How can innovation in regulatory science inform the regulatory process to facilitate the development of new vaccines?

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CBER
Strategic Plan for Research and Regulatory Science

Regulatory Science:

Development and use of the scientific knowledge, tools, standards, and approaches necessary for the assessment of medical product safety, efficacy, quality, potency, and performance.

http://www.fda.gov/downloads/BiologicsBloodVaccines/ScienceResearch/UCM303542.pdf

Case Study II: Use of Novel Cell Substrates for the Production of Viral Vaccines

- CBER regulates vaccines against diseases caused by viruses
- Cell culture systems ("cell substrates") are used to produce many viral and virus-vectored vaccines
  - Cell substrates are considered in the context of the entire manufacturing process
  - Cell substrates can be difficult to characterize-- thus, cell substrates have historically given rise to important regulatory considerations
  - Cell substrates play an important role in consideration of vaccine safety
- CBER regulatory science programs include the development of new tools to evaluate the safety of cell substrates used to produce viral vaccines
  - CBER's goal: address the issues in a scientifically rational manner, quantitatively when possible

Examples of Cell Substrates used for Licensed and Investigational Vaccine Production

- Animal tissue (eggs)
- Primary
  - Embryo fibroblasts
- Human diploid
  - MRC-5 (lung); WI-38 (lung)
- Avian stem cells
  - EB66 (duck)
- Insect-derived
  - Sf9, Hi-5
- Continuous cell lines
  - VERO

VRBPAC Discussions & Public Meetings on Use of Novel Cell Substrates for the Production of Vaccines

- 1998: Neoplastic and tumorigenic cells for vaccine manufacture
- 2000: Vero cells (non-tumorigenic passage) for live-attenuated vaccines
- 2001: *In vitro* transformed human cells
    - (293, PER.C6) for defective adenovirus-vectored vaccines
- 2005: Tumorigenic MDCK cells for inactivated influenza virus vaccine
- 2008: MDCK cells for live, influenza virus vaccine
- [2010: Porcine Circovirus in rotavirus vaccines]
- 2012: Human tumor-derived cell lines
- Numerous public meetings with academia, regulated industry to discuss the characterization of cell substrates, e.g.,
    - 2013: PDA/FDA Advanced Technologies for Virus Detection in the Evaluation of Biologicals

New Cell Substrates for Viral Vaccine Production

- Novel approaches required for the development of new vaccines, e.g.,
  - HIV, pandemic influenza, emerging infectious diseases (e.g., SARS)
- Expanding the repertoire of cell substrates
- Most mammalian cells considered are continuous cell lines
  - Some tumorigenic
  - Some derived from human tumors

New Cell Substrates for Viral Vaccine Production

- Virus growth advantage
- More rapid scale-up
- Ability to bank & thoroughly characterize cells
- Adaptation to serum-free growth and growth in suspension
- Examples of human tumor derived cells proposed for use of new cell substrates discussed at VRBPAC in 2012
  - A549 (lung adenocarcinoma): adenovirus-vectored vaccines
    - e.g., influenza, HIV, anthrax
  - CEM (lymphoblastic T cell leukemia): Inactivated HIV vaccine
  - HeLa (cervical carcinoma): AAV-vectored HIV vaccine

Factors that Could Potentially Convey Risk from Tumor-Derived Cells

- Cells
  - If cells were present in vaccine, they could retain their original phenotype
  - They still would be susceptible to rejection by the host immune system
- Cell DNA
  - Oncogenic activity: Tumor induction in animals (theoretical risk)
  - Infectious activity: Presence of infectious viral genomes (integrated or extrachromosomal) that may produce an infectious virus
- Adventitious agents
  - Potential presence of known, unknown or unexpected viruses
  - Increased risk due to more passages in history,
  - Potential ability of cell substrate to support growth of additional viruses
  - Potential for a virus to have been involved in tumor development
- Other?

Current FDA Recommendations For Cell Substrates

- 2010 Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases
- http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecompliancereguratoryinformation/guidances/vaccines/ucm202439.pdf

CBER Approaches to Assess the Safety of Tumorigenic Cell Lines

- Combining conventional assays with additional assays
- Cells
  - Complete removal of whole cells during manufacture
- DNA
  - Tumorigenicity assays
  - Cell DNA oncogenicity & infectivity testing
  - Reduction in amount and size of DNA
    - Fragmentation and removal during manufacture
  - Risk assessments are guided by experiments on DNA oncogenicity and infectivity
- Adventitious agents
  - Cell lysate oncogenicity testing
  - *in vitro* virus induction studies
  - New technologies for Adventitious Agent Detection (W.I.P.)
    - Virus microarrays
    - Broad-range PCR with mass spectrometry (PLEX-ID)
    - Massively parallel (deep) sequencing (MPS)

CBER Approach to Evaluating the Biological Activity of DNA

- Establish sensitive & quantitative assays to detect the activity
  - Cell DNA oncogenicity and infectivity assays
    - *Sheng et al, 2008, Biologicals 36: 184-197*
    - *Sheng-Fowler et al, 2009, Biologicals 37 (2209) 259-269*
- Use assay to quantify the activity to estimate safety/risk
- Use the assay to quantify the amount of reduction in biological DNA activity afforded by various treatments (chemical inactivation, nuclease digestion, etc.)
  - Reduction in amount and size of DNA
- Use data to estimate safety factors for a product with respect to the residual cell-substrate DNA in that product
  - the level of clearance needed to reach acceptable margins of safety, with respect to residual cell-substrate DNA, for vaccines

