

March 6, 2000 FDA Gene Therapy Letter, Discussion of Adenovirus Vectors continued from April 5.

The morning session of the April 5, 2001 BRMAC was devoted to an overview and discussions concerning responses to the March 6, 2000, FDA Gene Therapy Letter. Discussion concerning adenovirus vector titer measurements and RCA levels was postponed until the July 13, 2001 BRMAC meeting. In the interim, the briefing document, questions for discussion and accompanying materials have been revised and updated.

Gene therapy is a promising field of clinical research. However, there has recently been widespread concern about the state of gene therapy clinical trials. Some of the issues that fueled concerns about safety in gene therapy clinical trials included the death of a patient in an adenovirus gene therapy clinical protocol and the risk of transmission of infectious agents by inadequately tested products. The rapid development in science and technology underway in gene therapy has meant that many of the standards for product testing considered adequate by the agency even a few years ago are deficient by today's standards.

Annual reports from IND sponsors have not always included the information that the agency needed to determine that current good manufacturing practices and testing procedures for gene therapy products were in use. Without this information, it is very difficult to develop reasonable, scientifically sound policies. In order to obtain the data needed, a letter was issued to all sponsors of gene therapy INDs and master files requesting, among other things, all product testing and characterization data, test methods, specifications, information regarding other products produced in the facility, and quality control procedures (referred to as the March 6 Letter to Gene Therapy Sponsors, see items 1 through 5 in the letter also included in this package).

In requesting and reviewing this information, our goals were the following:

1. Ensure that all gene therapy products currently in clinical trials are adequately tested by contemporary standards,
2. Determine where testing requirements need to be made more stringent or relaxed,
3. Gather information to aid in development of additional guidance,
4. Gain information concerning product characterization and manufacturing processes and arrangements in order to move these products forward toward licensure,
5. Determine appropriate use of training resources,
6. Increase public confidence in the oversight of gene therapy products and clinical trials, and
7. Develop a mechanism to ensure that IND annual reports routinely contain updates of this information.

After a comprehensive review of the data provided in response to the March 6 Letter to Gene Therapy Sponsors, several issues were presented to the BRMAC for informational purposes or for scientific input towards development of new guidance during the

meeting of April 5-6, 2001. The July 13, 2001 meeting revisits this subject and will present the following issue on which we are seeking additional guidance: adenovirus vector titer measurements and RCA levels (briefing documents for each item below are included in this package). At the April 5 meeting, Dr. Stephen Chanock gave a presentation entitled: "Clinical Issues of Adenovirus Infection in Marrow Transplant Recipients". Transcripts and slides from Dr. Stephen Chanock's presentation have also been included in these briefing materials.

Adenovirus Vector Titer Measurements and RCA Levels

To be presented by Dr. Steven Bauer

This presentation will provide an update on developments in characterization of replication defective adenovirus vectors used for gene transfer experiments and is seeking guidance on FDA recommendations for acceptable levels of replication competent adenovirus in vector preparations. The presentation will include a description of a recent initiative to develop a reference material consisting of a wild-type adenovirus for use in determination of vector particles, infectious titer and presence of replication competent adenovirus. In addition FDA has made changes in recommendations for acceptable ratios of infectious to non-infectious particles and acceptable quantities of RCA in adenovirus vector preparations. Finally, FDA is seeking guidance on the RCA recommendation and will ask the committee to discuss whether or not the recommendation should differ depending on the patient population that receives replication defective adenovirus vectors.

Development of an adenovirus reference material

During characterization of adenovirus vectors, determination of the infectious titer, determination of the ratio of infectious to non-infectious vector particles, and detection of replication competent adenovirus recombinants are each important procedures related to safety and product consistency. Currently, FDA recommends that patient doses be calculated on the basis of total number of virus particles rather than infectious particles. There are two reasons for this recommendation: 1) determination of the particle number is more precise than infectious titer measurements since it is based on a physical measurement; and 2) a primary toxicity of adenovirus vectors is mediated by an innate immune response to the viral coat proteins largely independent of the transgene expressed by the vector.

