

Adenoviral Vector Testing Conferees Agree That Standard Is Needed; Working Group to Move Forward

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As the development of adenoviral vectors for gene delivery applications broadens and moves forward, the possibility of approved products is on the horizon. Fundamental to the drug development process, is the establishment of an adequate safety profile and demonstration of clinical efficacy. For new classes of drugs including gene therapy, the safety profile of a product is comprised of product specific data as well as collective data from products that share common elements in the platform technology (e.g., E1-deleted Ad vectors). However, when analysis of safety and efficacy across a product class relies on data generated by non-standardized methods and comparability between quantities such as viral particle are relatively imprecise (see below), confidence in safety assessments is lacking. In these cases, especially when efficacy has not been convincingly demonstrated, regulators often take a conservative approach and assume that the relative risk is high when making decisions about dose escalation and other risk/benefit decisions.

Currently, there are still many questions regarding the safety and efficacy of adenoviral vectors and thus drug developers and regulators must proceed cautiously until more information is obtained. Although variations in dosing regimens, routes of administration and disease applications can confound interpretation of collective data, the field has been further hindered by the inability to readily compare clinical safety data due to differences in characterization of clinical vector lots among various sponsors.

There are two primary safety issues with adenoviral vectors. The first is the toxicity of the vector particles themselves and the second is the risk associated with replication competent adenovirus (RCA). Although vector dosing is based on particle number, different methods are being used to define particle dose. The most common method, recommended in the 1998 FDA Guidance on Human Somatic Cell Therapy and Gene

Therapy, is to measure the absorbance at OD 260 nm after lysing the viral particles and convert that to particle number based on a published extinction coefficient. Values derived from this type of measurement cannot be directly compared because the composition of a given vector preparation (particle concentration, purity, formulation) can influence the absorbance of the sample. Values derived from analytical methods that may measure a sub-component of the vector preparation (e.g., DNA dye binding, intact particles by anion exchange HPLC, reverse-phase HPLC analysis of viral protein components) make comparisons even more difficult. The inability to precisely compare viral particle counts obtained by different manufacturers makes analysis of dose-related clinical safety effects difficult.

A third concern for adenovirus vectors relates to the infectious titer of preparations. Infectious titer can have an impact on both safety and efficacy. Currently, adenoviral vector lots with particle to infectious titer ratios of 100 to 1 or less can be used in clinical trials per the 1998 Guidance. This ratio is used to monitor production lot consistency and stability. However, the methods for determining vector infectious titer are not standardized across sponsors. There are also several issues with infectious titer determinations complicating their use currently. Since infectious titer is a biological assay it is a measure that carries considerable imprecision, often greater than 30% inter-day precision. By comparison, physical methods to determine particle number frequently have inter-day precision values of less than 5%. Additionally the methodology used in determining infectious titer rarely takes into account the slow diffusion of adenoviral vector particles and how that affects the number of particles that actually come into contact with the cells during the assay. This means that while infectious titer assays can be made reliable in performance, the calculations used to convert raw data into infectious titer typically overestimate the number of particles that are able to interact with the cells to create an infection event. In this way infectious titer assays can underestimate the actual number of infectious particles present in the sample. Thus until infectious titer assays become more standardized, comparing the safety or efficacy of doses that have the same particle number but vary in their infectious titer measurement presents a challenge for regulators in the field of adenovirus vector based gene transfer.

For RCA, testing is even less well defined and comparable. The amount or volume of a production lot that should be tested, what analytical methods to use to quantitatively measure RCA, and how to report RCA testing results are variable across drug developers. As a result there are limited data by which to determine the risk associated with the presence of specific amounts of RCA as an impurity in different clinical settings. Although it is understandable and acceptable to have differences in the specific analytical tools used by drug developers to assess their products, the lack of an international reference standard for dose and RCA assay hampers comparison analyses. The availability of a standard material for which particle and RCA concentration can be measured and assigned should facilitate development of the field.

The Williamsburg BioProcessing Foundation hosted a one day meeting on October 5 (Washington, D.C.) with representatives from industry, regulatory agencies, contract testing laboratories, academia, and standard setting organizations to discuss testing of adenoviral gene therapy vectors and whether and how to develop a standard. There were more than 115 attendees with representation from all sectors. The overall goal of the one day meeting was to: [1] determine if there was consensus that a standard was useful, [2] identify technical and practical issues related to the development of a standard(s) for adenoviral vector testing, and [3] determine how to proceed with the development of a standard.

