pharmaceuticals (Final June 2023)
  - Indication specific guidances
  - Timing of studies: ICHM3(R2): Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals (2013)

Examples of Therapeutic Proteins

- Growth factors
  - EGF, PDGF, erythropoietin, KGF
- Cytokines
  - Interleukins, Interferon-gamma, Interferon-beta
- Toxin fusion proteins
  - Diptheria toxin fused to IL-2
- Standard monoclonal antibodies (mAbs)
  - With or without mutations to modify Fc activity
- Bivalent mAbs
- Fc-fusion proteins
- Single chain antibodies (ex: camelids, diabodies)
- Antibody drug conjugates (ADCs)
- Immunocytokines, Immunotoxins
- Mixtures of 1 or more antibodies
- Antibody mimetics (anticalins, fynomers, etc)

Therapeutic Proteins: Nonclinical Programs

- Pharmacodynamic studies
  - Mechanism of action
  - Fc activities (ADCC, CDC, ADCP, other)
  - Cytokine release assay (if applicable)
  - Proof-of-concept
  - Selectivity/specificity
  - Relevant species assays
- Safety pharmacology studies
  - CNS, respiratory and cardiovascular endpoints- most often incorporated into the general toxicology studies (ICHS7a and ICHS6(R1))
  - hERG assay not performed

Therapeutic Proteins: Nonclinical Programs

- Pharmacokinetic Studies
  - Absorption studies (plasma/serum kinetics)
  - Tissue distribution studies- can be performed, but due to limitations with data interpretation not required
  - Metabolism/excretion studies not typically performed (catabolism)
- General toxicology studies
  - Program designed based on properties of the product
    - One rodent and one non-rodent toxicity study unless justified otherwise
  - Dosing durations: up to 6 months
- Human tissue cross-reactivity study (mAb)
  - Flexibility in the need for this study—depends on the product, indication, and Division of the US FDA

Therapeutic Proteins: Nonclinical Programs

- Local tolerance studies
  - Incorporated into the general toxicology studies
- Genotoxicity assays
  - Not typically performed, inability of product to enter living cells and interact with DNA (ICHS6(R1))
- Reproductive and developmental toxicology studies
  - Study designs dependent upon relevant species, immunogenicity, mechanism of action, embryo-fetal exposure

Therapeutic Proteins: Nonclinical Programs

- 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
- Other studies
  - Immunotoxicity Studies
  - Independent Immunogenicity Studies
  - Impurity Studies
  - Excipient Studies

Immunogenicity- Therapeutic Protein

- Immunogenicity= production of anti-product antibodies
- Types of anti-product antibodies
  - Binding
    - Minimal to no impact on product exposure
  - Clearing
    - Increase clearance of product, which results in decrease systemic exposure of product
  - Sustaining (rare)
    - Decrease clearance of product, which prolongs half-life/exposure of product

Immunogenicity-Therapeutic Proteins

- Types of anti-product antibodies (cont.)
  - Neutralizing
    - Neutralizes pharmacological activity
    - Can interfere with product binding to target
  - Cross-reactive with endogenous proteins
    - Neutralizes product activity and corresponding endogenous protein activity
    - Concern is neutralization of nonredundant systems (e.g., Pure red cell aplasia)

Immunogenicity- Therapeutic Proteins

- The affect of anti-product antibodies on the outcome of a toxicology study:
  - No effect
  - Decrease exposure by increasing clearance and/or neutralizing activity
    - Can interfere with interpretation of study
    - Can prevent longer duration toxicity studies
  - Sustain exposure by decreasing clearance
    - Potentially leads to or increases toxicity
  - Cause adverse effects not directly related to the product
    - Anaphylaxis/anaphylactoid reactions
    - Immune complex deposition
  - Cross react with endogenous protein and neutralize activity
    - Could induce severe toxicity

