

## Overview of March 6, 2000, FDA Gene Therapy Letter

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 include the death of a patient on a gene therapy clinical protocol, the risk of transmission of infectious agents by inadequately tested products, and numerous violations that the agency uncovered on directed inspections. 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 up to 10 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 the current good manufacturing practices and testing procedures for gene therapy products and to ensure compliance. 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, and
6. Increase public confidence in the oversight of gene therapy products and clinical trials.
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, we have identified the following issues that will be presented to the BRMAC for informational purposes or for scientific input towards development of new guidance:

- A. Informational issues:
  1. Definition of potency assays for gene transfer products
  2. Addition of in vitro virus testing of production lots of gene transfer products
  3. Sponsor responsibilities regarding Quality Assurance and Quality Control (question #5 of March 6 Letter)
  4. Issues specific to multi-use manufacturing facilities
- B. Issues on which we are seeking additional guidance (briefing documents for each item below are included in this package)
  1. Testing of plasmids as manufacturing intermediates in gene therapy products
  2. Generation of replication competent retrovirus and different packaging cells
  3. Adenovirus Vector and RCA Levels

## **Responses to Gene Therapy Letter – Multi-Use Facility, QA/QC Issues**

To be presented by Mary Malarkey

In the gene therapy letter of March 6, 2000, CBER asked sponsors to provide a summary of their product manufacturing quality assurance (QA) and quality control (QC) programs (see question #5). Many sponsors did not understand what was expected and did not provide adequate responses. CBER has been trying to clarify the expectations through a variety of educational outreach efforts, such as talks at conferences. As an additional such effort, an educational presentation will be made at the BRMAC meeting April 5.

An important topic that is often misunderstood is the division of responsibilities between an IND sponsor and a multi-use facility contracted to do some part of product manufacturing. Many gene therapy vectors are produced in multi-use facilities, including commercial contractors and also National Gene Vector Laboratories. Sponsors often assume that the contract lab will provide all necessary QA/QC, manufacturing and product testing information to CBER, and do not involve themselves sufficiently in design of the testing, examination of the data, and answering of CBER questions. However, sponsors are ultimately responsible for their products and submission of appropriate data. Also, contract labs typically perform only a certain range of tests, and some tests such as potency assays or activity/expression assays are done on a product-specific basis by the sponsor. Thus, QA/QC issues and reporting apply to the sponsor, too, not just to the contract lab.

An additional concern with multi-use facilities is the potential for cross-contamination of one product with a product made previously. The sponsor of a particular IND is not privy to a list of other products made in the facility, and cannot test for cross-contamination. The multi-use facility should do that, or validate the production and purification process to rule out cross-contamination. The sponsor is responsible for the safety and identity of the product and the contract facility should provide the sponsor appropriate information regarding controls to prevent and assess cross-contamination. Information that is considered proprietary, i.e. actual list of specific products, may be submitted to CBER by the contract manufacturer as a Type V Drug Master File.

## **Retroviral Vectors: Influence of Packaging Cell Lines and Generation of Replication Competent Retrovirus (RCR)**

To be presented by Dr. Carolyn Wilson

This section provides a discussion of factors influencing the frequency of contamination with replication competent retrovirus (RCR) in retroviral vectors, as well as a brief summary of current experience, so the committee may consider whether certain characteristics or types of vector production systems may be inherently less safe for clinical use. To date, we have not specified what vector producer cells (VPC) are acceptable for retroviral vector manufacture, only that the vectors be tested for RCR. We now are asking the committee to consider whether sufficient experience has been obtained to allow determination of those characteristics of VPC that would be deemed unacceptable for manufacture of retroviral vectors for clinical use.

### **Current CBER Recommendations**

The concern that exposure to RCR presents a risk to patients is based, in part, on a study reporting lymphoma and death in 8/10 immunosuppressed monkeys treated with cells transduced with a preparation of retroviral vector contaminated with RCR [1]. To minimize the potential for RCR exposure to patients, retroviral vectors used in clinical trials are subject to stringent tests for RCR contamination. As an additional safety net, we also recommend that sponsors monitor for evidence of RCR infection in patients exposed to retroviral vectors through either *ex vivo* or *in vivo* contact. For details on our current guidance, please refer to "Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors" (10/18/2000), available at the following web site: <http://www.fda.gov/cber/guidelines.htm>.

