
AMERICAN COLLEGE OF TOXICOLOGY
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Regulatory and Scientific Considerations for the Nonclinical Safety Assessment of Prophylactic and Therapeutic Vaccines

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Presentation Outline

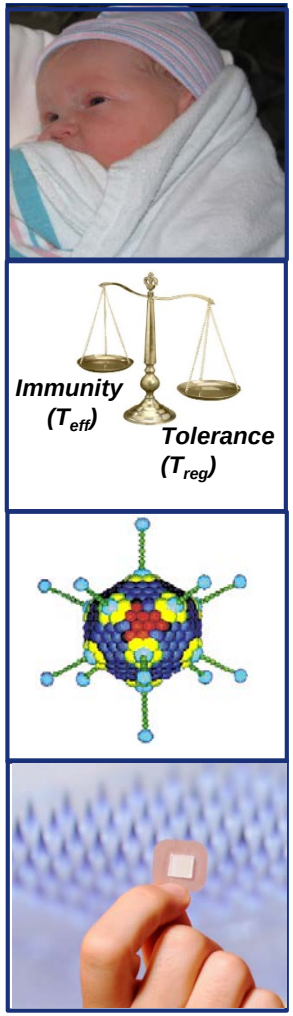
- Introduction to Safety Assessment of Prophylactic and Therapeutic Vaccines
  - Goals of Vaccine Safety Assessment
  - Regulatory Guidelines
  - Considerations for the Design of Toxicity Studies
  - Similarities and Differences in Study Designs for Prophylactic and Therapeutic Vaccines
- Case Studies
  - Allergy vaccine
  - Cancer vaccine
  - Infectious disease vaccine

Prophylactic and Therapeutic Vaccines

- Prophylactic Vaccines:
  - Stimulate specific immune responses to prevent the occurrence or severity of a disease.
  - Examples:
    - FLUAD® - for prevention against influenza disease.
    - PREVNAR 13® - for the prevention of invasive disease caused by *Streptococcus pneumoniae.*
    - VAQTA® - for the prevention of disease caused by Hepatitis A virus.

- Therapeutic Vaccines
  - Stimulate specific immune responses to control or ameliorate an active disease.
  - Examples:
    - IMLYGIC (talimogene laherparepvec)® - for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery.

Increased Emphasis on Nonclinical Safety Assessment of Vaccines



- Prophylactic vaccines are administered to healthy individuals, including infants
- Therapeutic vaccines may break immune tolerance to self proteins
- Novel vaccine types and adjuvants
- Novel delivery systems

Goals of nonclinical safety assessment for vaccines

- Assess the safety of doses proposed for the clinical studies in humans
- Identify potential target organs or systems at risk for toxicity, and reversibility; toxicity may result from:
  - Antigen / immunogen
  - Adjuvant(s) / immunomodulator(s)
  - Immune response
- Identify parameters for monitoring in clinical trials
- Caveats:
  - Rare sub-population toxicity can only be addressed in humans
  - Animal models are not always indicative of the effect in humans
    - Relevant species for therapeutic vaccines: Homology of the target between toxicology species and human must be considered

Vaccine Regulation by Food and Drug Administration

United States Food and Drug Administration (FDA)

- Center for Drug Evaluation and Research (CDER)
- Center for Biologics Evaluation and Research (CBER)
  - Office of Vaccines Review and Research (OVRR) — Regulates prophylactic and therapeutic vaccines for infectious disease indications
  - Office of Tissues and Advanced Therapies (OTAT) — Regulates therapeutic vaccines for cancer and chronic indications
  - Office of Blood Research and Review (OBRR)

Toxicology study plans can be discussed with FDA
[e.g. Type B or Type C meeting]

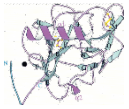
Regulatory Guidelines: Prophylactic and Therapeutic Vaccines for Infectious Diseases

- WHO Guidelines on Nonclinical Evaluation of Vaccines (2005)
- US FDA's Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (2006)
- US FDA's Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (2007)
- MHLW, Japan: Guideline for Nonclinical Studies of Vaccines for Preventing Infectious Diseases (2010)
- SFDA, China: Technical Guidelines for Preclinical Research on Preventative Vaccines (2010)
- WHO Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines (2013)

Regulatory Guidelines: Therapeutic Vaccines for Cancer and Chronic Conditions

- US FDA's Guidance for Industry: Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events (2006)
  - Provides specifics on preclinical biodistribution and persistence studies
- US FDA's Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines (2011)
  - One section on preclinical studies
- US FDA's Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (2013)
  - Mostly focused on cell and gene therapy products; one section on therapeutic vaccines
- EMA's Guideline on the Evaluation of Anticancer Medicinal Products in Man (currently under revision)
  - Two paragraphs on animal studies