Both physical measurements and infectious titer determinations have technical limitations. Currently, the most widely used measurement of adenovirus particle number is based on lysis of vector particles, followed by measurement of the absorbance at 260 nm. Using an agreed upon constant relating optical density to vector genomes, the number of particles can be calculated. However, the optical density measurement can be affected by the formulation of the vector product. In addition, results obtained by different laboratories are not always comparable. Infectious particle measurements are much less precise due to the effect various parameters have on the

efficiency of in vitro infectivity. The best inter-assay variability for infectious titers is on the order of 30%.

Due to these technical problems with determinations of adenovirus particle number and infectious titer, a consortium called the Adenovirus Reference Material Working Group has initiated an effort to produce a wild-type adenovirus preparation that can be used as a reference material for improving precision and accuracy of particle and infectious titers, and for allowing comparability of data from different labs (1).

Change in particle to PFU ratio

Until the April 5, 2001 BRMAC meeting, FDA recommended that preparations of adenovirus vector used in patients have less than 100 total viral particles per infectious unit (usually expressed as <100vp/iu). This recommendation was developed over five years ago and was based on vector product testing results from manufacturers of adenovirus vectors. Responses to the March 6 Gene Therapy Letter were used to review the vp/iu ratios actually obtained by adenovirus vector manufacturers. CBER review found that ratios less than 30vp/iu were routinely achieved. Therefore, FDA is changing the recommended specification for clinical lots of replication defective adenovirus vectors to <30vp/iu. The rationale is to minimize exposure of patients to inactive adenovirus particles within the practical limits currently observed in vector production by a variety of sponsors manufacturing different adenovirus vectors. In the future, this ratio will be expressed as percent infectious particles in a vector preparation so the current recommendation will be expressed as follows: minimum 3.3% infectious particles in a clinical lot.

Change in recommendation on RCA limit

Currently, the most common production method for replication defective adenovirus vectors uses the cell line HEK 293. This cell line contains integrated adenovirus sequences, including the E1 genes. During cloning of vectors, the E1 regions are deleted so that replication is defective in cells lacking E1. However, the integrated E1 genes in HEK 293 complement the E1 genes lacking in the vector, allowing manufacture of high titer replication defective adenovirus vectors. Unfortunately, homologous recombination between adenovirus sequences that are present in both the defective vector and in 293 cellular DNA can reintroduce E1 sequences into the vector and yield replication competent adenovirus (RCA). RCA can infect and replicate in many different cell types. The concern that RCA could lead to adverse events in patients led FDA to recommend limits on RCA levels in clinical lots of adenovirus vectors. Before 1998, FDA recommended that preparations of adenovirus vectors contain RCA at a concentration ≤ 1 iu/patient dose if for use in patients in whom adenovirus infection would be considered a potential risk (2). In the absence of quantitative data regarding risks associated with RCA administration, this recommendation had been made on the basis that it was a reasonably achievable value. As manufacturers developed methods to produce higher titer vector preparations FDA changed the recommendation to <1 RCA/ 10^9 iu of vector. This recommendation took into account pragmatic considerations regarding amounts of vector to be tested. In order to be consistent with the current FDA recommendation that patient doses be based on vector particle number and given the relative imprecision of infectious titer measurements, FDA is changing this recommendation to < 1 RCA/ 3×10^{10} vp. The quantity 3×10^{10} vp was derived by

multiplying the previous 10^9 iu by 30 which has been determined as a reasonable upper limit for vp/iu ratio.

