ANIMAL CELL SUBSTRATES

INTRODUCTION
Animal Cell Substrates for Biological Products

- Recurring focus of attention / anxieties for the past 50 years

- Recurring inter-linked issues
  - Safety
    - Transmissible agent (e.g., viruses)
      - Infectious disease
      - Cancer
      - Other diseases
    - Transmissible elements (e.g., oncogenes)
      - Cancer
      - Other diseases (e.g., encephalopathies)
  - Acceptability
    - State of knowledge / understanding of risks (e.g., cancer)
    - Ability to deal with identifiable risks
    - Ability to characterize cell extensively
    - Willingness to take calculated risks
ANIMAL CELL SUBSTRATES

DEFINITIONS
ANIMAL CELL SUBSTRATES

Phenotypic Characteristics of Cells Grown *in vitro*

- **Life potential**
  - Finite
  - Infinite

- **Tumorigenic potential (assay dependent)**
  - (+)
  - (-)

- **Chromosomal compliment**
  - Diploid
  - Heteroploid
Animal Cell Substrate Classification Scheme

- **Primary cells**
  - Examples: monkey kidney, hamster kidney, & chick embryo fibroblasts

- **Diploid cell lines (human and nonhuman primate)**
  - Finite life
  - Non-tumorigenic
  - Examples: WI-38, MRC-5, FRhL-2

- **Continuous cell lines**
  - Infinite life
  - Heteroploid
  - Tumorigenic (majority)
    - In vitro “transformation” during subculture (animal)
      - Examples: BSC-1, LLC-MK2, MDCK, & BHK-21
    - Transformed *in vitro* by whole virus or viral element(s) (animal and human)
      - Examples: 293, PerC.6
    - Derived from tumor tissue (human and animal)
      - Examples: Namalwa, HeLa, T-24
  - Non-tumorigenic (minority)
    - Example: VERO at passages <200, some rabbit cell lines
ANIMAL CELL SUBSTRATES

HISTORY
Animal Cell Substrates
Decisions & Developments

- 1950s
  - Human cancer cells (HeLa)
  - Primary monkey kidney cells
  - 1954 Armed Forces Epidemiology Board
Mammalian Cell Substrates

Decisions & Developments

- **1960s**
  - Human diploid cells (HDCs)
    - Risk of a theoretical latent oncogenic agent
      - No tests available for a theoretical agent; therefore unable to characterize cells to demonstrate its absence
    - 1967 NIH conference
    - Gradual acceptance of HDCs as substrate for vaccine production
      - First in Europe
      - Eventually in USA and elsewhere
Animal Cell Substrates

Decisions & Developments

- 1970s - Human cancer cells
  - Namalwa - lymphoblastoid cells for IFN
    - Virus (EBV)
    - DNA
  - IFN
    - Not a replicating agent
    - Therapeutic agent vs prophylactic
    - Purification & validation to demonstrate undetectability of EBV and cell DNA
- 1978 NIH conference
Animal Cell Substrates

Decisions & Developments

- 1980s - Animal cancer cells
  - Characteristics
    - High density
    - Rapid growth
    - High expression of product
  - Examples
    - CHO for rDNA
    - Hybridomas for MAbs

- 1984 NIH / FDA conference
  - DNA (10 pg/dose), viruses, transforming proteins

- 1986 WHO Study Group
  - DNA (100 pg/dose), viruses, (transforming proteins)
Animal Cell Substrates
Decisions & Developments

- 1990s – 2000s Cancer Cells
  - Examples of human CCLs for products in development
    - HeLa – HIV vaccines
    - Per.C6 – Influenza and HIV vaccines
    - 293ORF6 – HIV vaccines
  - Examples of other CCLs for products in development or approved
    - MDCK – influenza vaccines
    - Hi-5 – human papillomavirus vaccine (approved)
- 2004 NIAID / WHO / IABS conference
- 2006 WHO Study Group on Animal Cell Substrates
  - Revise WHO Requirements for animal cell substrates
# Animal Cell Substrates

## Real Risks: Infectious Agents Transmitted to Humans in Biological Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Cell / Tissue</th>
<th>Agent(s)</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polio vaccines</td>
<td>1° MK</td>
<td>SV40</td>
<td>?</td>
</tr>
<tr>
<td>PPF</td>
<td>Human plasma</td>
<td>Hepatitis B</td>
<td>+</td>
</tr>
<tr>
<td>Transplants</td>
<td>Human cornea dura mater</td>
<td>Rabies, Prions</td>
<td>+</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Human pituitary</td>
<td>Prions</td>
<td>+</td>
</tr>
<tr>
<td>Factors VIII &amp; IX</td>
<td>Human plasma</td>
<td>HIV, Hepatitis A, B, C</td>
<td>+</td>
</tr>
</tbody>
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Continuous Cell Lines

Summary

- Risks associated with CCLs are the same as those identified in 1954
  - Transmissible agents (e.g., viruses)
  - Cellular components (e.g., DNA)
- Scientific knowledge and technical abilities are significantly better than in 1954
- Data are now being generated to answer more specifically questions related to DNA risk
- Prospects are bright for a consensus on the criteria for acceptability of a wide range of CCLs