Study Designs Must Consider the Complexities of Each Vaccine Type

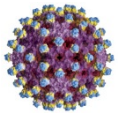

Recombinant Protein

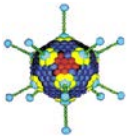

Synthetic Peptide

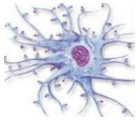

Virus Like Particle


Live Attenuated Microorganisms


Plasmid DNA


Inactivated Whole Microorganisms


Live viral vector


Modified dendritic or tumor cells

Many may also be administered using a device

Considerations in the Design of Toxicity Studies

- Selection of Animal Model
- Vaccine Formulation
- Route and Method of Administration
- Vaccine Doses
- Dosing Frequency
- Dose Groups
- Types of Nonclinical Toxicity Studies

Selection of Animal Model

- Single species is generally sufficient
  - One that elicits a strong immunogenic response against antigen and is responsive to the delivery vector (if one is used) and to any adjuvants used
  - For vaccines utilizing viral vectors, consider using a species that is permissive for the vector virus
  - For therapeutic vaccines:
    - Demonstration of a similar binding profile and effector function for the induced antibodies
    - Selected species should contain the target of interest
- Unlike most protein biotherapeutics, non-human primates (NHP) are not a default species
  - For prophylactic vaccines, NHP typically not used (rodent, rabbit)
  - For therapeutic vaccines, NHP may be required based on considerations of target, immune response, or adjuvant/immunomodulator cross-reactivity
- Disease models are typically not used for toxicology studies
  - For therapeutic vaccines, toxicology endpoints can be collected in pharmacology studies in disease models

Vaccine Formulation

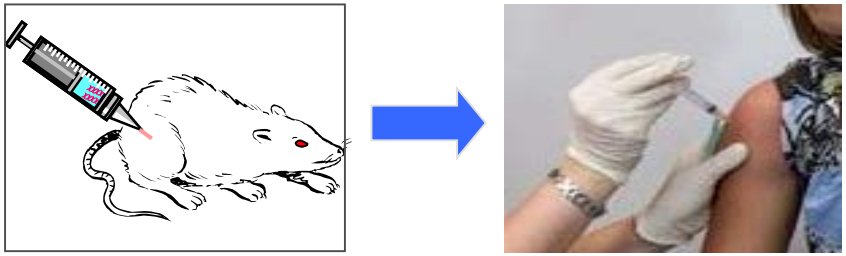
- GMP (clinical) lot preferred

- GLP lot equivalent to the clinical lot is also acceptable

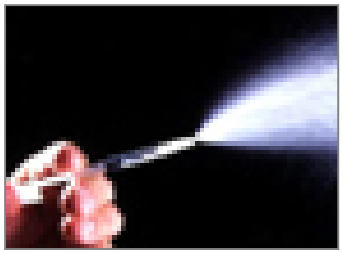
- Release, Characterization and Stability data required (Purity, Identity, and Potency)

Route and Method of Administration

- Use intended clinical route



- Use the same delivery device that is intended to be used in the clinic



Vaccine Doses

- Administration of a full-human dose equivalent is preferred
  - Total volume can be administered at more than one site
  - Alternatively, administer a dose that exceeds the human dose on a mg/kg basis
- For prophylactic vaccines, administer N+1 doses (N=number of clinical doses)
  - For example, if 3 doses are to be administered in humans, administer 4 doses in animals
- For therapeutic vaccines, administer at least N doses (N=number of clinical doses)
  - For example, if 3 doses are to be administered in humans, administer 3 doses in animals

Dosing Frequency

- Prophylactic vaccines:
  - Clinical dosing regimen generally involves large intervals (several months) between vaccine doses (e.g. months 0, 2, and 6)
  - Nonclinical dosing regimen: Interval between dosing should be based on the anticipated immune response (e.g. every 3 weeks)
- Therapeutic vaccines:
  - Clinical dosing regimen are usually more frequent (e.g. every 2-4 weeks)
  - Nonclinical dosing regimen should mimic the clinical dosing regimen
  - However, may require regular long term booster doses

Dose Groups

- Include a negative control group (e.g. saline-treated animals) for comparison with vaccine treated groups
- For adjuvanted vaccines, an adjuvant-alone control group is included (for adjuvants where limited toxicology data are available)
- Include the full-human vaccine dose level
- Can include lower vaccine dose levels, or alternative vaccine formulations that might be tested in clinical trials