### **Manufacture of Retroviral Vectors: Vector Producer Cell Design Elements**

Retroviral vectors are manufactured by use of mammalian cell lines (known as vector producer cell lines, VPCs) engineered to express *retroviral helper sequences* and *retroviral vector sequences*. The retroviral helper sequences encode retroviral structural and enzymatic proteins required to make a vector particle. The retroviral vector sequences have deletions in retroviral protein coding sequences, while retaining the *cis*-acting elements that are required for packaging the vector RNA into the vector, reverse transcription, integration, and transcription. VPC produce vector particles that are structurally similar to a retrovirus, but are genetically devoid of the helper sequences. Therefore, cells exposed to retroviral vectors cannot make progeny virions, i.e. the vectors are replication-defective.

Although the goal of using retroviral VPCs is to produce replication-defective vectors, the manufacturing process still carries the risk of generating RCR. RCR may result if recombination occurs between vector and helper sequences present in the VPC in a manner that generates a single viral genome fully constituted with all sequences necessary for viral replication. Several factors influence the frequency of recombination events, and different VPCs vary in the design elements incorporated to lower recombination frequencies.

#### **1. Reduction in amount of homology between vector and helper sequences.**

Homologous recombination of retroviruses occurs at a frequency at least 1000-fold greater than non-homologous recombination [2]. Therefore, vector producer systems have been designed with reduced lengths of homologous sequences between different elements present in the cells. Helper sequences may use heterologous promoters or poly-adenylation sequences in lieu of corresponding retroviral elements (long terminal repeats, or LTRs). Vector sequences may contain reduced amount of envelope coding sequence to minimize overlapping regions of homology.

2. Reduction in homology between vector/helper sequences and cellular DNA. Sequences endogenous to the cells being used may also contribute to the extent of homologous sequences. Murine cells carry endogenous retroviral sequences with homology to the retroviral vectors currently being used. Recombination between the exogenous retroviral vector genetic elements and the endogenous retroviral sequences may give rise to RCR (for example, such recombinant retroviruses were reported in the monkeys that developed lymphoma and died after exposure to RCR-contaminated retroviral vector [3]). Therefore, some recently developed VPC have used cells of different species (such as dog or human) to avoid the potential reservoir of cellular DNA as a substrate for homologous recombination. Alternatively, one VPC uses a non-murine retroviral envelope with reduced homology to cellular endogenous sequences compared to the murine retrovirus envelope commonly used.
3. Division of helper sequences to more than one expression cassette to increase the number of recombinations required to generate RCR. Early VPCs use a single expression cassette for all the retroviral helper functions. In this case, RCR generation can result from a single recombination event. Later VPC divide the helper functions into two expression cassettes, which means that a minimum of two recombination events would be required to generate RCR.
4. Other changes in the vector sequences. Additional design elements include changes in the vector sequences, such as introduction of stop codons into an open reading frame. If recombination events occur with this type of vector, retroviral proteins can not be expressed, preventing generation of RCR.

### **VPCs and Current Experience**

The responses to the March 6 letter allow CBER to gather data regarding RCR generation with different VPC based on current manufacturing experience. In particular, the sponsors' responses to question #4 allowed CBER to identify VPC used to produce clinical lots rejected due to RCR generation. While the review of sponsors' responses to question #4 provides some valuable data to analyze the correlation between VPC and RCR, the data somewhat flawed, as they likely under-represent the numbers of relevant events of RCR generation. For example, question #4 only asked for the list of rejected clinical lots and the reasons why. Therefore, if RCR were generated at earlier production stages (for example, in the Master Cell Bank), sponsors may not have reported those data in the response to question #4 (although some sponsors did). Of note, we did not request sponsors to report the total number of production lots manufactured. Therefore, the denominator is unknown. Despite the limitations of these data, CBER wanted to report to the BRMAC a summary of the VPC reported to have RCR identified in production of clinical lots (Table 1). The following VPC, all murine cell lines, had reports of RCR in at least one IND or Master File: PA317, AM12 and Psi-CRIP. No RCR contaminated lots were reported by sponsors using PG13 or human-based VPC. Please note that PG13 and human-based VPC have been used by fewer sponsors and for fewer years than PA317, AM12, and Psi-CRIP.

