Introduction to Adventitious Agent Issues
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Public confidence in vaccine safety
Maximal vaccine benefit

Adventitious Agent
An infectious agent that is extraneous to the product

Goal: Final products should not contain adventitious agents

OVRR approach to adventitious agent issues
- Identify potential issues, including theoretical ones
- Discuss issues in public
- Make decisions based on the best available science
- Ensure that potential issues are known to research subjects

Potential adventitious agents to be considered today
- Transmissible spongiform encephalopathy (TSE) agents
- Viruses
Adventitious agents: Examples

<table>
<thead>
<tr>
<th>Product</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow fever vaccine</td>
<td>Hepatitis B Virus, ALV</td>
</tr>
<tr>
<td>Measles vaccine</td>
<td>Pestivirus</td>
</tr>
<tr>
<td>Blood products</td>
<td>Hepatitis viruses, HIV</td>
</tr>
<tr>
<td>Urokinase</td>
<td>Reovirus</td>
</tr>
<tr>
<td>Growth hormone &amp; Dura mater grafts</td>
<td>CJD agent</td>
</tr>
<tr>
<td>Interferon.3</td>
<td>MVM</td>
</tr>
<tr>
<td>Polio &amp; adenovirus Vaccines</td>
<td>SV40</td>
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</tbody>
</table>

SV40 in vaccines

- Millions received SV40-contaminated pRhMK-produced polio and adenovirus vaccines in the late 50s and early 60s
- Cell supernatants caused tumors in laboratory animals and CPE in pCMK cells
- Vaccine seeds were treated with anti-SV40 neutralizing antibodies in the early 1960s
- Epidemiological studies suggest no adverse sequelae to vaccinated children
- SV40 DNA has been detected in some human malignancies by PCR

SV40: lessons learned

- Value of ensuring that products are free of adventitious agents
- Importance of ensuring freedom of oncogenic agents, especially for vaccines given to children

Viral Vaccine Production

Creating a quantitative framework for decision-making

- Need to estimate pre-test probability of a problem
- Need to consider number of doses (or dose-equivalents) that can be tested
- Need to understand sensitivity of assays
- Need to consider safety margins

Adventitious agent testing principles

- Should consider issues specific to material in question
- Value of quantitative validation
  - High sensitivity assays include amplification step
  - Controls
- Where possible, should use tests that have the potential to detect unsuspected agents
Factors that could influence adventitious agent risk

- **Species**
- **Cell type or tissue of origin**
  - previous exposures
    - fetal vs. adult origin
  - tumor association
    - knowledge of transforming event
  - ability to bank cells
- **Maintenance or passage history**

Vaccine cell substrates

- Whole animals
- Primary cells
- Diploid cell strains
- Neoplastic cell lines

Advantages of neoplastic cell substrates

- Host range
- Cell banking
- **Serum-free** growth
- Can express **complementing** genes

Issues associated with neoplastic cell substrates and oncogenic viruses

- Potential that an oncogenic virus was involved in the cell line’s neoplastic transformation
- Some of these cell lines are more likely to have uncertain histories
- Potential severe consequences that are difficult to evaluate in short-term clinical studies

Testing for adventitious viruses

<table>
<thead>
<tr>
<th>Test</th>
<th>Amplification</th>
<th>Potential to detect the unexpected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture (TC)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Egg inoculation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Animal inoculation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- death</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- weight loss</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Animal Ab production</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PCR-RT-PCR RT (RFLP)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TC + PHRT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Specific PCR</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Electron Microscopy</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
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PCR vs. Biological Assays

- **PCR**
  - More sensitive for small samples with low residual DNA
  - Very specific
  - Works independently of growth characteristics
  - May not represent live virus
- **Biological assays**
  - More sensitive for large samples
  - Greater potential to detect the unknown
  - Requires growth in a specific system
  - More relevant endpoint (e.g., only way to detect oncogenicity)
Methods used to discover viruses

- Animal inoculation
- Tissue culture
- Electron Microscopy
- Molecular methods

Some tumor viruses discovered using animal assays of cell lysates or supernatants