Types of Nonclinical Toxicity Studies

Repeat-Dose Toxicity	Comprehensive antemortem and postmortem analyses
Local Tolerance	• Single and repeat-dose evaluations • Limited in-life toxicity parameters • Histopathological assessment limited to injection site

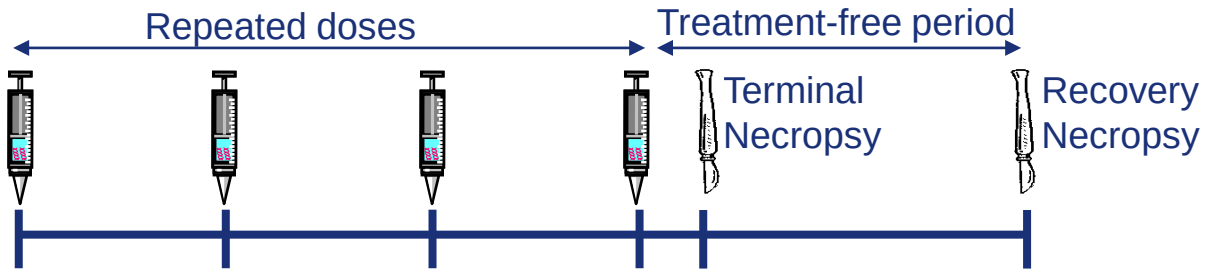
Note: Local tolerance evaluations can be included within a repeat-dose toxicity study

Types of Nonclinical Toxicity Studies (cont.)

Biodistribution and Integration Studies	For nucleic acid and viral-vector based vaccines
Genotoxicity and Carcinogenicity	Usually not needed for vaccines, but are needed for a new synthetic adjuvant that is considered to be a New Chemical Entity
Safety Pharmacology	May be needed if the vaccine is planned to be marketed in Japan or if there are findings in the repeat dose toxicity study that suggest impact to cardiovascular, CNS, respiratory or renal systems
Developmental And Reproductive Toxicity (DART)	Needed for vaccines administered to pregnant women or Women of Child-bearing Potential
Neurovirulence	Needed for vaccines that have the potential for reversion to virulence and neurotropic activity

Repeat-Dose Toxicity Studies

Conducted to examine the effects of repeated vaccine administration



General Principles:

- Conducted prior to clinical studies
- Performed in one relevant species
- If study is performed in rodents; both genders, N = 10/group
- Duration depends on the indication and clinical plan
- At least one dose level plus control groups (adjuvant-alone and PBS)
- Local tolerance and single-dose toxicity assessed within this study

Repeat-Dose Toxicity Studies (cont.)

Antemortem effects evaluated

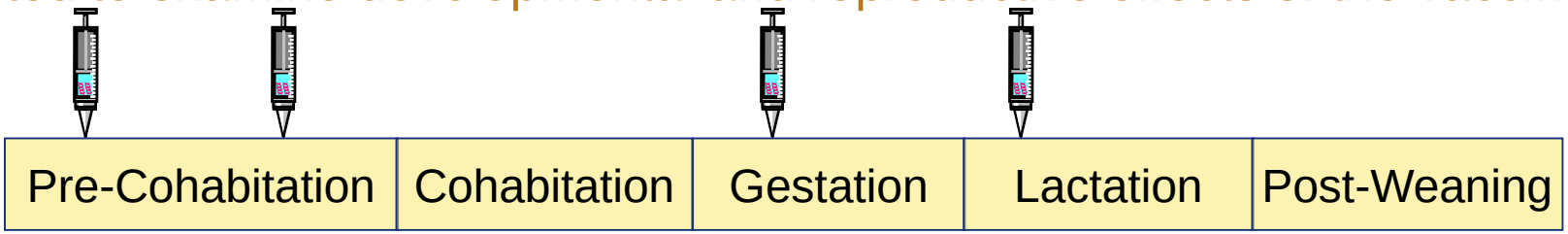
- Physical signs, body weight, food consumption
- Ophthalmic exams
- Clinical Pathology (after 1st and last dose and after recovery period): Hematology, serum chemistry, coagulation parameters, urinalysis
- Immunogenicity (prior to and 2-weeks after 1st and last doses)
- Draize scoring at injection site

Postmortem evaluation

- Necropsies: After the last dose, and after a treatment-free period
- Complete necropsy, organ weights, and tissue collection
- Complete histopathological evaluations
  - Routine tissue list
  - Includes immune system (local and distant lymph nodes, thymus, spleen, bone marrow, Peyer's patches)
  - Includes injection sites for local inflammatory reaction

