Plant-based Vaccines: Regulatory Considerations

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Overview

• Considerations for Plant-based Vaccines
  • Plant vaccine technology
  • Manufacturing considerations
    • Minimizing risks associated with adventitious agent
    • Safety considerations
  • Product considerations
    • Potency
    • Immunogenicity/adjuvant use

• Summary
• Reference Guidance Documents
Vaccine Manufacturing Systems

Cell-based expression systems: Mammalian, insect, yeast, plants

Plant-based vaccines:
- May eliminate live virus culture and use of animal-based components, which are often part of the mammalian or insect cell-based systems
- May permit large scale, transient gene expression
- May be adaptable to rapid scale-up of biomass and maintenance of surge capacity for response to disease outbreak
- May permit multiple antigen variants and formulations to be produced rapidly
Cloning of target gene into “Launch Vector” system (Agrobacteria binary plasmid with a virus expression vector)

Hydroponic infiltration of Agrobacteria containing target genes into plants

Accumulation of expressed target protein in plants

Musiychuk et al, Influenza Other Respi Vir, 2007
Plant-based Vaccine Technologies

- Antigen expressed in *Agrobacterium* through plasmid
- Bacteria used to infect plants and achieve transient expression of antigen, some of which assemble into virus like particles (VLPs)
- Purified plant-derived antigens are currently in preclinical and clinical development
- Rapid production
  Theoretically high yields (continuous harvest)
Virus Like Particles (VLPs)

• Non-infectious particles
  ➢ resemble virions with no genetic material

• Proteins self-assemble into VLPs

• Broadened immune response (antigen presentation, cellular immunity) vs. recombinant protein

Plant-based VLPs
• Rapid production and response time
  ➢ Theoretically high yields (continuous harvest)
Considerations for Plant-Based Products

- Consider the potential environmental impact of all aspects of the manufacturing process, including but not limited to transport of seeds and plants, planting, growing, harvesting, processing, purifying, packaging, storage, and disposal.

- You must receive a permit from APHIS/BRS prior to engaging (7 CFR 340).

Scope of this talk will be limited to the contained facilities.
Considerations for Plant-Based Products

- Banking of the plant lines for genetic stability and product consistency
- Storage and germination of Master Seed Stock (MSS) and Working Seed Stock
  - Germination rate as stability indicator
  - Reducing possibility of cross pollination
- Health status at harvest
- Bioburden
Considerations for Plant-Based Products

- Residual host cell material in final product
  - Host cell protein contamination
  - Host cellular DNA contamination

- Post-translational modification
  - Different glycosylation pattern than mammalian system
  - Effect on immunogenicity?

- Purification strategy
  - Evaluate buffering conditions and their effect on VLP integrity
  - Removal of adventitious agents
Considerations for Plant-Based Products

• Safety-related considerations
  - Consistency of manufacture
  - Purity (adventitious agent and residual host cell contaminant testing)
  - Immune response (allergenicity)

• Formulation and potency evaluation
  - Often need specific potency reagents/methods to standardize and assess stability
• Minimize the introduction of contaminating adventitious agents
  ▪ Use qualified critical biological raw materials

• Evaluate where in the manufacturing process there is potential for introduction of adventitious agents

• Ensure a controlled manufacturing process in which adventitious agents are removed
Evaluation of Adventitious Agents

- Evaluation of Adventitious Agents
- Homogenization
- Antigen Purification
- Virus clearance studies, if feasible
- Adventitious agent testing on bulk
- Bulk
- Phase 1 clinical trial material
Viral Clearance Studies

Viral clearance strategy for the plant-based products is the same as for any product manufactured in other cell substrates.

- Viral clearance
  - Removal of contaminating viruses
  - Perform spiking studies to estimate the clearance afforded by each step
  - Multiple uses of similar steps (e.g., filtering with the same type of filter twice) does not lead to increased viral clearance

- Studies should support 6 logs of virus clearance above the contaminating virus concentration per dose \((10^6/\text{dose} \rightarrow \text{need 12 logs of clearance/dose})\)

- For VLP products, viral clearance may be difficult

Adventitious Agents Testing

When the viral clearance studies are not feasible, adventitious agent testing of the bulk needs to be undertaken in same way as performed for products manufactured in other cell substrates.

