

## **STRUCTURAL CHARACTERIZATION OF GENE TRANSFER VECTORS**

### **Background Information**

#### **Introduction**

Currently there are 187 active gene transfer INDs under CBER regulatory authority. Almost equal numbers utilize ex vivo transduction of cells or in vivo administration of gene transfer vectors. The three most commonly used gene transfer vectors are retrovirus (65 total), adenovirus (55 total) and plasmid DNA (48 total) (see figure 1). Additional clinical trials are underway involving other vector classes based on poxvirus, adeno-associated virus, and herpesvirus (Figure 1). In addition, the vectors in use will continue to evolve either through continued improvements in existing systems or development of novel vector systems, such as lentivirus.

One property of gene transfer vectors that causes concern is that genetic information of the vector product for a gene transfer trial may be inadvertently altered. This genetic change may occur during construction or production. Often the procedures used to generate gene transfer vectors may be responsible for inducing genetic changes, depending upon the accuracy of the processes (Table 1). Alternatively, vectors based upon viruses may be subject to genetic change during production as a consequence of the normal biology of the virus being used (Table 1). Some of the viruses in use are prone to undergo genetic alteration through mechanisms such as DNA recombination or via transcription by error prone polymerases (e.g., reverse transcriptase of retroviruses). Genetic changes in a vector may range from relatively minor changes such as single nucleotide substitutions, to insertions or deletion of larger regions (up to several hundred base pairs). Unfortunately, intermediates used in construction of vectors are not always carefully characterized nor documented, and unexpected sequences may be present in the final vector product if full characterization has not been done. As the field progresses, and sponsors proceed with later stage clinical trials and treatment of larger numbers of subjects, the manufacture of vectors will be on a larger scale, and these problems may become more common.

Unexpected nucleotides in the genetic sequence may impact the safety and function of the gene transfer vector or the expressed product. In some instances, alterations of the genetic sequences may have no biological consequence. Other genetic changes may alter the gene expression or vector function in expected (i.e., introduction of stop codon to make truncated protein) or unexpected ways (i.e., exogenous sequences introduced may contain transcriptional control elements or open reading frames). The time at which mutations occur during vector production can influence the ability to detect changes that may nonetheless influence product safety and function. A mutation occurring during early stages of vector production may be much easier to detect than one occurring during the last step in a large-scale vector manufacturing process. Given the many uncertainties regarding the range and influence of genetic changes, CBER realizes gene transfer products may need more stringent

structural characterization; hence CBER is considering changing its current guidance on this issue. CBER is seeking guidance from the committee regarding three major areas:

- 1) When should structural characterization of gene transfer vectors be undertaken (both in terms of phase of clinical development and manufacturing scheme)?
- 2) How much structural characterization of gene transfer vectors should be undertaken?
- 3) How should the genetic sequence information of gene transfer vectors be used?

## **Current Recommendations**

Current published guidance on this topic can be found in the "CBER Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (3/98)". In section V, "Production, Characterization and Release Testing of Vectors for Gene Therapy", the following recommendation is provided:

Early in product development, vector characterization consisting of sequence data of appropriate portions of vectors and/or restriction mapping supplemented by protein characterization is acceptable. For later phases of product development and licensure, more extensive sequence information should be provided. When sequencing of the entire vector is not feasible due to the size of the construct, it may be sufficient to sequence the genetic insert plus flanking regions and any significant modifications to the vector backbone or sites known to be vulnerable to alteration during the molecular manipulations. Vector sequences which modulate vector-host interactions should be described if known, and stability of the host cell/vector system considered.

## **Problems Encountered**

The limited scope of our current guidance has resulted in partial structural characterization of most gene transfer vectors used in clinical trials. More recently, due in part to product development, and in part to increased accessibility to high throughput DNA sequence technology, some sponsors have submitted more complete sequence analysis of gene transfer vectors. With more complete sequence analysis of gene transfer vectors, unexpected genetic changes are sometimes identified.

Here, we summarize several examples of cases identifying genetic deviations. In at least one instance, the unexpected DNA was a consequence of "leftover" sequences from previous versions of a vector that were not well documented. However, the extraneous sequences did contain an open reading frame. In another case a commonly used adenovirus vector with deletions in E1 and E3 contained exogenous DNA elements derived from salmon DNA sequences. Published investigations speculate that these sequences were most likely introduced when salmon DNA was used as a carrier in transfection experiments used to generate the original Ad5 *d*309 mutant virus. This mutant virus was used to generate the pJM17 shuttle plasmid that forms the backbone

of most currently used Ad5 vectors [1]; [2]. In another case, we have received information showing that in large-scale vector production lots, genetic alterations may be present in a small percentage of the vector. In such cases, DNA sequencing of template prepared from the large-scale vector is not sensitive enough to detect deviations present at low levels. Although no adverse consequences have been correlated with presence of low level deviations, they could impact safety or efficacy – at a minimum, they indicate that the product manufacturing process is not robust, and therefore constitute a concern from the FDA perspective.