DART with Post-Natal Evaluation

Conducted to examine developmental and reproductive effects of the vaccine



- Required if pregnant women or Women of Child-bearing Potential in target population
  - For vaccines indicated for maternal immunization, data need to be available prior to enrolling pregnant women in any clinical trial
  - For vaccines indicated for Women of Child-bearing potential, generally conducted concurrent with Ph III to support marketing application
- Typically conducted in species in which immune response demonstrated
  - If conducted in rodents, need at least 40 animals per group (20 C-section; 20 natural delivery)
- Vaccine administration:
  - Prior to mating (to elicit peak antibody production during early organogenesis)
  - During the period of organogenesis and in late gestation; during lactation
- Assesses: Maternal toxicity, fertility, embryo-fetal development, late gestation and lactation, and juvenile development

Repeat-dose Toxicity Study Designs: Similarities and Differences between Prophylactic & Therapeutic Vaccines

Prophylactic Vaccine (or Infectious Disease Therapeutic Vaccine)

- N*+1 doses administered
- Mimic clinical route of administration
- Full Human Dose administered
- Compressed dosing schedule

Therapeutic Vaccine

- Usually N* doses is sufficient
- Mimic clinical route of administration
- Dose levels should bracket and exceed the proposed clinical dose levels
- Mimic clinical regimen as closely as possible

*N = number of clinical doses

Case Studies

- Prophylactic vaccine case studies have been published* and previously presented in other forums
- The case studies presented here will focus on the following **therapeutic vaccines:**

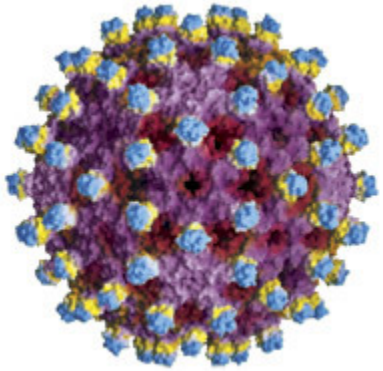
1. Allergy vaccine – David Clarke
2. Cancer vaccine – David Clarke
3. Infectious disease vaccine – Karissa Adkins

*Prophylactic Vaccine Examples:

- Zoster Vaccine (gE/AS01): G. Giordano et al. J Appl Toxicol. 2017 Feb;37(2):132-141.
- Malaria Vaccine: L. Segal et al. Regul Toxicol Pharmacol. 2015 Mar;71(2):269-78.
- HPV Vaccine: L.D. Wise et a. Birth Defects Res B Dev Reprod Toxicol. 2008 Dec;83(6):561-72.

Case Study #1

Considerations in the Design and Toxicology Strategy for a Vaccine Designed to Treat Moderate-Severe Asthma or Allergic Rhinitis



The Use of Vaccines for Chronic Diseases

- The use of mono-clonal antibodies in the control of chronic diseases is well established
  - Xolair® is currently used to treat individuals with moderate to very severe allergic asthma, poorly controlled by inhaled corticosteroids, or moderate to severe allergic rhinitis
- Potential to use a vaccine to elicit a polyclonal antibody to response that will have similar efficacy as the established mAb
  - Develop and anti-IgE vaccine that elicits antibodies that bind similar to Xolair®

