Well-Characterized Adenoviral Vector-Based Vaccines: Are We There Yet?

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Merck & Co., Inc.
"This looks like a good time to help ourselves to a snack."
Merck’s Adenovirus-Based Vaccines

• Replication-incompetent adenovirus
  – E1 deficient
  – Propagated in E1-complementing cell line (PER.C6™)

• For HIV Vaccine, incorporates transgenes for HIV-1 \textit{gag}, \textit{pol}, and \textit{nef}
  – Could incorporate any gene of interest

• Monovalent or multivalent
  – Single construct or multiple constructs
  – Mono-gene or multi-gene

Fuller et al., \textit{Cell} 1991
Adenovirus reconstruction from Cryo-EM
How Was it Designed to Work?

- No functionality
- Polypeptide sequence **only** is critical

Presented by MHC to T-Cells ➔ Immune Response
Hope and Disappointment in HIV Vaccine Development at Merck

- Phase I studies showed strong immune response in recipients
  - Animal studies had shown reduction in viral load

- Phase II studies, initiated in 2004, were designed to test if the immune response elicited was sufficient for efficacy

- Disappointingly, the results from Phase II (Sept. 2007) suggested that the measured ELISPOT was not indicative of efficacy
Why Discuss it Further?

• Hopefully others can learn from this, and speed development of other HIV vaccines
  – "If I have seen further, it is by standing on the shoulders of giants.”
    --Sir Isaac Newton, 1675
  – Other Adenovirus-based vaccines are being developed

• Merck was preparing for Phase III and potential launch
  – This is a good case study for how to move toward “well-characterized” live virus vaccines
Ensuring Quality Through “Cradle-to-Grave” Analytics

Viral Seeds → Cell Bank (PER.C6) → In-Process Monitoring → Drug Substance → Drug Product → Stability

“Well-Characterized” should reflect the entire process, not just the end product

*Raw material testing is also important at each stage
What Should This Talk Convey?

1. The extensive analytical complexity of this program as a whole

2. How we have been able to get our arms around this complexity

   Attention to detailed science makes a difference in true understanding and control
Unique Challenges in Adenovirus-Based Analytics: Sub-Populations

**Total Virus Particles**
Safety?

**Full Particles**
Safety? Efficacy?

**Infectious Particles**
Efficacy? Productivity in manufacturing.

**Antigen Expressing Particles**
Efficacy? Residuals in manufacturing.
Critical Analytical Characterization

Need to assess these attributes for all sub-populations of viral particles
Breadth and Depth

• Each of the subsequent slides could have 20 slides describing the story behind it.

• The goal of this talk is not to “wow” you with detailed data, but to illustrate the variation and complexity (and sheer numbers) of problems we faced and how we overcame them.

“Gee, Mr. Wilson...even the tooth fairy leaves more than that!”
General Manufacturing Scheme

GROWTH IN PER.C6 CELLS
↓
(AUTO)LYSIS
↓
CLARIFY
↓
NUCLEASE
↓
UF
↓
AEX
↓
2nd CHROMATOGRAPHY
↓
UF

The end product is a purified preparation of Adenovirus particles

Sources: Huyghe, 1995; Misc Patent Applications (Introgen, Aventis, Canji, Schering)
Identity and Purity
Challenges With Identity/Purity

A seemingly simple observation kicked off ~12 months of detailed investigation, ultimately leading to a critical conclusion.

- Unexpected bands observed in all digests suggesting possible deletions
- Led to cloning of fragments, sequence identification, ultimately potentially to re-design of original plasmid construct
Purity: Unique Challenges with PER.C6 Cells

Neuronal cell type from human fetus previously unlicensed for use in vaccines

Prior cell expansion with bovine serum

Transformed and non-adherent phenotype

Recombinant E1-encoding expression system

Duncan et al
Purity: Extensive Testing to Detect Potentially Contaminating Viruses

- Tests in cell lines
- Test in eggs and animals
- Product-enhanced reverse transcriptase
- Electron microscopy
- PCR

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Duncan et al
Tracking clearance through the process gave good confidence in the end product purity.
Purity: Host Impurity Clearance

- Approximately 7 log clearance in host cell DNA after purification
- Final product is largely free of host contaminants
Purity: Quantification of Empty Capsids

Disc Centrifugation

CsCl Gradient Preparation

• Three methods developed for this critical parameter.

• Orthogonal assays led to increased understanding of the process and the assays themselves.

Sweeney et al (in prep)
Strength and Potency
Assays for Strength (i.e., Dose)

• Three methods developed:
  – Lowry
  – UV-SDS\(^1\)
  – Genome Quantitation\(^2\)

• Key assay attributes
  – Accurate, precise, specific, and reproducible
  – Flexible for multiple constructs
  – High throughput, short turn-around time

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Strength: Aggregation Issues

Vaccine aggregates at 37ºC

Relatively straight-forward measures of aggregation (DLS) proved useful for this vaccine

Sweeney et al
Potency Assay Development

Gained precision and specificity in an infectivity assay, but vaccine does not replicate in the vaccinee (Biological Relevance?)

"Progressive" TCID$_{50}$

Quantitative PCR-Based Potency Assay (QPA)

Potency: In Vitro Antigen Expression (IVAE)

**Advantages:**
- Likely the most relevant measure of vaccine activity; Specific; Precise

**Disadvantages:**
- Choosing appropriate antibodies was complex and time-consuming
- Consistency and availability of reagents moving forward is challenging
Concordance of Infectivity and Antigen Expression Assays

- Infectivity and antigen expression assay results were similar
- Variability was slightly lower in the IVAE
- Both assays appear appropriate as the key potency assay

MERCK
In both the clinical and IVAE data, trivalent vaccine generated slightly stronger responses (not statistically significant clinically)

- Only true for “high Ad5” clinical population
- Suggested IVAE might be the more clinically relevant assay
- IVAE has operational disadvantages
Investment in Assay Development

Total Virus Particles
- Lowry Protein Assay
- Dynamic Light Scattering

Full Particles
- Genome Quantitation Assay (GQA)
- Reverse-Phase HPLC Assay
- CsCl Gradient Analysis (% full)
- UV Absorbance Assay (UV-SDS)
- Disc Centrifugation

Infectious Particles
- TCID\textsubscript{50}
- Q-PCR Based Potency Assay (QPA)

Antigen Expressing Particles
- Silver stain/Western blot
- In vitro Antigen Expression Assay (IVAE)

Transgene-encoded Antigen
Ensuring Quality Through “Cradle-to-Grave” Analytics

Viral Seeds (MVS, WS) → Cell Bank (PER.C6) → In-Process Monitoring → Drug Substance → Drug Product → Stability

- Genetic stability
- Manufacture in a permissive cell-line
- Never-before used in human trials
- A lot of safety testing
- DNA digestion
- Protein clearance
- Aggregation
- Defining the “active” units of safety and efficacy
- 2-8 ºC stable
- Narrow window for release
- Measuring purity
- Biophysical charac.

There are analytical challenges at each stage.
Are We There Yet?

- Significant understanding of the cellular and molecular biology, biophysics, and physical chemistry of the vaccine has been attained.

- Most assays are suitable for “platform” testing.

- The missing link is clinical efficacy.

Defining “well-characterized” depends on the application.
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Many, many others within and outside of Merck
“We try never to forget that medicine is for the people--it is not for the profits. The profits follow, and if we have remembered that, they have never failed to appear.”

--George W. Merck, December, 1950