The National Gene Vector Laboratory responsible for producing retroviral vectors (Indiana University Vector Production Facility) has provided permission to CBER to summarize their experience with the RCR detection during vector production using different VPC. The summary provided in Table 2 allows one to analyze the incidence of RCR positive lots from all the clinical production lots prepared. The NGVL experience demonstrates a higher incidence of RCR in PA317 compared with the other VPC used. Presumably the design of PA317 with a single expression cassette is a contributor to this finding.

#### ***DRAFT* Question for the Committee:**

Based on currently available VPCs and the data available regarding RCR identification during vector manufacture, is it reasonable to discourage use of VPCs with a single expression cassette for the helper sequences, such as PA317? Are there sufficient data to support discontinuation of the use of certain VPCs for manufacture of clinical-grade retroviral vectors?

## References

1. Donahue, R.E., et al., *Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer*. J. Exp. Med., 1992. **176**: p. 1125-1135.
  2. Zhang, J. and H.M. Temin, *Rate and mechanism of nonhomologous recombination during a single cycle of retroviral replication*. Science, 1993. **259**: p. 234-238.
  3. Purcell, D.F.J., et al., *An array of murine leukemia virus-related elements is transmitted and expressed in a primate recipient of retroviral gene transfer*. Journal of Virology, 1996. **70**(2): p. 887-897.
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**Table 1. Summary of VPC Characteristics and RCR Generation**

<b>VPC</b>	<b>Cell Line</b>	<b>Design of Helper Sequences</b>	<b>Envelope</b>	<b>RCR Observed</b>
PA317	Murine	One Expression Cassette 5' LTR, heterologous polyA	Amphotropic murine leukemia virus	Yes, fairly common
AM-12	Murine	Two Expression Cassettes 5' LTR, heterologous polyA	Amphotropic murine leukemia virus	Yes
Psi-CRIP	Murine	Two Expression Cassettes 5' LTR, heterologous polyA	Amphotropic murine leukemia virus	Yes
PG13	Murine	Two Expression Cassettes 5' LTR, heterologous polyA	Gibbon ape leukemia virus	No
Other	Human	Two Expression Cassettes	Amphotropic murine leukemia virus	No

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**Table 2. Summary Data from Indiana University Vector Production Facility**

<b>VPC</b>	<b>Number Production Lots</b>	<b>Number RCR Positive Production Lots*</b>	<b>Incidence of RCR</b>
PA317	4	3	75%
AM-12	12	3	25%
Psi-CRIP	2	0	0%
PG13	14	0	0%

\*In each of the cases reported, the RCR was only observed in the production lot, not the Master Cell Bank.

## Testing of Plasmids as Manufacturing Intermediates in Gene Therapy Products

To be presented by Dr. Suzanne Epstein

Plasmid DNA as a final product for administration directly to patients is subject to lot release. In contrast, plasmids used in early stages of deriving vectors are regulated not as biological products but only as reagents used in manufacturing. In the latter case, a plasmid may be used in the derivation of a construct and never again, certainly not on a lot-by-lot basis.

There is an intermediate category which we think calls for a different approach. In some systems plasmids are used transiently in every production run for transfection of cells to make *ex vivo* cell products, or to generate retroviruses, AAV, or other vectors in cell cultures. In such cases, the plasmid is used over and over again from a bank, it is used late in product manufacturing, and the quality of the plasmid preparation can have a direct impact on product safety, consistency, and efficacy. For these reasons, we propose that such plasmid preparations be subject to quality control testing and acceptance criteria. We think that these uses of plasmids are analogous to use of retroviral vectors to transduce cells for *ex vivo* gene therapy. The transduced cells are the products administered to patients in that case, but CBER has required extensive quality control testing of the retroviral vector preparations.