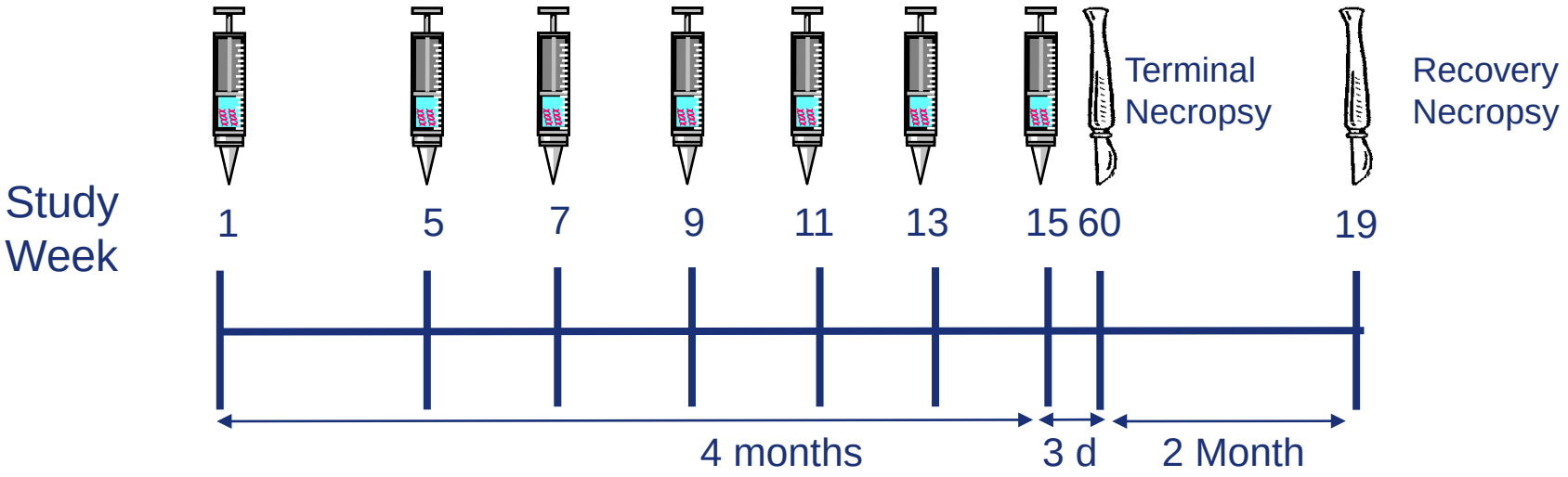
Vaccine Composition

- Vaccine composed of peptides designed to generate antibodies that bind specifically to the FcεRI-binding region of IgE
  - Multiple peptides screened for anti-IgE response
- Short peptides used to minimize potential for self-IgE T-cell recognition
- Virus-like particles (VLPs) used as carrier for the peptides
  - Also provides T-cell help for the antibody responses
- 2 separate peptides included to enhance breadth of coverage
- Formulated with $Al(OH)_3$ ± TLR agonist
  - To enhance antibody titer and avidity

Challenges in Designing the Non-Clinical Program

- Species Selection: Peptides only homologous with NHP and Human
  - Need to use NHP for the Toxicology Program
- Limit Pharmacology Models of asthma or allergic rhinitis in NHP
  - Develop mouse-mimic vaccine for use in mouse asthma models
- Clinical Regimen May Require Chronic Dosing
  - Non-Clinical data suggested needed 4+ doses to establish Ab titers
    - Potential to require boost doses every 3-6 months
  - How to design the repeat-dose toxicity study (N, N+1, 6 month, 9 month)

Repeat-Dose Toxicity Study



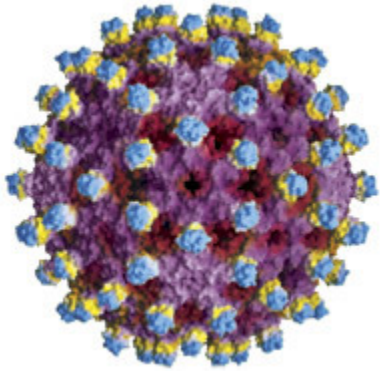
Parameter	Clinical Regimen	Tox Study Design
Number of doses	4 doses + Additional boosts	7 doses
Route	IM	IM
Dose level	100 μg of each of 2 antigens in vaccine	100 μg of each of 2 antigens in vaccine
Frequency	1, 5, 9, and 24 weeks + additional boosts as required	1, 5, 7 ,9, 11, 13 and 15 weeks

DART Study – strategy

- Necessary for inclusion of WoCBP and licensure
- Species selection
  - Usually same species used for repeat-dose toxicity study – NHP
- Dose evaluated
  - Clinical dose administered by clinical route
- Dose groups and design
  - Saline control
  - Vaccine (1 formulation only)
- Endpoints
  - Maternal toxicity, pre- and post-natal development
  - Immune response (pre-study, prior to mating, necropsy)
- Challenges
  - Vaccine DART Study in NHP
    - Sufficient time for Ab response during gestation for gestation-only dosing
    - If pre-mating dosing needed – increases size and cost of the study