Immunogenicity-Therapeutic Proteins

- In toxicology studies, the primary concern is for the development of clearing and/or neutralizing anti-product antibodies
  - Lower exposure of the target organs to therapeutic protein
  - Generation of misleading toxicity data
- In general, immunogenicity response to a human protein in an animal is not predictive of a human response
- Immunogenicity response to a foreign protein likely represents the type of antibody response that may be mounted in humans
- Production of antibodies against a product is not in itself a toxicity; however, it can lead to toxicities

Immunotoxicity-Therapeutic Proteins

- ICHS6(R1) states “Immunotoxicological testing strategies may require screening studies followed by mechanistic studies to clarify such issues. Routine tiered testing approaches or standard testing batteries, however, are not recommended for biotechnology-derived pharmaceuticals.”
- Amount of immune toxicity testing for a therapeutic protein depends primarily on the pharmacology of the product (mechanism of action)
  - Example: Neutralizing an infectious agent or binding and inhibiting non-immune receptor vs binding and activating or inhibiting immune cells
- Also consider indication and patient population to determine appropriate level of immune toxicity testing.
- Routine general toxicity endpoints performed for all protein products may be all that is needed

General Toxicity Studies - Routine Endpoints

- Clinical observations
  - Increased infections
- Clinical pathology
  - Hematology: Total and differential leukocyte count
  - Clinical chemistry and coagulation
    - Acute phase inflammatory markers: decrease albumin and A:G ratio, increase in globulins and fibrinogen
- Organ weights, gross and histopathology
  - Changes in lymphoid tissue
    - Spleen, lymph nodes, bone marrow, thymus, etc

Immune modulators

- Immune modulator
  - FDA 2023 immunotoxicity guidance: pharmaceutical that is intended to alter the performance of a discrete component of the immune system to either stimulate or suppress specific immune system activities.
- Examples of mechanisms:
  - Directly bind to and stimulate or inhibit an immune cell activity

    OKT3: binds to CD3 on T cells and blocks function. Binding results in early activation of T cells, which leads to cytokine release, followed by blocking T cell functions.

Immune modulators

- Examples of mechanisms (cont):
  - Bind to and inhibit or stimulate a cytokine or other secondary molecule, which leads to immune cell modulation
    - Vedolizumab: binds to α4β7 integrin and blocks the interaction of α4β7 integrin with mucosal addressin cell adhesion molecule-1 (MAdCAM-1), inhibiting migration of memory T-lymphocytes across the endothelium into inflamed gastrointestinal parenchymal tissue
    - Certolizumab pegol: neutralize membrane-associated and soluble human TNFα
  - Bind to and block a receptor that releases the inhibition of immune cell response (e.g., check point inhibitor)
    - Nivolumab binds programmed death receptor-1 (PD-1) and blocks its interaction with PD-L1 and PD-L2, thereby releasing PD-1 pathway-mediated inhibition of the immune response, enhancing T cell activity

Immune Toxicity Specialized Endpoints

- In Vitro Assays
  - Assays to establish the pharmacological activity of the product
    - Examples: Immune activation/inhibition assays, cell migration assays, receptor occupancy, immune cell proliferation assay, ADCC, CDC, etc
  - Cytokine release assay
    - Unstimulated human immune cells (typically PBMCs, can use whole blood)
      - Plate-bound and soluble formats
- General Toxicity Studies
  - Immunophenotyping (blood)
  - Cytokine release (blood)
    - Proinflammatory: IL-1, IL-2, IL-6, TNFα, IFNγ
    - Interpret with caution as some species (monkeys) are known to be less sensitive to cytokine levels than humans

Immune Toxicity Specialized Endpoints

- General Toxicity Studies (cont.)
  - Immunohistochemistry of tissue sections
    - Identifies specific cell types in tissues
    - Defines immune-mediated pathology (immune complex deposition)

Other Immune Function Assays/Studies

- Other potential studies/tests
  - T-cell dependent antibody response (TDAR)
    - Assesses specific immunity to demonstrate immune competence
    - Primary IgM and IgG response to known antigen (e.g., keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT), sheep erythrocytes)
    - Secondary immune response upon boost
  - Delayed-type hypersensitivity (DTH)
    - Assesses cell-mediated immunity (Type IV delayed hypersensitivity reaction)
    - Antigen-specific reaction (e.g., TT)
    - CD4 and CD8 T cells and antigen presenting cells (macrophage and dendritic cells)