As additional nucleotide sequence information is gained, CBER suspects that these types of observations will not be exceptional. Many gene transfer vectors are constructed in academic laboratories where there is often an absence of documentation and characterization necessary to prevent introduction of inadvertent sequence elements. In addition, the properties of the vector construction and production schemes make many gene transfer vectors prone to introduction of genetic changes.

### **Current Thinking**

Recent identification of unexpected genetic alterations in gene transfer vectors has resulted in CBER's reconsideration of our current guidance [found in "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (3/98)"]. Analyses of sequence data support the need for more detailed structural characterization of gene transfer vectors prior to initiation of phase I clinical trials. One proposal under consideration would recommend that full genetic sequence information be provided for vectors of 40 KB or less prior to initiation of phase I clinical trials. Since full sequence analysis of larger vectors may be too great a burden for entering phase I clinical trials, vectors greater than this size would not be subject to full sequence determination until prior to initiation of phase II clinical trials. In these cases, CBER is considering that at a minimum, coding sequence of the gene or cDNA of interest, transcriptional control regions, and regions including and flanking any sequences manipulated during production of the vector be determined prior to initiation of phase I clinical trials.

CBER is requesting guidance to determine when during production and what material should be analyzed for identification of the genetic sequence, and whether the analysis should be performed as a one-time determination or perhaps on several production lots. In addition, once a sponsor determines the genetic sequence of a gene transfer vector, the sequence should be subject to database analysis. At a minimum, it should be compared to the expected sequence, but additional sequence analysis may need to also be performed, especially in those instances where segments of exogenous, unexpected sequences are identified.

CBER is also seeking guidance on the appropriate regulatory actions to consider when genetic changes are observed. One response may be to redesign the gene transfer vector to eliminate the unexpected elements. In those cases that include use of gene transfer vectors with cumbersome construction methods, such as in the generation of an adenoviral vector, it may not be reasonable to request vector reconstruction. In those cases, additional data will be needed to support regulatory decisions. In particular, it may be necessary for a sponsor to determine whether the unexpected

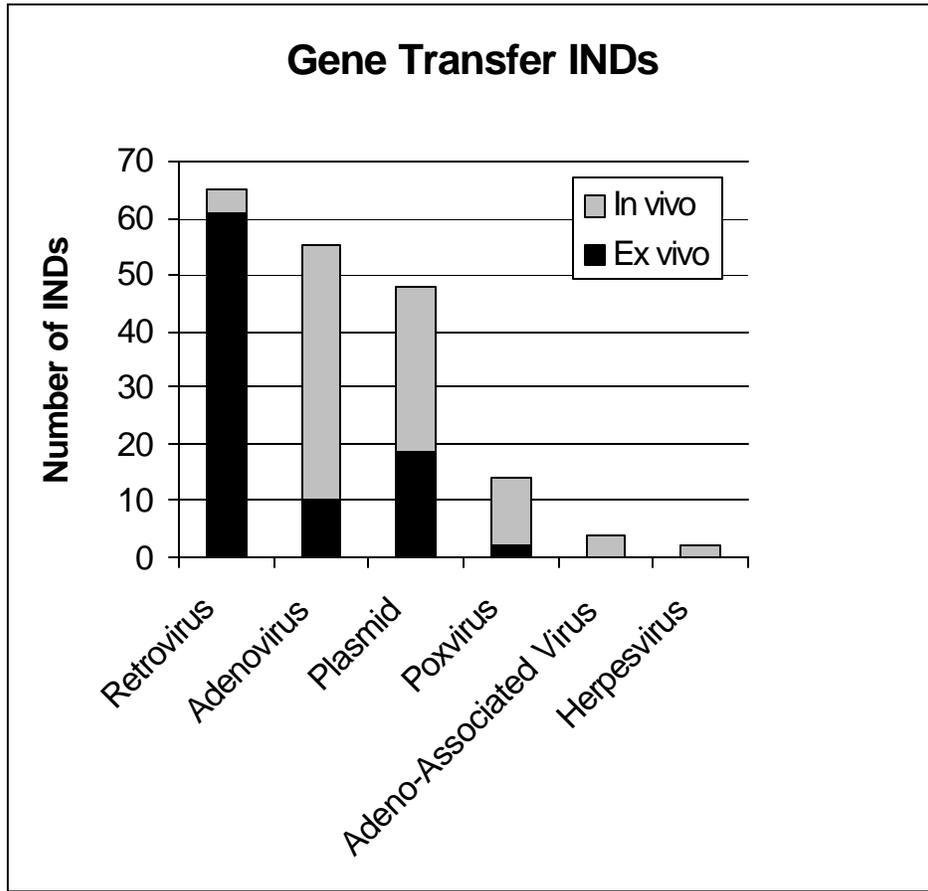
structural elements impact on vector function, vector stability, expression of the transgene, or potentially, expression of cellular genes.

