Utility of a Recombinant Adeno-Associated Viral Vector Reference Standard

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Recombinant Adeno-associated viral (rAAV) vectors are known to be efficient vehicles for gene transfer in animal models. The attractive feature of this vector system consists primarily of long-term gene expression with little or no associated toxicities following administration to a variety of tissues.1-11 Previous and ongoing clinical trials in humans demonstrate a very good overall safety profile, but problems persist due to the lack of any systematic method for normalizing doses administered to animals and humans.12-14 To date, most of the work involves AAV serotype 2 vectors, but vector systems based on other AAV serotypes continue to develop rapidly.15-21 Administered doses are usually based on titer, but the defective nature of AAV makes determining vector infectious units difficult. Titering methods based on vector genomes (using hybridization, real-time PCR, or spectrophotometry) are more reliable, but give no information as to the infectivity of the vector. Determining infectious titer is critical, as the ratio of infectious virions to vector genome-containing virions helps to determine the dose, potency, and strength of the vector preparation.

To date, some work has been performed to address potential long-term toxicities, such as insertional mutagenesis and germ-line transmission.22,23 It was recognized early on that toxicology studies addressing these issues would require large numbers of animals and resources beyond those available to academic centers and small enterprises involved in the treatment of rare genetic diseases. Discussions began in May 1999 at a joint FDA/NIH workshop. Members of the rAAV gene therapy community from academia, industry, and the federal government discussed the potential for developing a shared body of preclinical data to address vector-related safety issues. It was generally accepted that in order to share and compare nonclinical and clinical studies performed by laboratories using different vector-transgene combinations, it would be necessary to determine vector dose, strength, and potency in terms of equivalent titer units. It was further recommended that a highly characterized RSS of rAAV would be required to facilitate these comparisons. The RSS would allow researchers to normalize their titer values to the common standard, thus allowing each group to state their titers in units comparable to other studies. To facilitate the effort, the proposed RSS would be produced, characterized by several labs, and distributed freely to all members of the research community.

Recently, this effort for generating a rAAV reference standard material received renewed attention. Following the example set by the adenoviral reference material working group (ARMWG) for developing the adenovirus reference material (ARM), the FDA is encouraging the AAV community to develop a high-quality AAV RSS. 24 Lentiviral vector and retroviral reference standards are also being established (see www.wilbio.com). At an ICH Gene Therapy Workshop sponsored by PhRMA on September 9, 2002, discussions took place about the importance of establishing vector reference standards for all vector systems. A major point made by Stephanie Simek of

CBER was to use reference standards to validate each laboratory's own reference material working group (ARMWG) for developing the adenovirus reference material (ARM), the FDA is encouraging the AAV community to develop a high-quality AAV RSS. 24 Lentiviral vector and retroviral reference standards are also being established (see www.wilbio.com). At an ICH Gene Therapy Workshop sponsored by PhRMA on September 9, 2002, discussions took place about the importance of establishing vector reference standards for all vector systems. A major point made by Stephanie Simek of CBER was to use reference standards to validate each laboratory's own reference standard and test methods. This would facilitate comparisons among nonclinical or clinical studies, aid in the manufacture of more consistent and higher quality vectors, and ultimately help formulate regulatory policy.

Recently, the AAV reference standard working group (AAVRSWG), a volunteer organization comprised of members of the AAV community, held a teleconference to discuss the effort required to create an AAV RSS. The AAVRSWG includes members from five industrial organizations, 13 universities, FDA/CBER, NIH, and WilBio, and represents four countries: US, France, Germany, and Japan. FDA and NIH representatives abstain during voting. The consensus was that the RSS should be used primarily to standardize measurements made at different locations to facilitate the inter-relations among doses used in different nonclinical and clinical studies. When reporting titers in the literature, or to the FDA, the relationship to the RSS would be included. It was agreed that the RSS would be made available to the entire gene therapy community in a form suitable for nonclinical and clinical data support, and that the information regarding RSS development would be freely accessible. It was recognized that generation of the RSS would be a major undertaking, because it must be supplied in sufficient quantity to each requestor for use in all necessary tests at each location, be of high quality, and remain stable for an extended period of time. A profile of the RSS was established, and it included a proposed titer of 1 x 1012 vg/mL. There would be 5,000-10,000 vials with 0.5mL containing between 1 x 1015 and 5 x 1015 vg. Characterization would include confirmation of the serotype with a neutralizing antibody (Nab) assay, evaluation of the purity (nucleic acid, protein, rcAAV), determination of the titer (infectious, particle, capsid), measurement of safety parameters (sterility, mycoplasma, endotoxin, and adventitious virus), and a full sequencing of the vector. A consensus was reached for the manufacturing system, and it was agreed that a helpervirus-free system would be preferred, however, a helpervirus would be acceptable only if the helpervirus and its proteins could be removed. It was further agreed that the production method should be cost-effective and use chromatographic purification methods.

Organizationally, the AAVRSWG decided upon four committees to facilitate the effort: Manufacturing, Quality Control, Donations, and Executive. The initial responsibility of the subgroups is to make further investigations and report information back to the group. The group at large will reach consensus or approve recommendations made by the subgroups.

During the teleconference, several hurdles facing the AAV RSS effort became apparent. The primary hurdle is motivation. There is a lack of urgency in the AAV community to

develop an AAV RSS. This is in contrast to the high-quality adenoviral reference standard that was developed out of necessity following the unfortunate death of a patient during an adenovirus gene therapy trial in 1999.24,25 The adenovirus community worked cooperatively to generate and characterize the ARM, and many groups donated time, reagents, and space to this effort.

The other hurdles are mainly procedural. A major point of discussion was whether the RSS should be a well-characterized research-grade material or one that is manufactured under GMP. Most participants agreed that producing the RSS under cGMP conditions would satisfy most of the requirements outlined above; however, the cost for cGMP was a major concern. In addition, the number of facilities capable of producing the RSS under cGMPs is very limited, which could be a burden if only a few members participate in production. Additionally, resources are limited due to the small number of companies involved in AAV gene therapy. Open questions regarding choice of vector serotype and transgene.

The AAV community has an important opportunity to define potential toxicities and maximum tolerated dose associated with the use of an AAV vector system delivering therapeutic drugs. At this time, the best path may be to create a highly characterized AAV2 RSS under controlled manufacturing conditions that meet the spirit of the GMPs, but may fall short of full compliance. This would allow the AAVRSWG to move forward with their plans to produce a reference standard, plus build an operational structure to facilitate production of RSSs for other AAV serotypes. The Williamsburg BioProcessing Foundation has generously offered to help obtain donations of raw materials, hardware, consumables, and testing services, as well as post information on its web site. The organization will also coordinate meetings and conference calls pertaining to the RSS, and participate directly in the effort.

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