Application of RCA recommendation

Adenovirus vectors are used in current gene therapy trials for many different clinical indications in a variety of patient populations (Table 1). The RCA limits discussed above are intended to ensure limited exposure of patients to replicating adenovirus in the products used in clinical trials. The current FDA approach is to apply these recommendations to all adenovirus vector products, regardless of clinical indication or mode of administration. In current clinical trials, doses can be as high as 10^{13} vp by intra-arterial injection. For such doses, potential exposure to RCA could be as high as 330 infectious particles, using the new recommended limit. In the past, some clinical trial doses were as high as 10^{13} iu. RCA exposure per dose in these cases could have been on the order of 10,000 RCA. (1×10^{13} iu/ <1 RCA per 10^9 iu).

The clinical consequences of adenovirus infection (serotypes 2 and 5) in healthy individuals are generally thought to be relatively mild, cold-like symptoms. In contrast, in immunosuppressed individuals, clinical experience in the arena of bone marrow transplantation suggests that adenovirus infection can lead to severe adverse events, including death (3,4,5, 6). Currently, adenovirus serotypes 2 and 5 have been adapted for gene transfer vectors. Both of these serotypes have been isolated from bone marrow recipients with adenovirus infections. Recent studies also suggest that the presence of adenovirus genomes, particularly adenovirus serotype 2, in the myocardium of pediatric cardiac transplant recipients is associated with increased adverse events including coronary vasculopathy and graft loss (7). It may be that infections in immunocompromised hosts are in part due to reactivation of persistent adenovirus (6). Given the range of potential responses to adenovirus infection in patients with different clinical statuses, FDA is seeking advice on whether we should apply current RCA limits to adenovirus vector products for all patient populations.

Table 1: Active Gene Transfer INDs with Adenovirus Vectors

Clinical Indication	Route of Administration	# of INDs
Cancer	Intratumoral/Intralesional	29
	Ex vivo transduction	13
	Intravascular	2
	Intraperitoneal/Intrapleural	2
	Oral	1
*Coronary/Vascular	Myocardial	3
	Intramuscular	2
	Intracoronary	1
#Genetic defect	Respiratory tract	3
	Intravascular	1
+Normal	Intradermal	1
		58 total

* Angina, peripheral vascular disease, coronary artery disease(2 INDs), critical limb ischemia, cardiac ischemia

Cystic fibrosis (3 INDs), hemophilia A

+Evaluation of immune response in normal volunteers

Reference List

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3. Foster, C.B., Choi, E.H., and Chanock, S.J. 2000. Adenovirus and marrow transplantation in children. *Ped. Path Molec Med*. 19:97-114.
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6. Flomenberg, P., Gutierrez, E., Piaskowski, V., and Casper, J.T. 1997. Detection of adenovirus DNA in peripheral blood mononuclear cells by polymerase chain reaction assay. *J. Med Virol*. 51: 182-188.5.
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DRAFT Questions for discussion by the committee

Adenovirus-based gene transfer products are used in a variety of clinical indications by a variety of routes of administration. FDA is seeking advice regarding RCA levels in adenovirus gene transfer products. For this meeting, FDA is not requesting votes on these issues. Currently the recommendation that adenovirus vector products should contain less than 1 RCA per 3×10^{10} vp is applied to all lots regardless of the clinical use.

1) Should recommendations regarding acceptable levels of RCA in adenovirus gene transfer products be the same for all clinical uses? Considering the following patient populations, please discuss the relative risks for RCA exposure:

- a) severely immunosuppressed or immunocompromised patients such as: patients with HIV, pediatric bone marrow or cardiac transplant recipients, adult cardiac or other whole organ transplant recipients, cancer patients after myeloblastic chemotherapy;
- b) mildly immunosuppressed or immunocompromised patients such as cancer patients;
- c) patients with genetic defects such as hemophilia, cystic fibrosis, or other.

2) Please discuss the sorts of experiments or data that should be used to set acceptable limits for RCA exposure. For example, are there measures of immune competency that would be adequate to determine whether or not exposure to RCA might pose a risk?

3) When adenovirus is used for ex-vivo transduction of target cells, should RCA measurements be performed on the transduced cells to help assess the risk in this setting? Or is it sufficient that vector preparations meet the standard requirements?