- Retroviruses
  - Rous sarcoma virus (1911)
  - Feline leukemia virus (1964)
- Poxviruses
  - Rabbit fibroma (1932)
- Papovaviruses
  - Rabbit papilloma virus (1933)
  - Polyomavirus (1953-57)
  - SV40 (1960-62)
- Adenoviruses
  - Ad 12 (1962)

Viral induction of tumors in animal assays

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>H</th>
<th>M</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rous &amp; murine sarcoma viruses</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Murine leukemia viruses</td>
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<td></td>
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<tr>
<td>Polyoma virus</td>
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<tr>
<td>SV40, BK, JC viruses</td>
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<td></td>
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<tr>
<td>Adenovirus 12, 18, 31</td>
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<tr>
<td>Adenovirus 9</td>
<td></td>
<td></td>
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<tr>
<td>SA7 (AGMK adenovirus)</td>
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<tr>
<td>CELO (chicken adenovirus)</td>
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<tr>
<td>Human herpesviruses and papillomaviruses</td>
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Animal tests for oncogenic viruses

- Could be used in cases where additional confidence that a product is free of adventitious oncogenic viruses is desired
- Many tumor viruses are 3-associated
- Inoculating 2 animal models with cell-free lysates of cell substrates, followed by observation for 5-6 months, would lead to maximum sensitivity

In vivo testing of cell substrates for oncogenic adventitious viruses

- Value for ensuring product consistency
- Best for ensuring absence of potential viral interactions
- Required to apply principles of clearance
- Lysate vs. supematant

Issues with using final product for animal oncogenic adventitious virus testing

- Potential for interference
  - Cell killing
  - Inflammatory response
  - Effect on apoptosis (especially E4+ viruses)
- Testing of final product would give assurance that the vector itself is non-oncogenic
New broadly-specific molecular approaches to virus detection

- Use consensus PCR primers to detect related viruses
- Molecular subtraction
- Non-specifically amplify viral nucleic acids

Potential benefit of the product

- Consider the intended recipient
- Some risks from viral adventitious agents are theoretic and must be placed into the context of the benefit of the product

Potential approach to TSE testing of Ad5 transformed human “Designer” Cell Substrates for vaccine production

- Consider
  - Cell type & potential exposures to BSE
- Tests to consider
  - Sequence PrP-encoding gene
  - Western blot
  - Add newer, more sensitive tests as they become available
- Informed consent, investigator brochure

Potential approach to virus testing of Ad5 transformed human “Designer” Cell Substrates for vaccine production

- Perform standard testing, including extensive tissue culture and electron microscopy
  - Ensure tests would detect any agents based on
    - fetal origin of cells
    - cell type
    - cell history
- Although mechanism of transformation is likely Ad5 genes, do extensive testing for potential oncogenic adventitious agents
  - Cell lysate oncogenicity testing
  - Other tests as they become available
- Informed consent, investigator brochure
TSE and Cell Substrates

- Issue of PrP (PRNP) genotype in cell donor
- Consequences of exposure to serum from countries where BSE or the risk of BSE exists
- Other factors that could increase risk of TSE infection (?PrP expression levels, neuronai or retinal origin of cells)

Current approaches to TSE issues

- Where possible, determine family & medical history of cell donor with respect to TSE risk factors.
- Sequence PrP (PRNP) gene
- Perform Western blot for the presence of protease resistant PrP.
- Determine if exposure to FBS from countries with BSE could have occurred.
- If possible exposure to FBS of unknown origin has occurred, document that cells cannot support replication of BSE agent.

OVRR approach to TSE issues

- Use existing technically feasible strategies to evaluate cells
- Informed consent & investigator brochure
- Present issues to VRBPAC for initial discussion
- Present issues to TSE advisory committee for more comprehensive discussion

TSE and Neoplastic Cells

- Potential effect of genomic instability on normal PrP gene
- Potential role of apoptosis in preventing TSE infection

Evolving approaches to TSE issues

- If possible exposure to FBS of unknown origin has occurred, document that cells cannot support replication of BSE agent.
- Evaluate level of PrP expression.
- Evaluate for the presence of infectious TSE agent by animal inoculation.
- Once new assays for detection of TSE agents become available, introduce them for cell substrate testing as soon as feasible.

Adventitious Agent Testing of Neoplastic Cell Substrates

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