- Non-Specific Methods – known/unknown agents
  - *in vivo* (animals)
  - *in vitro* (cell culture)
  - physical/biochemical/molecular
- Species-specific – known agents*
  - Assays for known viruses
- Cultivatable and non-cultivatable mycoplasmas (and spiroplasmas, if appropriate)

*Molecular/biochemical
Considerations for Plant-Based Products

Potency

• Specific capacity to effect a given result
• Measured by *in vivo* or *in vitro* assays
• Measure of manufacturing consistency and stability
• May not directly correlate with clinical efficacy
• Sponsors may propose a wide variety of assays as long as they:
  • make sense scientifically
  • are suitable for the intended purpose

Immunogenicity

• Need for an adjuvant?
Adjuvant - an agent that is added to a vaccine antigen to augment or potentiate (and possibly target) the specific immune response to the antigen

Adjuvants are added to vaccines:

- to enhance the immune response to the vaccine antigens
- to reduce the dosing frequency
- to induce cross-protective effects
- to direct the immune response or
- to achieve antigen sparing.
Use of Novel Adjuvants

21 CFR 610.15 Constituent materials
a) Ingredients, preservatives, diluents, adjuvants

- “All ingredients ... shall meet generally accepted standards of purity and quality”
- “An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product”

- Vaccine adjuvants are not licensed alone
- Specific vaccine/adjuvant formulation is licensed
Considerations for Novel Adjuvanted Vaccines

- **Adjuvant and adjuvanted vaccine characterization**
  - Identity, purity data
  - Stability data may include:
    - degree of adsorption (aluminum salts)
    - particle size and distribution (for emulsions/liposomes)

- Mechanism of action of adjuvant and mode of action of adjuvanted vaccine, if known

- Proof-of-concept studies (in animals) prior to the initiation of clinical trials

- Data from preclinical safety assessment of adjuvant and adjuvanted vaccine in animals

- If available, data from any clinical trials on safety of novel adjuvant
Preclinical Toxicology Evaluation

- Nonclinical toxicology studies in relevant animal model to support entry into clinical trials
  - Mimic clinical trial in terms of dose, regimen, route, use of device, etc.
  - Provide support for a choice of safe clinical dose
  - Identify potential toxicities and target organs
  - Identify safety parameters for clinical monitoring

- Conduct Toxicology studies in compliance with Good Laboratory Practices (GLP), as specified in 21 CFR 58

- Test articles used in GLP studies should ideally be from lots manufactured with the same production process, formulation, and release specifications as the lots intended for clinical use

- Evaluation of vaccine stability recommended prior to toxicology studies
Product Development

Identification of vaccine candidate

Manufacturing process development

Preclinical studies
- Product characterization
- Immunogenicity

Preliminary information:
- Dose finding
- Route of administration

Pre-IND Meeting:
- Manufacturing issues
- Product testing
- Animal safety testing
- Phase 1 protocol

IND:
- Phase 1 Clinical trial

Highly recommended
Summary

- Plants provide an alternative to current fermentation technology for production of recombinant proteins and VLPs.
- Regulatory pathway for the development of plant-based vaccines is the same as for other preventive vaccines.
- Careful consideration of manufacturing processes, preclinical studies, optimization of immunogenicity and close safety monitoring during clinical trials.
Some Relevant Regulatory Documents (1)

Code of Federal Regulations (CFR):

21 CFR Parts 600s – Biologics Licensing Regulations

21 CFR Part 25 - Environmental Impact Considerations

21 CFR Parts 210, 211, 225, & 226 - Good Manufacturing Practices for Drugs and Biologics

21 CFR Parts 312 & 314 - IND, DMF, and NDA Regulations
Some Relevant Regulatory Documents (2)

- PTC Characterization of Cell Lines Used to Produce Biologicals (1993)
- Guidance for Industry for Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (2010)
- ICH Q5D (R1) Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products Q5A (R1) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin
- WHO Guideline on Nonclinical Safety Evaluation of Vaccines
- WHO Guideline on Clinical Evaluation of Vaccines
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Public Access to CBER

CBER website:  
http://www.fda.gov/BiologicsBloodVaccines/default.htm

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