## References

1. Bett, A.J., V. Krougliak, and F.L. Graham, *DNA sequence of the deletion/insertion in early region 3 of Ad5 dl309*. *Virus Research*, 1995. **39**: p. 75-82.
2. Gingras, M.-C., P. Arevalo, and E. Aguilar-Cordova, *Potential salmon sperm origin of the E3 region insert of the adenovirus 5 dl309 mutant*. *Cancer Gene Therapy*, 1996. **3**(3): p. 151-154.
3. *Fields Virology*, 3<sup>rd</sup> Edition. Editors-in-Chief Fields, B.N., Knipe, D. M., Howley, P.M. 1996. Lippincott-Raven Publishers, Philadelphia, PA (consulted for information in Table 1).

Selected additional references provided in the committee briefing package include recent review articles on virus vectors used in gene transfer clinical trials.

Figure 1.



**Table 1. Vector Classes: The Basics**

<b>Vector Class</b>	<b>Genome</b>	<b>Envelope</b>	<b>Replication Properties</b>	<b>Vector Generation (Common)</b>
Plasmid	Variable	Not Appl.	Episomal in E. coli	Standard recombinant DNA methods
Retroviral Vector (Gamma)	~8-10KB, single-stranded RNA	Yes	RNA transcribed into dsDNA, which integrates into host cell genomic DNA. Prone to recombination and high mutation rates	Standard recombinant DNA methods to generate vector genome-containing plasmid which is then introduced into retroviral vector packaging cell lines (express viral structural proteins and enzymes)
Adenovirus Vector	36-38 kbp, ds DNA	No	DNA replicates in cytoplasm. Naturally prone to recombination (mechanism responsible for diversity of serotypes)	Homologous recombination in 293 cells between shuttle plasmid carrying adenovirus sequences plus transgene and recombination vector
Adeno-associated virus vector Parvovirus	~5 KB, ssDNA	No	In the absence of helper virus, wildtype virus known to preferentially integrate into q arm of chromosome 19. Replication requires helper virus, either adenovirus or herpesvirus	a) cotransfection of ITR-containing expression cassette and AAV packaging plasmid along with infection by adenovirus b) cotransfection of ITR containing expression cassette, AAV packaging plasmid and Adenovirus helper plasmid (VA, E4, E2A)
Herpesvirus vector	124-135 kbp, ds DNA	Yes	DNA replicates in nucleus in episomal form. Not known to integrate. Naturally prone to recombination	Replication-selective mutants generated via phenotypic selection mechanisms in tissue culture
Poxvirus	130-375 kbp, ds DNA	Yes	DNA replicates in cytoplasm. Prone to high rates of recombination, elimination of direct repeats.	Homologous recombination via transfection of shuttle plasmid containing regions of homology to vaccinia and transgene and infection with vaccinia virus into chicken embryonic dermal primary cells or other cell lines.

**DRAFT QUESTIONS FOR THE COMMITTEE**  
**SESSION I: Structural Characterization of Gene Transfer Vectors**

1. For vectors up to 40 KB in size, we propose that the full sequence (coding and non-coding) should be determined prior to initiation of a phase I clinical trial. Do you agree with this proposal?
2. For vectors greater than 40 KB in size, we propose that coding sequence of the gene of interest, transcriptional control regions, and regions including and flanking any sequences that are altered during production of the vector should be determined prior to initiation of a phase I clinical trial. For this group of vectors, we further propose that the full sequence should be determined prior to initiation of a phase II clinical trial. Do you agree with these proposals? Please comment on the following:
  - a) Extent of flanking sequence determination that would be adequate.
  - b) Upper limit on the size of vector that would be subject to full sequence analysis. For example, herpes virus and pox virus vectors may be as large as 200-280 KB.
3. Given that during construction, propagation and production of certain vectors, genetic instability has been observed, what material(s) and what characterization should be used for determination of genetic stability? Characterizations to consider include restriction mapping, gene expression profile, PCR analysis, partial sequence analysis, and full sequence identification. How many times should this analysis be done (i.e., several production lots)? Some issues for consideration are listed below.
  - a) For detection of low level genetic changes, what is the most appropriate method(s)? Please consider the level of mutation having a significant impact on vector safety or function.
  - b) For retroviral vectors, which of the following materials should be analyzed and to what extent: input plasmid, integrated vector provirus in the vector producer cells, vector RNA in the final product?
  - c) For adenovirus vectors, which of the following materials should be analyzed and to what extent: shuttle plasmid, vector seed stock, final product?
  - d) For plasmids, which of the following materials should be analyzed and to what extent: plasmid isolated from the master cell bank vs. final production lot(s)?
4. What analyses of the vector sequence should be performed? We propose that the analyses include at least each of the following:
  - a) Comparison to expected sequence.
  - b) Analysis for open reading frames.
  - c) Analysis using currently available public DNA and protein databases and sequence analysis software.

Please comment.

5. If unexpected sequence or open reading frames are identified during the analysis of the vector sequence, what additional steps do you recommend? For example,
  - a) Expression analysis of the open reading frame for RNA and protein.
  - b) Analysis of subjects when the open reading frames are identified after completion of phase I investigation (for example, for antibody response to the potential protein).
  - c) Expression pattern of the vector sequences, if sequences identified include transcriptional control elements.