MAMMALIAN CELL SUBSTRATES

INTRODUCTION

Mammalian Cell Substrates for Biological Products

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

- Recurring inter-linked issues
  - Safety (infection, cancer, other diseases)
    - Transmissble agent (e.g., viruses)
    - Transmissible elements (e.g., oncogenes)
  - Acceptability

MAMMALIAN CELL SUBSTRATES

DEFINITIONS

MAMMALIAN CELL SUBSTRATES

Phenotypic Characteristics of Cells Grown in vitro

- Life potential
  - Finite
  - Infinite
- Tumorigenic potential (assay dependent)
  - (+)
  - (-)
- Chromosomal compliment
  - Diploid
  - Heteroploid

Mammalian 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
    - 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 (animal)
    Example: VERO at passages <200, some rabbit cell lines

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HISTORY

Mammalian Cell Substrates
Decisions & Developments

- 1950s
  - Human cancer cells (HeLa)
    vs
  - Primary monkey kidney cells

Mammalian Cell Substrates
Decisions & Developments

- 1960s
  - Human diploid cells (HDCs)
    - Risk of a theoretical latent oncogenic agent
      - No tests available for a theoretical agent
    - Gradual acceptance of HDCs

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Decisions & Developments

- 1970s - Human cancer cells
  - Namalwa - lymphoblastoid cells for IFN
    - Virus (EBV)
    - DNA
  - IFN
    - Not a replicating agent
    - Purification & validation

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Decisions & Developments

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

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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
    - MDCK – influenza vaccines
    - SF9 – human papilomavirus vaccine

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Decisions & Developments

1954 - 2006

Year	Meeting	Major Outcome
1954	AF Epidemiology Board	1^{o} monkey kidney
1967	NIH	Consider human diploid cells
1978	NIH	Consider alternate cell substrates (e.g., Namalwa for Interferon)
1984	NIH/FDA	DNA, viruses, transforming proteins 10pg DNA/dose
1986	WHO Study Group	DNA, viruses, transforming proteins. 100pg DNA/dose
1996	WHO ECBS	10 ng DNA/dose
1999	FDA, NIH, WHO, IABS	DNA risk issues unresolved
2004	FDA, NIAID, WHO, IABS	Consensus statements
2006	WHO	Start revision of WHO Requirements for cell substrates

Mammalian Cell Substrates
1986 WHO Study Group

- CCLs acceptable in principle
- Primary concern is viral safety
  - Emphasis should be on the elimination of potential viruses pathogenic for humans
- DNA is of lesser concern – 100 pg
- Transforming proteins are not a realistic concern
- Validation & wide margin of safety (~$1/10^6$)

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Infectious Agents Transmitted to Humans in Biological Products

Polio vaccines	1º MK	SV40 / ?
PPF	Human plasma	Hepatitis B
Transplants	Human cornea & dura mater	Rabies Prions / CJD
Growth hormone	Human pituitary	Prions / CJD
Factors VIII, IX	Human plasma	HIV / AIDS Hepatitis A, B, C

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CELLULAR DNA

Why Worry About DNA?

- Cells may contain
  - Cancer cell genes
  - Integrated viral genes
- Cellular DNA may be carried over into products
- DNA in products may be transferred to patients
  - Oncogenic event
  - Pathology

ELEMENTS of DNA RISK

- Infection
- Tumor induction
  - Expression of oncogene
  - Activation of proto-oncogene(s)
  - Inactivation of tumor suppressor gene(s)
- Insertional mutagenesis:
  - activation, inactivation, up-regulation, down-regulation
  - Generally considered to be of negligible risk based on gene therapy studies

Risk estimates of the potential for DNA to cause an oncogenic or infectious event

Source for Estimate of Risk	Type of Risk	Estimated Level of Risk
1986 WHO Study Group	Oncogenic	1 in $2x10^{10}$
1987 Petricciani and Regan	Oncogenic	1 in $1x10^{10}$
1990 Temin	Oncogenic	1 in $1x10^{12}$
1995 Kurth	Oncogenic	1 in $1x10^{12}$
1997 Dortant	Oncogenic	1 in $5x10^{8}$
1999 Krause and Lewis	Infectious	1 in $4x10^{9}$

2004 IABS Conference
Consensus Points

- No published study to date has demonstrated that tumor cell DNA can cause tumors in animal models or in humans. Quantitative risk estimates by five groups, based on various assumptions, suggest that if there is a risk of oncogenesis from cell substrate DNA in biological products, it is extremely low.

2004 IABS Conference
Consensus Points

- The manufacturing process should address risk by achieving a level of cellular DNA well below a theoretical point at which DNA might be expected to be oncogenic.
- The acceptable level of residual cellular DNA per human dose has evolved as the assessment of risks has evolved
  - 10 pg (FDA 1984)
  - 100 pg (WHO 1986)
  - 10 ng (WHO 1994)

2004 IABS Conference
Consensus Points

- Risk of residual cell substrate DNA can be reduced to negligible levels when:
  - a DNA-inactivating method or a nucleic acid fragmentation method is used in the manufacturing process of vaccines
  - data are available to:
    - validate these methods
    - demonstrate the consistency of the manufacturing process

2004 IABS Conference
Consensus Points

- WHO should establish a working group to recommend studies designed to answer specific questions relating to theoretical risks associated with residual cellular DNA

2004 IABS Conference
Recommended studies

- Platform studies to address the risk of oncogensis by residual cellular DNA in appropriate models, including the use of relevant positive controls.

- Dose-response studies to determine the relationship of DNA dose to biological activity.

- Studies to determine whether there is less risk from DNA derived from non-tumorigenic continuous cell lines than from those that are tumorigenic.

2004 IABS Conference
Recommended studies

- Risk reduction strategy studies
  - Determine the impact of reducing the size of DNA fragments to various lengths
  - assess the effect, if any, of the configuration of the DNA (naked DNA, chromatin DNA, etc.)

2004 IABS Conference
Consensus Points

- WHO could facilitate reaching a consensus on this point and should be encouraged to do so by undertaking a review of the DNA issue, as was done by the 1986 Study Group and the 1994 ECBS, when sufficient new data become available to warrant such a meeting.

- Agreement among major regulatory agencies and WHO should be reached on levels of cellular DNA that can be considered risk-free.

WHO 2006 Study Group

- Response to 2004 IABS conference recommendations
- 1st meeting in May 2006
  - Review scope of current WHO Requirements
  - Focus on CCLs
    - DNA
      - Review results of studies in progress (CBER/NIAID)
    - Tumorigenicity
      - Review current WHO guidance and draft an update
    - Oncogenicity
      - Consider developing WHO guidance for Requirements
    - New cell systems (e.g., insect cell lines)
      - Consider developing WHO guidance on broad acceptability issues

WHO 2006 Study Group

- 2nd meeting in April 2007
  - Review draft revision to tumorigenicity guidelines
  - Review draft tumorigenicity protocol
  - Review current results of DNA studies
  - Consider updating requirements for adventitious agent tests
- Estimated completion in 2008
- WHO/IABS conference in 2009
  - Public presentation of data and conclusions of Study Group
  - Develop consensus on revisions to WHO Guidelines

Continuous Cell Lines
Summary

- Risks associated with CCLs are the same as those identified in 1954
  - Transmissble agent (e.g., viruses)
  - Cellular component (e.g., DNA)
- Scientific knowledge and technical abilities are significantly improved since 1954
- Data is now being generated to answer specific questions related to risk
- Prospects are bright for a consensus on the criteria for acceptability of a wide range of CCLs