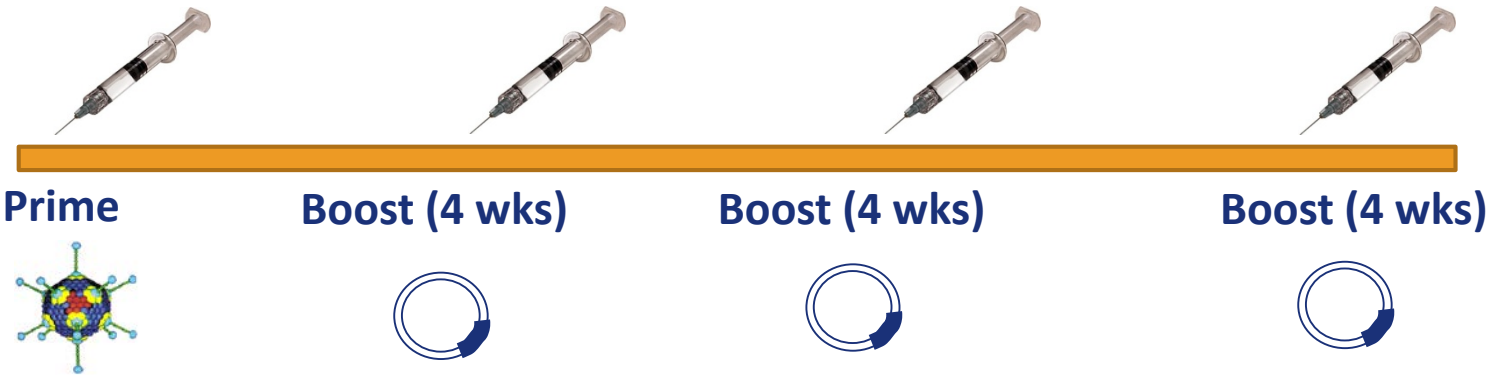
Case Study #2

Designing a Toxicology Strategy for a Vaccine Based Immunotherapeutic Regimen to Treat Prostate Cancer



Overview of the Vaccine

- Indication: Treatment
- Vaccine: A novel, non-replicating viral vector or pDNA encoding 3 distinct prostate antigens
- Adjuvant: N/A – but vaccine components administered with immune checkpoint inhibitors
- Clinical Regimen: Prime with adenovirus followed by 3 boosts with pDNA/EP



Nonclinical Safety Strategy For Development

- Repeat-dose toxicity study
- Biodistribution study
- In silico analysis for protein cross-reactivity

Vaccine is designed to elicit an immune response to a self antigen - safety concern is potential for more extensive toxicity

Potential Safety Concerns

- Vaccine intended to break tolerance to a self-antigen
  - Need understanding homology of the proteins encoded
    - Human protein administered to NHP
  - Distribution of the protein in humans and NHP
    - Potential for off-tumor toxicity
  - Additionally, identify potential human protein sequences that have homology with the protein sequence
    - Step-wise evaluation of 8 to 14-mers for potential cross-reactive epitopes (100% homology)
  - Correlation with any unexpected findings in the repeat-dose toxicity studies?
- Potential for persistence of DNA (adenovirus or pDNA) components of the vaccine

Repeat-Dose Toxicity Study

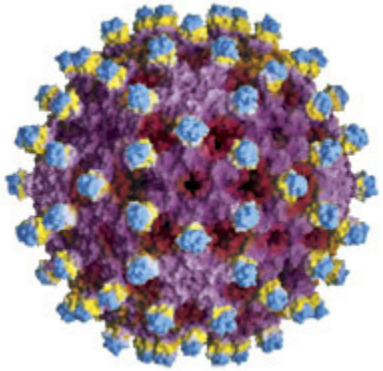
- Species selection
  - High protein homology between NHP and Human (>90% for all 3 antigens)
  - Generate a robust immune response to antigens
- Dose evaluated
  - Highest planned clinical dose administered by planned clinical route
- Dose groups
  - Saline control
  - Vehicle control (formulations for both proteins and virus)
  - Adenovirus only
  - Vaccine
- Regimen
  - Adenovirus prime dose followed by 3 boost doses with pDNA
    - Matches 1 clinical cycle of dosing
- Endpoints
  - Standard toxicology parameters
  - Humoral and cellular immune response
    - Ability to measure cellular immunity may influence species selection

Biodistribution Study

- Necessary for novel pDNA or viral vectors; required for IND filing
- Species selection
  - Permissive host for the virus vector (primary consideration)
- Dose groups
  - Vehicle
  - Viral vector encoding prostate antigens
- Regimen
  - Single dose using clinical route (IM) of administration
- Endpoints
  - Tissues collected on Days 2 (early time point to capture peak exposure to vector), 30 and 90 for evaluation of viral copies (QPCR)
    - Vaccine employs a non-replicating virus so it is not necessary to assess viral shedding in urine, feces, vaccine in blood was determined
  - Clinical observations, injection site evaluation
- pDNA backbone biodistribution was assessed in a separate program with different antigens – not considered necessary to repeat