Examples:

### 1. *Ex vivo* transfected cells

Certain therapies are based on fibroblasts, lymphocytes, or other cells transfected with a plasmid to express a therapeutic protein, and then implanted into the patient. In such cases, the product administered to the patient is the cellular product (1;2).

### 2. AAV vectors

Plasmids are used for generating AAV vectors in tissue culture. In a review by Büeler (3), Fig. 2 shows two ways of generating AAV, one using adenovirus as a helper virus and the other using only plasmids. In a three plasmid system, one plasmid contains the AAV vector, one the rep and cap genes for packaging, and one the adenovirus helper functions. Cells are transfected and AAV is produced in lysates. Two plasmid systems have also been developed. In such a system (4), one plasmid contains the vector, and the other has both rep/cap and adenovirus helper functions.

### 3. Retroviral vectors

Retroviral vectors are often produced in packaging cell lines stably expressing gag-pol and env genes. However, an alternative is transient transfection of cells with plasmids expressing gag-pol and env, as well as a plasmid containing the sequences for the retroviral vector genome (5). The packaging genes, gag-pol and env, can be expressed from two separate plasmids to reduce the chance of generating replicating virus. In this approach, plasmids are used every time a lot of retrovirus-containing supernatant is made, rather than once to establish a producer cell line.

In these cases, it is important to perform quality control testing of the plasmid preparations, and to establish acceptance criteria. This will avoid contaminated cultures that lead to rejection of products for patient administration, and will also improve consistency of transfections and provide a more efficient manufacturing process.

### List for BRMAC discussion of tests for plasmids used as intermediates:

sterility (no growth at 14 days in CFR or USP test)  
residual levels of toxic process reagents used in production, for example, solvents  
endotoxin (LAL) Note: endotoxin contamination can reduce transfection efficiency.  
identity (examples: plasmid size, restriction map)  
purity (examples: UV absorbance, agarose gel electrophoresis)  
concentration (example: UV absorbance)  
activity/gene expression (product-specific assays)  
possible additional tests; see question 2

Additional testing is recommended for plasmids for direct administration to patients, for example, more stringent purity testing including analysis for residual *E. coli* DNA, RNA, and protein. In addition, full plasmid DNA sequence determination and homology searching is recommended, potency assays should be developed prior to phase III trials, and a stability program should be developed.

### DRAFT Questions for the committee:

We propose to establish testing recommendations for plasmids when used as intermediates for transfection to produce each lot of a gene therapy vector or cellular gene therapy product. Appropriate acceptance criteria should then be established by sponsors in consultation with CBER. Such testing would avoid contamination of cell cultures and help achieve consistency of manufacturing. Please comment on the following questions:

1. Are the tests listed necessary and appropriate for this purpose?
2. Are the tests listed sufficient, or should any other tests be added, for example full sequence and homology analysis, potency, and stability?

### Reference List

1. Jensen, M., G. Tan, S. Forman, A.M. Wu, and A. Raubitschek. 1998. CD20 is a molecular target for scFvFc: receptor redirected T cells: implications for cellular immunotherapy of CD20+ malignancies. *Biol. Bone Marrow Transplant.* 4:75.
2. Jensen, M.C., P. Clarke, G. Tan, C. Wright, C.C. Wen, T.N. Clark, F.Y. Zhang, M.L. Slovak, A.M. Wu, S.J. Forman, and A. Raubitschek. 2000. Human T lymphocyte genetic modification with naked DNA. *Molecular Therapy* 1:49.
3. Büeler, H. 1999. Adeno-associated viral vectors for gene transfer and gene therapy. *Biol.Chem.* 380:613.
4. Grimm, D., A. Kern, K. Rittner, and J.A. Kleinschmidt. 1998. Novel tools for production and purification of recombinant adenoassociated virus vectors. *Human Gene Therapy* 9:2745.
5. Soneoka, Y., P.M. Cannon, E.E. Ramsdale, J.C. Griffiths, G. Romano, S.M. Kingsman, and A.J. Kingsman. 1995. A transient three-plasmid expression system for the production of high titer retroviral vectors. *Nucleic Acids Res.* 23:628.

