

OPEN SESSION

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Introduction to the Session on "Designer" Cell Substrates

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Definitions

- "Neoplastic cells" - is used in its broadest sense to include spontaneously transformed cells, virus-transformed cells, or other types of immortalized cells lines that may be either tumorigenic or nontumorigenic.
- ◆ Transformation - the process by which normal cells are changed by viral or cellular oncogenes or spontaneous events to become immortal neoplastic cells.
- Tumorigenicity - ability of neoplastic cells growing in tissue culture to multiply and develop into tumors when injected into animals.
- ◆ Oncogenicity - ability of a virus or viral/cellular genes to convert the cells of an injected animal into tumor cells

Summary of the OVR Approach

- ◆ Identify the issues
- ◆ Develop appropriate models to evaluate each issue
- ◆ Develop the necessary data to establish the validity of the models used for issue evaluation
- ◆ Develop criteria to consider levels of risk
- ◆ Discuss the approach in public forums and meetings

Two Fold Purpose

- ◆ Review the status of the OVR approach to the use of neoplastic cells as substrates for viral vaccine development
- ◆ Introduce "Designer" Cell Substrates and the issues associated with their use for vaccine manufacture

Factors Contributing to the Need to Reconsider Neoplastic Cells as Substrates for Vaccine Development

- Cell lines capable of complementing the growth of defective viral vectors used as antigen delivery systems
- Development of viral-vectored HIV vaccines
- ◆ Progress in understanding carcinogenesis and in defecting adventitious agents
- ◆ Successful experience with highly-purified biologicals derived from tumor cells

Issues (and Related Concerns) Associated with the Use of Neoplastic Cells as Substrates for Vaccine Manufacture

- ◆ Issue 1 - Tumor cell contamination (possible induction of tumor allografts)
- ◆ Issue 2 - Adventitious agent contamination (possible transfer of known and/or unknown viruses, TSE agents)
- ◆ Issue 3 - Residual cell substrate DNA contamination (possible transfer of activated oncogenic and/or infectious-genetic information)
- ◆ Issue 4 - Residual cell substrate protein contamination (possibility of injection site reactions and/or transfer of prions)
- ◆ Issue 5 - Viral-viral and viral-cell interactions (possible transfer of novel or recombinant viruses)
- ◆ Issue 6 - Genomic instability (possible activation of endogenous or latent viruses)

Defined-Risks Evaluation

- ◆ Assess quantitatively where possible the risk posed by the issues
- ◆ Establish the probability of a worst case scenario for plausible issues
- ◆ Use available data to evaluate plausible risks individually and cumulatively
- ◆ Use cumulative data to assess the relative risk of the product

Categories of Neoplastic Cell Substrates

- Category 1 - Human cells used for vaccine manufacture transformed by known mechanisms: Hypothetical examples- WI-38 cells, MRC-5 cells immortalized by hTERT (human telomerase gene).
- ◆ Category 2 - Early passage human (diploid) cells transformed by known mechanisms: Examples - 293 cells, PER.C6 cells.
- ◆ Category 3-5 - Non-human primate cells transformed spontaneously (VERO, CV-1, BSC-1), all cell substrates derived from tumors of any species, and those cell substrates not covered in Categories 1-4: Examples - HeLa cells, HUT-73 cells.

Factors Promoting the Use "Designer" Cells as Vaccine Substrates

- ◆ Development of cells to complement the replication of bioengineered viral vectors
- ◆ Increasing experience with viral vectors in gene therapy and the production of biologically active proteins
- ◆ Development of HIV vaccines

Public Discussions and Meetings

- ◆ November 1998 Advisory Committee
- ◆ September 1999 Workshop on Neoplastic Cell Substrates
- ◆ May 2000 Advisory Committee Meeting

"Designer" Cell Substrates Defined

"Designer" Cell Substrates are defined as normal human cells that are neoplastically transformed by known viral or cellular oncogenes or by immortalizing cellular genes.

"Designer" Cell Substrates Under Consideration Today

- ◆ 293 cells - human embryonic kidney cells transformed by a restriction enzyme cleaved fragment of the adenovirus 5 genome (Graham et al. J. Gen. Virol. 36: 59-74 (1977)).
- ◆ PER.C6 cells - human embryonic retinal cells transformed by a cloned fragment of the adenovirus 5 genome. (Fallaux et al. Human Gene Therapy 9: 1909-1917 (1998)).

Regulatory Issues Associated with the Use of "Designer" Cell Substrates

- ◆ Tumorigenicity
- ◆ Residual cell substrate DNA contamination
- ◆ Adventitious agent contamination

Tumorigenicity as an Issue in the Regulatory Management of "Designer" Cell Substrates

- ◆ Tumorigenicity has been perceived to be a trait associated with high risk due to the possibility of transferring cell components (cell DNA, cell proteins, viruses) with oncogenic activity to vaccine recipients.
- ◆ Proteins from tumor cells are unable to sustain neoplastic development and to transform cells, leaving cell DNA and oncogenic viruses as the risk factors associated with cell substrates that are tumorigenic.

Residual DNA as an Issue in the Management of Designer Cell Substrates

- ◆ DNA from neoplastic cells can contain activated oncogenes, viral oncogenes, the genomes of oncogenic viruses, latent viruses, as well as retrovirus proviruses.
- ◆ Cloned cellular oncogenes can induce tumors in rodents.
- ◆ DNA from oncogenic viruses and cloned viral oncogenes can induce tumors in rodents.
- ◆ Latent viral genomes and retrovirus proviruses sequestered in cell DNA can be infectious.

Defining Tumorigenicity Again

- ◆ Tumorigenicity is the ability of neoplastically transformed cells growing in tissue culture to grow into tumors when injected into rodents.

The Tumorigenicity of 293 Cells in Nude Mice

293 Cells Graham et al. 1977	293 Cells Lewis et al. Unpub.	A549 Cells Lewis et al. Unpub.
10 ⁷ /mouse = NT	10 ⁷ /mouse = 4/4	10 ⁷ /mouse = NT
10 ⁷ /mouse = 3/20	10 ⁷ /mouse = 4/4	10 ⁷ /mouse = 4/4
10 ⁷ /mouse = NT	10 ⁷ /mouse = 0/4	10 ⁷ /mouse = 4/4
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TPD ₅₀ (est) = 10 ^{7.4}	TPD ₅₀ = 10 ^{6.5}	TPD ₅₀ = 10 ^{7.3}

Adventitious Agents as an Issue in the Management of "Designer" Cell Substrates

- ◆ All cell substrates are subjected to possible contamination with adventitious agents.
- ◆ Due to their laboratory origins, Designer Cell Substrates might represent a risk of adventitious agent contamination.
- ◆ Because they are neoplastically transformed and may be tumorigenic, Designer Cell Substrates might represent a risk of contamination with unknown, possibly latent, oncogenic agents.