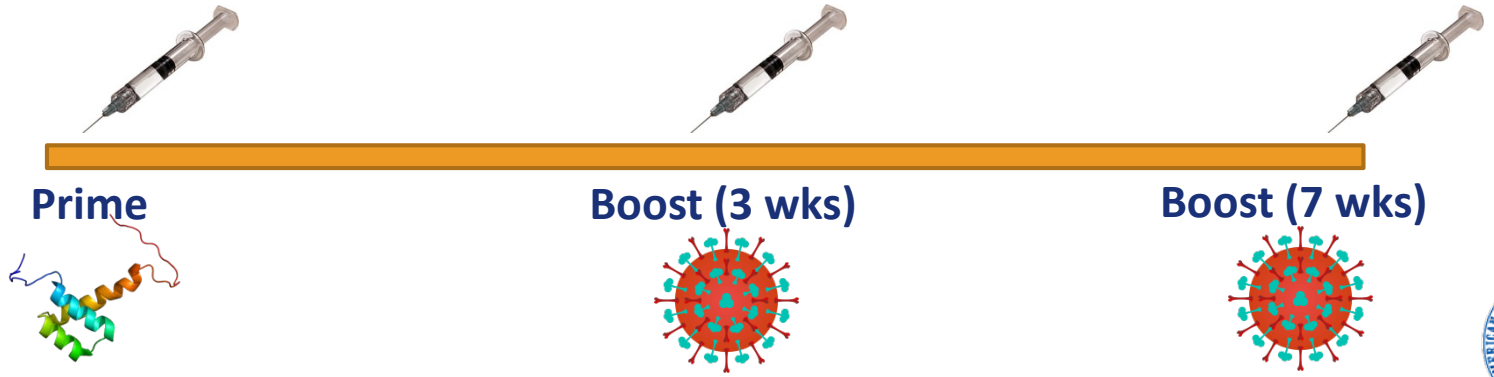
Case Study #3

Designing a Toxicology Strategy for a Vaccine Designed to Treat Chronic Hepatitis B Virus (HBV) Infection



Overview of the Vaccine

- Indication: Treatment of people ≥18 to 65 yrs chronically infected with hepatitis B virus (HBV) following antiviral therapy
- Vaccine: A novel, replicating viral vector encoding HBV core, envelope, and polymerase proteins in combination with recombinant HBV proteins
- Adjuvant: Aluminum hydroxide (AlOH) adjuvant used in the recombinant protein formulation
- Clinical Regimen: Prime with recombinant proteins followed by 2 boosts with viral vector



Nonclinical Safety Strategy For Development

- Repeat-dose toxicity study
- Biodistribution study
- In silico analysis for protein cross-reactivity
- Fertility and developmental toxicity study (DART)

Repeat-Dose Toxicity Study

- Necessary for IND filing
- Species selected should…
  - Generate a robust immune response to HBV antigens (primary consideration)
  - Be a permissive host for the target virus (secondary consideration)
- Dose evaluated
  - Highest planned clinical dose administered by planned clinical route
- Dose groups
  - Saline control
  - Vehicle control (formulations for both proteins and virus)
  - Vaccine
- Regimen
  - Protein prime dose followed by 3 boost doses with the virus (“N+1”)
- Endpoints
  - Standard toxicology parameters
  - Humoral and cellular immune response
    - Ability to measure cellular immunity may influence species selection

Biodistribution Study

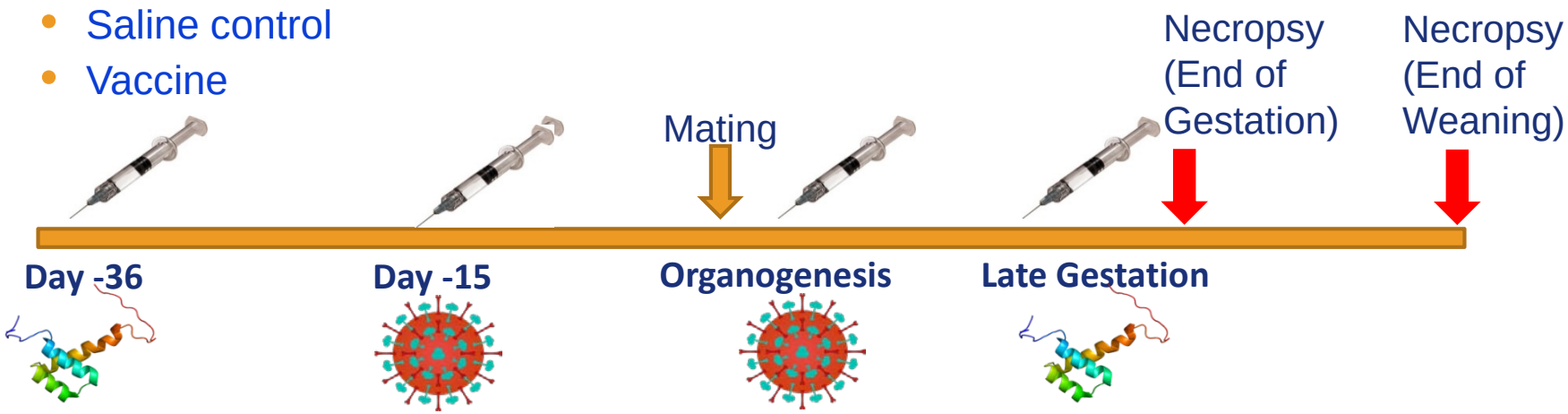
- Necessary for novel viral vectors; required for IND filing
- Species selection
  - Permissive host for the virus that the vector is derived from (primary consideration)
- Dose groups
  - Vehicle
  - Viral vector encoding HBV antigens
- Regimen
  - Single dose using clinical route of administration
- Endpoints
  - Tissues collected on Days 2 (to capture peak exposure at injection site), 5 (to capture peak viremia in blood/tissues), 30 and 90 for evaluation of viral copies (QPCR)
    - Vaccine employs a replicating virus so viral shedding should be evaluated in blood, urine, feces, and saliva
  - Clinical observations, injection site evaluation