Manufacturers need to determine and document DNA clearance

New Technologies for Broad/Novel Virus Detection

- Microarrays
  - Array consists of virus-specific oligos based upon known and related virus sequences
- Broad-range PCR with mass spectrometry (PLEX-ID)
  - Long PCR primers that are specific for virus families
  - Amplicons are detected and sized by mass spectrometry (MS)
- Massively parallel (deep) sequencing (MPS)
  - Sequencing without prior knowledge of sequences for known and novel viruses
- Emerging tools used to investigate cells and source material in vaccine manufacture as well as testing commercially available vaccines
  - Technical challenges to be addressed include standardization & validation, development of appropriate controls
  - Need for follow-up strategies to determine the biological relevance of a positive result
  - Currently not been recommended for use in GMP manufacturing
- CBER research programs are investigating advantages and limitations of these novel technologies to help identify appropriate regulatory uses of these new technologies

Public Meeting: Vaccines and Related Biological Products Advisory Committee (VRBPAC) 2012

Human tumor-derived cell lines could be an important addition to the repertoire of currently available cell substrates

•Risk mitigation strategies are the same for vaccines generated using human tumor-derived cell lines as for other cell substrates

VRBPAC

•agreed that CBER had adequately addressed the safety concerns associated with tumorigenic cells substrates and human tumor derived cell substrates

- i.e., by combining conventional assays with additional assays such as *in vivo* assays to measure DNA oncogenicity*, in vitro* assays to measure DNA infectivity

•encouraged the use of new virus-detection technologies in the characterization of human tumor-derived cell substrates and other cell substrates

•encouraged discussion with the international community on the use of emerging technologies and to discuss risk-mitigation strategies

Panel Question: What is the Potential of Science to Advance Regulation ?

- CBER's regulatory science program
  - Fundamental to CBER's ability to provide effective regulatory review of biological products
  - Provides CBER with scientific expertise, tools, and data to support scientific based regulatory decision making and policy development
  - Addresses scientific aspects of regulatory issues and evaluates and implements, when applicable, innovative technologies to improve test methods for currently licensed products and those under development
  - Facilitates development & licensure of vaccines including vaccines against emerging infectious diseases
    - E.g. products manufactured using novel cell substrates
      - Flucelvax (made in MDCK cells), Flublok (made in Sf9 cells)

Panel Question: How can Regulatory Science Enhance Global Access to Vaccines for Emerging Infectious Diseases?

- International collaboration
  - Scientific and regulatory information sharing with foreign regulatory counterparts & international health agencies
  - Training
  - Collaborative research, e.g.,
    - Develop panels, reagents, methods to detect EIDs
    - Develop and evaluate pre-clinical models to study pathogenesis and protective immunity & to identify correlates of immunity that facilitates development of vaccines for the developing world
    - Encourage research supporting the development of new vaccines to treat infectious diseases affecting millions globally
  - Collaboration with not-for-profit NGOs and PDPs
    - E.g., CBER PATH-MVI to develop improved laboratory tests for predicting the level of safety and effectiveness of exp. Malaria vaccines

Back-up Slides

Quantitative Assay to Asses Oncogenic DNA Activity

- Oncogenicity of DNA in vivo: establishment of an *in vivo* model that can be used to estimate the risk of an oncogenic event by DNA
  - Evaluation of tumor induction in mice strains with expressions plasmids for the human H-ras oncogene and the murine c-myc oncogene
  - Data indicate that amounts of the *ras/myc* dual-expression plasmid of 1 ng are capable of forming tumors in mice
  - Results demonstrate that cellular oncogenes can induce tumors following SC inoculation
- Possible way of evaluating and estimating the theoretical oncogenic risk posed by residual cell-substrate DNA in vaccines

  - *Sheng et al, 2008, Biologicals 36: 184-197*

Quantitative Assay to asses Infectious DNA activity

- Infectivity of DNA *in vitro:* establishment of an *in vitro* transfection/co-cultivation assay to determine the quantity of a retroviral provirus in cellular DNA that can establish a productive infection
  - Capability to recover infectious virus from 1 pg of cloned HIV DNA and from 2 ug of cellular DNA from HIV infected cells.
  - Infectivity could be reduced to below detectable levels by treatment of the DNA (digestion or inactivation)
- Assay was used to determine
  - the specific activity (infectivity) of a DNA copy of a retroviral genome
  - the level of clearance of cell-substrate DNA achieved by digestion or chemical inactivation
  - the level of clearance needed to reach acceptable margins of safety, with respect to residual cell-substrate DNA, for vaccines

❑ *Sheng-Fowler et al, 2009, Biologicals 37 (2209) 259-269*

Adventitious Agent Detection:
Chemical Induction for Latent Virus Detection

- Treatment of cells with different inducers under optimized conditions
  - Endogenous Retroviruses: 5'-iodo-2'-deoxyuridine (IUdR) and 5-azacytidine (AzaC) are known inducers of endogenous retroviruses
  - Latent DNA viruses: 12-*O*-tetradecanoly phorbol-13-acetate (TPA) and sodium butyrate (NaBut) can induce various latent DNA viruses
- Follow-up in case of a positive result
  - Determine origin of PERT or PCR signal (*viral vs. cellular*)
  - Characterization of induced viruses (if any)
  - Investigations of potential risk
  - Infectivity/coculture studies using various target cells including nonhuman and human
- These methods provide additional approaches for characterizing potential virus risk in cell substrates

  - ***Khan, AS et al, Biologicals, 2009 37: 196-201; Ma, W et al., Biologicals, 2011 39: 158-66 Ma, H et al., J Virol, 2011 85: 6579-88***