## **Adenovirus Vector Titer Measurements and RCA Levels**

To be presented by Dr. Steven Bauer

This section provides an update on developments in characterization of replication defective adenovirus vectors used for gene transfer experiments and seeks guidance on FDA recommendations for acceptable levels of replication competent adenovirus in vector preparations. This 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**

Particle number, infectious titer and the presence of replication competent adenovirus are important product characteristics for adenovirus vectors. During characterization of adenovirus vectors, determination of the infectious titer, the ratio of infectious to non-infectious vector particles, and detection of replication competent adenovirus recombinants are each important measurements 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. One reason is that determination of the particle number is more precise since it is based on a physical measurement. The other reason is that 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.

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. This measurement can be affected by the formulation of the vector product and it is not clear whether results obtained by different laboratories are 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 are 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 of particle and infectious titers. (1).

### **Change in particle to PFU ratio**

Currently, FDA recommends that preparations of adenovirus vector used in patients have less than 100 total viral particles per infectious particle (usually expressed as <100vp/pfu). This recommendation was developed over five years ago and was based on vector product testing results from manufacturers of adenovirus vectors. Review of the March 6 letter responses from numerous adenovirus vector manufacturers suggests that ratios less than 30vp/pfu are routinely achieved. Therefore, FDA is changing the recommended specification for clinical lots of replication defective adenovirus vectors to <30vp/pfu. 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.

### **Change in recommendation on RCA limit**

The most common production method for adenovirus vectors uses the cell line HEK 293. This cell line contains integrated adenovirus sequences that complement the E1A regions which are not present in the vector. The cell line thus supports replication of otherwise defective adenovirus vectors. However, recombination between sequences in the defective vector and homologous E1A sequences present in 293 cellular DNA may yield replication competent adenovirus (RCA). RCA can infect and replicate in many different cell types. The possibility that

RCA could lead to adverse events in patients has led to FDA recommendations that RCA in adenovirus vector preparations for clinical use be limited. Before 1998, FDA recommended that preparations of adenovirus vectors contain RCA at a concentration  $\leq 1$  pfu/patient dose if for use in patients in whom adenovirus infection would be considered a potential risk (5). In the absence of quantitative data regarding risks associated with RCA administration, this recommendation had been made on the basis of a reasonably achievable value. As technology progressed to permit higher titer vector preparations and recognizing pragmatic considerations regarding amounts of vector to be tested, FDA changed the recommendation to  $< 1 \text{RCA}/10^9 \text{pfu}$  of vector. Due to the current FDA recommendation that patient dosing should be based on vector particles and given the relative imprecision of infectious titer measurements, FDA is changing this recommendation to  $< 1 \text{RCA}/3 \times 10^{10} \text{vp}$ . The amount of  $3 \times 10^{10} \text{vp}$  was derived by multiplying the previous  $10^9$  pfu by 30 which has been determined as a reasonable upper limit for vp/pfu ratio.

### **Application of RCA recommendation**

Adenovirus vectors have been used to study many different clinical indications in a variety of patient populations. The RCA limits discussed above are intended to ensure limited exposure of patients to replicating adenovirus in the products used in clinical trials. In clinical trials, doses can be as high as  $10^{13} \text{vp}$  by intra-arterial injection. For such doses, potential exposure to RCA could be as high as 330 infectious particles, using the new recommended limit.

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 (2,3,4). 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. Given the range of potential responses to adenovirus infection in patients with different clinical status, FDA is seeking advice on whether or not the current RCA recommendation should be applied to adenovirus vector products for all patient populations.

### **DRAFT Question for the committee**

Currently the recommendation that adenovirus vector products should contain less than 1 RCA per  $3 \times 10^{10}$  vp is applied to all lots regardless of the clinical use.

- ◆ Are there clinical indications for which this recommendation would be too stringent?
- ◆ Is this recommendation sufficiently stringent for immunocompromised patients? For example, patients with HIV infection, bone marrow transplant recipients, cancer patients after myeloablative chemotherapy, recipients of whole organ transplants?

### **Reference List**

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2. 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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5. Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy - 3/30/98.