In Silico Cross-Reactivity Evaluation

- Goal is to identify potential human protein sequences that have homology with the viral protein sequence
- Step-wise evaluation of 8 to 14-mers for potential cross-reactive epitopes (100% homology)
  - Based on average peptide size for presentation to MHC
- Proteins with 100% homology are further evaluated for biological plausibility
  - Intracellular? Surface protein available for antibody binding? Limited expression?
  - Correlation with any unexpected findings in the repeat-dose toxicity or DART studies?

DART Study

- Necessary for licensure
- Species selection
  - Usually same species used for repeat-dose toxicity study
- Dose evaluated
  - Clinical dose administered by clinical route
- Dose groups and design
  - Saline control
  - Vaccine



- Endpoints:
  - Maternal toxicity, maternal fertility, pre- and post-natal development
  - Immune response (pre-dose, prior to mating, necropsy)

Conclusions

- Nonclinical safety assessment of vaccines is an important component of vaccine development and helps address potential safety concerns
- Design of nonclinical toxicology programs should be consistent with regulatory expectations
- Toxicology programs are also designed to address specific vaccine concerns (e.g. new adjuvants or delivery systems, breaking tolerance) on a case-by-case basis
- The design of toxicity studies depends on how the vaccine is to be used in the clinic

Biography: Jayanthi Wolf, Ph.D.

- Jayanthi Wolf is an immunologist with more than fifteen years of experience in the development of vaccines and biotherapeutics.
- Jayanthi is currently a Director in Regulatory Affairs at Merck in North Wales, Pennsylvania, where she provides regulatory leadership for project teams by developing and implementing global regulatory strategies.
- Prior to her current role, Jayanthi held various scientific and managerial positions in Safety Assessment and Bioprocess Development at Merck in West Point, Pennsylvania. She has contributed to the discovery and development of several vaccines and biological products.
- Jayanthi earned her Ph.D. degree in Molecular Biology and Immunology from Princeton University prior to joining Merck in 2001. She is a member of the Society of Toxicology, Regulatory Affairs Professionals Society, and the Biotechnology Innovation Organization (BIO) BioSafe's Specialty Biologics Expert Working Group.

Biography: Karissa Adkins, Ph.D.

- Karissa Adkins is a toxicologist with more than fifteen years of experience in the development of vaccines and biotherapeutics.
- Karissa is currently the head of vaccine safety at Takeda in Cambridge, Massachusetts, where she is responsible for developing and overseeing the toxicology strategies to advance vaccines in development.
- Prior to her current role, Karissa was a Director in the Investigative Toxicology group at Pfizer in Groton, Connecticut, and Wyeth in Andover, Massachusetts. She has contributed to the development of several vaccines and biological products at Pfizer and Wyeth.
- Karissa earned her Ph.D. degree in Pharmacology and Toxicology from the University of Arizona. She is a member of the Society of Toxicology and the Biotechnology Innovation Organization (BIO) BioSafe's Specialty Biologics Expert Working Group.

Biography: David Clarke, Ph.D., DABT

- David Clarke is a toxicologist with more than 25 years of experience in the pharmaceutical industry.
- David is currently the Pfizer Drug Safety R&D Therapeutic Area Lead for Vaccines, located in Pearl River NY, supporting the vaccine projects for infectious diseases as well as cancer vaccines within Vaccines Research and Development.
- Prior to his current role, David had several scientific and managerial roles in Pfizer, Wyeth, Nycomed Pharma, and Parke-Davis.
- David has a Ph.D. in Pharmacology and Toxicology from Queen's University, Kingston, Canada and is a diplomat of the American Board of Toxicology.