Other Assays

- Other potential studies/tests
  - Complement activation (blood)
  - T cell activity (ELISpot)
  - Natural killer (NK) cell cytotoxicity assay
  - Other innate immune cell activation assays
  - Immunophenotyping of spleen, thymus
  - Developmental immune toxicity studies

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Vaccines

General Information for Vaccines

- Primary Guidances
  - WHO: Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (2013)
  - Annex 1 WHO guidelines on nonclinical evaluation of vaccines (2005)
  - FDA guidance: Considerations for plasmid DNA vaccines for infectious disease indications (2007)
  - FDA guidance: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (2006)
  - WHO: Evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases: regulatory considerations (2020)

Vaccines

- Manufactured by living cells
  - Recombinant proteins, attenuated viruses, attenuated bacteria, plasmids, viral vectors
- Synthetic molecules
  - mRNA, DNA, peptides
- Adjuvants
  - MF59, Alum, CpG oligonucleotide, GMCSF, etc
- Carriers
  - Lipid nanoparticle, virus-like particles, etc
- Stimulation of humoral and/or T cell-mediated immune responses

Vaccine Nonclinical Development

- Pharmacology
  - Immunogenicity studies
    - Immune response characterization
    - Immune response durability studies
    - Interference studies if vaccine has more than one antigen
  - Challenge studies
  - Adjuvant studies
- Safety Pharmacology studies
  - Not typically performed, may be needed for novel adjuvants

Vaccine Nonclinical Development

- Distribution
  - Limited to specific product types
    - Ex: mRNA-LNP, Viral vector vaccines, live attenuated viruses administered by inhalation, novel adjuvants
    - Not performed for many vaccines
  - Pharmacokinetic, metabolism and excretion studies not typically performed

Vaccine Nonclinical Development

- Toxicology Studies
  - General toxicity study in one species
    - Relevant species should be used: Typical toxicity species is rabbits, but other species can be used depending on the product characteristics
      - Ex: ferret for influenza, hamster for Hantan, rat for CpG adjuvanted vaccines
    - Evaluate same absolute dose (mg) and same administration volume as human dose
    - N+1 duration
  - Novel adjuvant studies
    - Similar development to drugs or therapeutic proteins
  - Novel delivery device tolerability and toxicity studies

Vaccine Nonclinical Development

- Reproductive and Developmental Studies
  - One study that encompasses embryo-fetal and pre and post-natal endpoints (can also include fertility)
    - Typical species is rabbits, but other species can be used
    - Evaluate same absolute dose (mg) and same administration volume as human dose
    - Typically needed for licensure
- Other studies specific to product type of vaccine
  - Shedding, transmission, reversion (reassortment) potential, integration

Immunogenicity and Immunotoxicity- Vaccines

- Immunogenicity is the primary mode of action
  - Typically, humoral response but some stimulate T cell mediated responses
- Toxicity endpoints for immune toxicity (modulation)
  - Clinical observations
  - Administration site local tolerance
  - Body temperature
  - Hematology
  - Clinical chemistry and coagulation
    - Acute phase inflammatory markers: albumin, A:G ratio, globulins and fibrinogen
    - Special parameters: C-reactive protein (rabbits), others as needed (complement)
- Organ weights, gross and histopathology
  - Changes in lymphoid tissue
    - Spleen, lymph nodes, bone marrow, thymus, etc

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Cell and Gene Therapies

General Information for Cell and Gene Therapies

- Primary Guidances
  - FDA guidance: Preclinical assessment of investigational cellular and gene therapy products (2013)
  - FDA guidance: Long term follow-up after administration of human gene therapy products (2020)
  - Other FDA product and indication specific guidances
  - ICHS12 guidance: Nonclinical biodistribution considerations for gene therapy (March 2023)
- Nonclinical programs are tailored to the product characteristics, patient population and clinical development plans

