NEW HIV VACCINES UNDER DEVELOPMENT

- **Viral Vectors:**
  - Pox viruses: **Causapox; MVA (modified vaccinia Ankara); NYVAC, Recombinant vaccinia.**
  - **Adenovirus 5:** replication competent; non-replicating vectors
  - Adeno-associated virus: non-replicating vectors
  - Venezuelan equine encephalitis: non-replicating vectors
  - **Semliki forest virus:** non-replicating vectors
  - **Herpes virus:** non-replicating vectors

**NEW HIV VACCINES AND ADJUVANTS UNDER DEVELOPMENT**

- **Inactivated HIV**
  - Recombinant plasmid DNA vaccines
  - Purified proteins, peptides, lipopeptides
  - **Bacterial vectors:**
    - Attenuated *Salmonella* delivering DNA vaccine
    - Attenuated *Shigella* delivering DNA vaccine
    - Recombinant BCG vectors
  - **Novel adjuvants, cytokines, co-stimulatory proteins.**

**NEW HIV VACCINES MAY REQUIRE NOVEL CELL SUBSTRATES**

- Complementing cell lines for *am*-replicating viral vectored vaccines
- Optimal production of recombinant live attenuated viruses
- Production of inactivated HIV vaccines.

**RESIGNER CELL SUBSTRATES: ADVANTAGES**

- Derived from primary cells or from well-characterized diploid cell lines.
- Cellular immortalization is achieved with known transforming genes (either viral or cellular)
- Absence of immortalizing genes and/or their products can be closely monitored during vaccine production and final product characterization.
### Adenovirus 5E1-transformed Designer Cell Substrates: Advantages

- Adenovirus 5 is non-oncogenic in humans.
- Adenovirus (or Ad5 E1a/lb)-transformed cells are not tumorigenic in immune-competent animal models:
  - Ad5 E1-expressing cells are killed by cytotoxic T cells and by natural killer (NK) cells
  - Ad5 E1-expressing cells are highly sensitive to mediators of apoptosis (i.e., TNFalpha)

### Designer Cell Substrates for Vaccine Development: OVRR Approach

- Extensive safety testing should be conducted on the new Master Cell Banks (MCB) independently of the vaccine vector. Rationale:
  - The same MCB can be used for production of multiple products.
  - If an adventitious agent is detected in the MCB, it is important to document its removal during product processing/purification and its absence in the final product.
  - Some viral vectors may interfere or reduce the sensitivity of certain safety assays.

### Adenovirus 5 E1-Transformed Designer Cell Substrates: Advantages

- Ad5-transformed cells are only weakly tumorigenic in immunodeficient (athymic) nude mice:
  - TPD: 6.5 x 10⁶ cells

- Ad5-transformed HEK cells (293) have been used in the production of adenovirus-based vectors for gene therapy.

### Designer Cell Substrates for Vaccine Development: OVRR Approach

- Sponsors should be encouraged to place the results of the MCB studies in the public domain in order to increase public confidence in the safety of the new cell substrate

### Proposed Testing of Novel Designer Cell Substrates for Vaccine Development

- MCB Tumorigenicity/oncogenicity studies:
  - Intact cells: Use several cell doses, observe nude mice for 5-6 months
  - DNA (high MW): To establish the inability of oncogenic sequences (viral or cellular-derived) to cause tumors in animal models

- Incomplete medical history of the original tissue and/or incomplete documentation of the tissue culture ingredients used in the propagation of the cell substrate
- Long passage history of immortalized cells may result in exposure to adventitious agents, and potentially to TSE/BSE agents due to undocumented bovine ingredients in the culture medium
Proposed Testing of Designer Cell Substrates for Vaccine Development

Adventitious agent testing:
- In addition to the standard assays, incorporate new state-of-the-art assays for detection of agents that can infect human cells (as needed).
- Cell lysates: To detect occult oncogenic viruses; inoculate two animal species (i.e., newborn hamsters and rats); observe for 5-6 months
- Sequence the PrP gene of the MCB.
- Test for the presence of protease-resistant PrP protein by sensitive Western Blots.

Residual DNA:
- A concerted effort should be made to reduce the amount of cell-substrate-derived DNA in the final product to ≤ 10 ng per human dose.
- For vaccine administration via the oral route: higher levels of residual cellular DNA may be allowed, especially if studies demonstrated no oncogenic potential.

Designer Cell Substrates for Vaccine Development: Discussion Points for VRBPAC
- Please discuss the adequacy of OVRR approach to the evaluation of “Designer Cell Substrates” for use in the manufacturing of viral vaccines:
  - Tumorigenicity/oncogenicity studies
  - Residual cell substrate DNA
  - Potential contamination with adventitious agents including occult oncogenic viruses and TSE/BSE agents.
- Please discuss any additional safety concerns.