



FDA Perspectives on the Development and Use of the Adenoviral Reference Material

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Background/History

- 1999 Death of a subject enrolled in Adenovirus Gene therapy clinical trial
- 1999 Development of RAC AdSAT working group
- 1999 RAC Safety Symposium
- Oct. 5th 2000 Williamsburg BioProcessing Foundation meeting
- Federal Register Notice 2/1/01
- Development of ARMWG



Recommendations of RAC AdSAT Working Group

- Development of qualitative, quantitative vector standards - Adenovirus
 - determine particle number
 - determine infectious titer
- Allow comparison of toxicities observed in different studies
 - preclinical
 - clinical



Leveraging Agreements

- Feb 1 meeting Co-Sponsorship agreement
 - Allowed for public discussion and input
- WBF-CBER Partnership Agreement
 - Allowed for partnership between FDA/WBF/industry/Academia
 - Identify relevant criteria in production, and distribution of adenoviral reference material
 - Improve ability to evaluate safety of adenoviral GT products



FDA's Role in ARMWG

- Review Proposals for vector production
- Make recommendation for selection of appropriate group(s) to manufacture, characterize and distribute reference material.
- Set testing qualifications for reference material
- Collate data from reference material testing
- Provide guidance to WG



Perspectives/Issues

- Concern over precision and accuracy of adenoviral titers
 - particle counts (multiple methods used)
 - infectious units (inconsistency between assays)
- Sharp threshold effect in dose/toxicity curve



Perspectives/Issue (cont.)

- Consistency in clinical dosing
 - dose control
 - closer approach to maximum tolerated dose
 - smaller dose increments
 - analysis of dose related adverse events
- Safety/Contamination concerns
 - RCA: how much is present
 - toxicity of vector particle



Approaches

- Reference Material Development
 - physical: particle counts
 - biological: infectious particle titer
 - procedural: validate laboratory methods



Importance of Reference Material

- Production of more consistent, safer, quality adenoviral vectors
- Allow comparability between preclinical studies
- Allow comparability between clinical studies
- Development of regulatory policy



Current FDA Recommendations

- Clinical dosing by viral particle number
- RCA levels $< 1\text{RCA}$ in 3×10^{10} vp
- Infectious units:vp ratio $< 30\text{VP}/1$ IU
- Complete sequence of all vectors $< 40\text{kb}$.



What is the Next Step?



Expected Use of Adenoviral Reference Material

- Used to define infectious titer and virus particle concentration for adenoviral GT vectors
- Used to validate an internal reference
- ARM can be used to validate internal analytical methods for virus particle and infectious titer
- Will allow for analysis of safety and efficacy based on similar unit measurements
 - RCA
 - Dosing



What is Not Expected

- Standardization of specific assay methods
- Endorsement of specific production, purification methods
- Duplicate ARM titer & particle values
 - Values were obtained using a specific method, using different methods will give different values
 - Accurate values will depend on number of replicates and variance of assay



How to use the ARM in infectivity titer assay

- Assay may be any titer assay (endpoint dilution, fluorescent focus, plaque assay, ARMWG assay)
- Assay should be validated to make sure it is being used in the linear range
- Variance of assay should be measured; this will determine how many replicates need to be done for an accurate result
- Each time your assay is run, the ARM (or a calibrated internal lab standard) must also be run



How to use the ARM in infectivity titer assay (cont.)

- When designing your assay, must take into account that wild-type ARM and E1-deleted adenoviruses may behave differently
- Each new virus must be validated using different dilutions in your assay to ensure parallelism with the ARM



Conclusions

- Use of ARM will allow comparison of data from different studies using different adenoviral vectors
 - Improve accuracy of assays - titer, particle & RCA
- Use of ARM will improve safety and efficacy
 - Control of clinical doses
 - Comparability between clinical trials
 - Comparability between preclinical trials
- Result in policy development



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ARMWG membership, production and characterization participants



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