Cell Therapies

- Manufactured by living cells or in vitro transcription
- Examples of cell therapies
  - Stem cell-derived cells
    - Adult (hematopoietic, mesenchymal, cardiac, neuronal, adipose)
    - Perinatal (placental, umbilical cord blood)
    - Fetal (amniotic fluid, neuronal)
    - Embryonic
    - Induced pluripotent stem cells (iPS cells)
  - Functionally mature/differentiated human or xenogeneic cell
    - Chondrocytes,
    - Pancreatic islet cells
    - Hepatocytes
    - Retinal pigment epithelial cells
    - Neuronal cells
    - Various immune cells

Gene Therapies

- Examples of gene therapies
  - Replication deficient viral vectors expressing various transgenes
    - Retrovirus, adenovirus, AAV, vaccinia virus, HSV, lentivirus, viral particles
  - Replication competent oncolytic vectors- many expressing transgenes
  - Non-viral vectors expressing various transgenes
  - Genetically engineered microorganisms expressing various transgenes
    - *Listeria, salmonella, clostridium, bacteriophage*, etc
  - *Ex vivo* genetically modified cells
    - CAR T cells, other immune cells, etc

Cell & Gene Therapy Nonclinical Development

- Pharmacology Studies
  - In vitro and/or in vivo mechanism/mode of action
    - Transgene expression kinetics/activity/durability
    - Cell differentiation, residual undifferentiated / partially differentiated cells
    - Functional cells vs other cells
    - Cell markers
    - Secreted molecules
    - Off target cutting
  - Proof-of-Concept Studies(typically in vivo)
    - Establish pharmacologically effective dose(s)
    - Optimize route of administration/dosing regimen
  - Safety pharmacology studies
    - Not typically done. Certain endpoints can be incorporated in animal studies if there is cause for concern
    - In vitro cell cytotoxicity assays with primary cultures or organoids

Cell & Gene Therapy Nonclinical Development

- Distribution and Persistence Studies
  - Vector/cell distribution and persistence
  - Cell phenotype and proliferation in tissues, cell migration/trafficking
- Toxicology (Safety)
  - General toxicity
    - One pharmacologically relevant species
    - Many programs are a mixture of safety endpoints in animal models of disease and/or toxicity studies in healthy animals
    - Duration of studies depends on the persistence of the product
  - Genotoxicity
    - insertional mutagenesis
  - Tumorigenicity/carcinogenicity
    - In vitro assays or in vivo animal study
  - Other studies
    - Delivery modality/route of administration
      - Invasive delivery procedure
      - Device compatibility

Immunogenicity and Immunotoxicity- Cell Therapies

- Cell Therapies
  - Immune-mediated cell rejection is a limitation for animal studies
  - Most animal studies are performed with chemically immune suppressed or genetically immune compromised animals
  - Some cell types do not stimulate an immune response (or use of analogous cell product) or carrier allows for assessment in immune competent animals
  - If an animal toxicity study can be performed
    - humoral responses against the product are evaluated (if possible), primarily to interpret the study
      - Parameters evaluated to determine effects on immune system similar to therapeutic proteins
        - Clinical observations, hematology, clinical chemistry, coagulation (when possible), gross and histopathology of lymphoid tissues, other parameters (as appropriate)

Immunogenicity and Immunotoxicity- Gene Therapies

- Gene Therapies
  - For genetically modified immune cells that modulate the immune system
    - Direct and indirect effects of the product's activity on the immune system is evaluated in vitro with human cells and/or in humanized hematopoietic mice
    - If an animal toxicity study can be performed (typically immune compromised animal)
      - Standard parameters evaluated: Clinical observations, hematology, clinical chemistry, coagulation (when possible), gross and histopathology of lymphoid tissues
    - Immunogenicity against the transgene is a concern
      - In many instances, risk assessments are performed and immunogenicity monitored in clinical trials

