Multi-epitope vaccines:Potency assays

Hana Golding

Division of Viral Products

CBER, FDA

Multi-epitope vaccines: Types of epitopes

- B cell epitopes
- T-Helper epitopes (HLA class II- restricted)
- CTL epitopes (HLA Class I- restricted)
- Mixture of all three

Multi-epitope vaccines: Delivery systems

- **Peptides: *simple, branched, lipopeptides***
- **fusion proteins**
- **Plasmid DNA**
- **Viral vectors**
- **Bacterial vectors**

Multi-epitope Vaccines: potency assays

- **Physical properties:**
  - **Peptides:*RP-HPLC, aa sequence***
  - **Plasmids: *% supercoil vs. closed circular vs. linear forms***
  - **Viral vectors: *particle number, density, infectious units***
- **Gene expression *in vitro:***
  - **Plasmids, viral vectors: *Western blots of cell extracts***
- **Immunogenicity:**
  - **In vitro assays**
  - **Small animals**

Multi-epitope Vaccine Potency Assays: B-cell epitopes

The inclusion of B-cell epitopes may be adventitious for both *in vitro* and and *in vivo* assays:

- **For Western blots of transfected or infected cells**
- **For immunogenicity studies in small animals**

Antibody Binding assays:

- **Stability/immunogenicity indicating dose range**
- **Serial dilutions of immune sera are recommended**

Antibody functional assays:

- **Desirable for advanced trials**
- **Help to establish correlates of protection**

Multi-epitope Vaccine Potency Assays: Th-cell and CTL epitopes

- **Challenges (I):**
  - **Cross-presentation of HLA class-I or class II restricted epitopes following uptake of exogenous peptides must be demonstrated**
  - **Intracellular processing and presentation of multi-epitopes cassettes or peptides may differ from that of intact proteins expressed by bacterially- or virally- infected cells**

Multi-epitope Vaccine Potency Assays: Th-cell and CTL epitopes

- **Challenges (II):**
  - **Western blots of transfected/infected cells cannot be conducted (no antibody recognition)**
  - **Most epitopes recognized by human HLA class I and class II molecules are not presented by murine MHC molecules**

However:

- **Several transgenic mouse strains expressing human HLA class I and class II are available**

Multi-epitope Vaccine Potency Assays: Development guidelines

- **Potency assays may vary from one product to another**
- **Potency assays commonly evolve as products move from phase I/II to phase III trials, and eventually through licensure.**
- **The ultimate potency assays used for release criteria of licensed products should be validated, quantitative, and predictive of the vaccine protective activity in humans.**

Multi-epitope Vaccine Potency Assays: Development guidelines

Potential *in vitro* assays for phase I/II trials of plasmids or viral and bacterial vectors:

- **Transcription of foreign gene containing epitopes**
- **Western blots of transfected/infected cells using antibodies against foreign gene product**
- **Recognition of of class-I or class-II restricted epitopes by antigen-specific cell lines established from infected individuals or vaccinated animals**

Multi-epitope Vaccine Potency Assays: Development guidelines

***In vivo* immunogenicity in animals (Phase II/III, licensure)**

- **Establish positive/negative response criteria early on**
- **Number of animals per group should be large enough to accommodate anticipated response rate**
- **It is important that the epitope(s) recognized by the transgenic mice are located in the C-terminus of the multi-epitope peptide or gene cluster.**
- **All assays should include negative and positive controls for antigen presentation (*i.e., flu M1 CTL epitope known to be presented by the HLA A0*201 trasgenic mice*)**