The goal of the meeting's morning agenda was to highlight the perspectives of those involved in, and impacted by, standards development. Representatives from industry, academia, FDA, U.S. Pharmacopeia (USP), the UK's National Institute for Biological Standards and Control (NIBSC), ATCC and the Biotechnology Division of the National Institute of Standards and Technology (NIST) described their perspectives, interests and possible roles in the development of an adenoviral testing standard. Representatives from 10 different companies then made short technical presentations that highlighted specific issues in determination of particle concentration, infectivity measurements, and in detection and quantification of RCA. All meeting participants then separated into small

groups to discuss in further detail and identify the issues related to developing standards for adenoviral vector testing. Appointed discussion group leaders presented the key points identified by each group when the entire conference reconvened for the afternoon session. The conference ended with consensus reached on several points after further discussions regarding RCA testing and limits, potency assays, and use of the term MOI.

The end of the conference reached consensus reached on the following points:

- 1) Development of a well characterized adenoviral standard is a good idea.
- 2) The development of a standard must proceed rapidly to re-gain public trust.
- 3) FDA should take responsibility for leading the process using a working group approach to accomplish the goal.
- 4) The Working Group will be responsible for identifying the process to evaluate and to select appropriate group(s) to manufacture, characterize, and distribute the standard.
- 5) Decisions will be made available for public comment via websites, journals, and meetings.
- 6) A wild type adenovirus should be the primary standard. Development of a second standard that is replication defective and tied to the first standard will be acceptable for physical characterization assays.
- 7) The standard must be characterized using procedures, the details of which are made available to all participants.
- 8) There will not be any standardization of specific methods at this time.
- 9) The standard vial label will list both particle concentration as well as infectious units. An orthogonal approach was recommended to establishing particle concentration including analyses by reverse-phase HPLC, quantitative real-time PCR, Pico Green DNA dye binding assay, absorbance at 260 nm, and anion ion exchange HPLC.
- 10) A specific method for using absorbance at 260 nm to determine the particle concentration from the new standard will be made available; *i.e.*, a new extinction coefficient will be determined for adenovirus based on the standard and a specific particle lysis method.

CBER/FDA made several points of clarification. First, in reference to the 1998 Guidance which states "that patient doses should contain no more than 1 pfu of RCA or equivalent," this recommendation was made by the agency based on what were typical dose levels in the mid-1990's, 10^9 particles. Since dose levels commonly rise above this level today, the levels of RCA that are acceptable on a per dose basis require a risk benefit analysis. The 1998 Guidance addresses this point stating that "if sponsors wish to propose a different specification, data should be provided demonstrating that the level of RCA present represents an acceptable risk for the intended patient population, route of administration, and dose." However until commonly agreed upon units of quantifying RCA are available, the agency will not be readily able to complete this assessment. The FDA considers RCA to be a safety concern and until there are enough data to establish the true risk of this agent, the agency has a current recommendation of <1 RCA in 10^9 infectious units of adenoviral vector product. When asked whether the agency will standardize the amount to be tested for RCA following a similar model as that developed for RCR testing, *i.e.*, a statistical confidence-based method, it was stated that the FDA is discussing this internally but that no specifics had yet been decided.

A second point made by the FDA was that a measure of infectious titer does not constitute a potency assay. Potency assays must quantify vector function. This typically means quantification of the biological function of the specific transgene. In the case of adenoviruses that do not express an exogenous transgene, it should still be possible to develop a potency assay based on the intended clinical effect. This does not necessarily imply that the mechanism of action of the vector is completely understood. The purpose of a potency assay is to enable one to distinguish lots that are too potent or not potent enough in terms of inducing the intended clinical effect. When asked how the FDA views the requirement for potency assays when the genes are species specific, the agency commented that they would encourage discussions with sponsors early in clinical development so that they could provide guidance in the development of an appropriate method.

An intense discussion point of the meeting was the use of the term MOI. This term is frequently used in describing assay conditions. It was agreed that MOI might not be a scientifically valid way to standardize infection efficiency across different procedures. However MOI can be a shorthand way to standardize assay input amounts across samples within one procedure (standard assay volumes and infection time) and to scale samples within one procedure (e.g., infecting cells in a flask versus a roller bottle). An example was given that interference and toxicity can be seen in RCA bioassays at "high MOIs". It was pointed out by several meeting participants that if toxicity is truly associated with the number of infection events, then infection efficiency is a function of virus concentration and infection time and not related to MOI (virus to cell ratio). A consensus was reached in that use of the term MOI should be in conjunction with other information, specifically virus concentration in particles/mL and infection time. The probability of an infection event can then be calculated based on a diffusion model.

Those who wish to participate in the Working Group should contact Keith Carson at the Williamsburg BioProcessing Foundation. The Foundation has agreed to act as a clearinghouse for the time being. One can reach them at: wbf@wilbio.com or 757-423-8823. The Working Group currently consists of more than 25 people from industry (both U.S. and Europe), FDA, testing companies, NIBSC, ATCC, academia, and the Williamsburg BioProcessing Foundation.

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