Immunogenicity and Immunotoxicity- Gene Therapies

- Gene Therapies (such as AAV, other viral/bacterial vectors)
  - Immune competent animals used for toxicity studies
    - Animals screened for pre-existing antibodies to the vector
    - Binding and/or neutralizing antibodies to the vector and binding antibodies and T-cell mediated responses to the transgene protein are monitored.
      - Primarily used to interpret the toxicity studies but may be useful to understand possible clinical effects
    - Parameters evaluated to determine effects on immune system similar to therapeutic proteins
      - Clinical observations, hematology, clinical chemistry, coagulation (when possible), gross and histopathology of lymphoid tissues
    - Additional parameters included as appropriate:
      - Cytokines, immune phenotyping, immunohistochemistry

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Blood Products

General Information for Blood Products

- Types of products regulated:
  - Hemoglobin-based oxygen carriers
  - Polyclonal immunoglobulin products to a single target
  - Fractionated plasma products
    - Alpha-1-Proteinase Inhibitor, Antihemophilic Factor, Antithrombin, C1 Esterase Inhibitor, Fibrinogen, Immune Globulins, Protein C, botulism antitoxin, etc.
  - Recombinant plasma proteins
    - coagulation factors, thrombin, fibrin, etc
  - Collection of blood and blood components used for transfusion

General Information for Blood Products

- Products used for therapeutic uses:
  - Follow general principles outlined in ICHS6(R1): Preclinical safety evaluation of biotechnology-derived pharmaceuticals (2011)
  - In general, nonclinical programs can be similar to those for therapeutic proteins (particularly for recombinant products)
  - Depending on product type focus can be on pharmacology and safety
  - Immunogenicity concerns in toxicity studies are the same as therapeutic proteins
  - Fractionated purified products- Common for robust immunogenicity to limit duration of toxicity studies
  - Changes in manufacturing routinely result in studies to determine if changes result in neoepitopes

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Summary

Summary

- Biologic products include blood-derived components, vaccines (prophylactic and therapeutic), allergens, immunoglobulins (monoclonal, polyclonal and fractionated), proteins, cell and gene therapies and live microorganisms
- Most biologics are regulated in CBER, with the exception of most therapeutic proteins which are regulated in CDER
- Primary guideline for nonclinical programs for therapeutic proteins is ICHS6(R1) (2011)
- Primary guideline for nonclinical programs for vaccines a WHO guidelines on nonclinical evaluation of vaccines (2005, 2013)
- Primary guideline for cell and gene therapies is Preclinical Assessment of Investigational Cellular and Gene Therapy Products (2013)

Summary

- Nonclinical programs for biologics consist of pharmacology, pharmacokinetic (primarily blood kinetics and tissue biodistribution) and toxicology studies
- Toxicology studies should be performed in a pharmacological/biological relevant species
  - Otherwise, results may be mis-leading
  - Alternative models may be needed to adequately evaluate potential toxicities
- The production of anti-product antibodies is an important consideration for all biologics
- In general, immunogenicity of a human biologic in animals does not predict immunogenic potential in humans
  - It can provide insight to the type of reactions that may occur
- Immunogenicity against a non-human biologic in animals is more likely to predict immune response in humans

Summary

- Primary utility of immunogenicity assessments for biologics, other than vaccines, in toxicology studies is for interpretation of the study
- Immunogenicity effect on a product: none, reduce exposure by increasing clearance or neutralizing activity or sustain exposure
- Immunogenicity can cause adverse effects in animals such as anaphylactoid reactions and immune complex deposition
  - Will not necessarily occur in humans
- Anti-product antibodies that cross-react against a non-redundant endogenous protein can have severe toxicological consequences
  - This may have clinical consequence depending on the protein identity of animal to human protein

Summary

- Evaluation of standard toxicity parameters may be sufficient to evaluate effects on immune system
- For products that modulate or target the immune system, additional studies may include:
  - Characterization of the intended pharmacological effects on the immune system
  - In vitro assessments with human immune cells, evaluating innate and adaptive effects
    - May not be primary part of the intended pharmacology but may be expected consequence based on product type
  - Inclusion of additional parameters in toxicity studies (body temperature, proinflammatory cytokines, immune phenotyping, etc)
- Nonclinical programs for biological products are developed on a case by case basis, depending on the product characteristics and indication