CHAPTER 21

Testing of Adenoviral Vector Gene Transfer Products: FDA Expectations

Steven R. Bauer*, Anne M. Pilaro†, and Karen D. Weiss†
*Division of Cellular and Gene Therapies and
†Division of Clinical Trial Design and Analysis
CBER Food and Drug Administration
Rockville, Maryland

I. Introduction

Adenovirus vectors that contain gene transfer products are biological products subject to Food and Drug Administration (FDA) regulation through the Center for Biologics Evaluation and Research (CBER) [1]. Sponsors of biologicals subject to FDA regulation that are not yet approved for marketing must file a "Notice of Claimed Investigational Exemption for a New Drug," which is abbreviated as "IND" for Investigational New Drug Application.

Adenoviral vector products have been studied in clinical trials under IND since 1993. As of December 2000, approximately 75 INDs involving administration of an adenoviral vector product have been filed with the FDA, with slightly more than 50% currently active. Each IND contains one or more clinical protocols. The vast majority (>90%) of the adenoviral gene therapy INDs target patients with cancer. The clinical studies contained in the remainder of the INDs target patients with vascular disease (coronary artery or peripheral) or genetic/metabolic diseases.

The sponsor is the entity or individual that holds and maintains the IND. The clinical investigator is the individual responsible for the care and welfare of the study participants at his or her site. Sponsors and investigators involved in FDA regulated research must be in compliance with federal regulations, described in the following sections of this chapter. In addition, investigators who receive federal funding for gene transfer clinical research or who conduct clinical studies at an institution that receives federal funds for recombinant DNA research must register the clinical protocol with the National Institute of

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Health (NIH) Office of Biotechnology Activities (OBA) and be in compliance with the NIH Guidelines [2].

The FDA's assessment of safety and ultimately effectiveness of adenovirus-containing products involves thorough evaluation of the information contained in the IND, and any supporting information cross-referenced to another IND or drug master file [3]. The type of information contained in an IND is set forth in 21 CFR 312.20, subpart B.

The following sections describe many of the agency requirements and guidances regarding drug development of adenoviral-containing products.

II. Manufacturing Control and Product Characterization

A. Purity, Safety, and Potency

When an adenovirus-based vector is used for the first time in humans, a major goal of FDA oversight is to ensure the safety of patients who receive the investigational product. A crucial component of safety and effectiveness is careful attention to the details of manufacturing and product characterization. The extent and quality of this information allows an assessment of the purity of the final vector preparation that will be administered to patients. Assessment of purity involves biological and biochemical characterization of the vector preparation and assessment of how completely the formulated product conforms to expected characteristics. For adenovirus vectors assessment of purity includes structural and biochemical information about the vector itself as well as demonstration of freedom from unexpected and potentially harmful agents such as viruses, fungi, and bacteria or bacterial toxins.

Another important goal of product characterization is assessment of the potency of adenovirus vector preparations. Potency measurements are intended to determine the extent to which a particular vector preparation has a desired biological activity. A vector preparation with insufficient potency has little chance of behaving as desired in a clinical trial. Although infectious titer has been proposed as a measurement of potency, this is currently not considered sufficient since the correlation between *in vitro* infectious assays and biologic effects has not been established. While potency is related to safety and efficacy, it is also an indicator of product manufacturing consistency.

Direct measurement of potency for a new adenovirus vector product is often challenging due to lack of an appropriate *in vitro* or *in vivo* system to measure potency. Therefore, in initial phases of product development, demonstration of transgene activity by enzymatic means is often adequate for initiation of clinical trials. Development of a *bona fide* potency assay for vector lot release will be required before FDA can license an adenovirus product. It is generally expected that a potency assay will be in place before Phase 3. Thus, as with all biological therapeutic products, assessment of the purity, safety, and potency of adenovirus vectors is a crucial part of product development.

B. Regulation of Process as Well as Product

The complexity of adenovirus vector manufacturing as well as inherent biological properties of the production system warrants oversight of the production process as well as the final product. Indeed, as with all complex biological products, in order to assure the purity, potency, and safety of adenovirus vectors, regulation of the manufacturing process is as important as characterization and testing of the final product. Therefore, there should be thorough characterization of starting materials and product intermediates in order to assure that the final vector product is acceptable for administration to humans.

Initial development of a new adenovirus vector involves manipulation and cloning of a transgene cassette with the desired gene and appropriate transcriptional regulatory elements. In a commonly used approach to vector production, an appropriate cell line is then cotransfected with the transgene cassette and a backbone shuttle vector that supplies the remaining components of the adenovirus genome. An appropriate cell line allows homologous recombination between the transgene and backbone plasmids and then supports synthesis of replication-defective adenovirus particles. It is the ability to mediate homologous recombination that allows assembly of the desired vector, but this ability also can lead to unintended structural changes. Thus it is crucial to select a vector clone that is fully characterized and has the intended structure. Since the same cell line is then also used to propagate vector for production of virus banks and for large-scale production, it is important to monitor the structure of the vector through several stages of manufacturing.

The cell lines used for production of adenovirus vectors add another complex, biological component to the manufacturing process. The characterization of the cell lines, including master cell banks and working cell banks is described in detail in section VI.

C. Current Good Manufacturing Practices

The principles of current Good Manufacturing Practice (cGMP) as per 21 CFR 210 and 211 apply to adenovirus gene transfer products. However, implementation of cGMPs may be staged according to the phase of product development, but there should always be appropriate documentation of manufacturing and of quality oversight. For Phase 1, this includes appropriate written protocols for each stage of product manufacturing and characterization. At later stages of product development, appropriate documentation of manufacturing should employ standard operating procedures (SOPs) and capture all important information relating to vector production. Quality oversight always involves quality control (QC) and quality assurance (QA) mechanisms, regardless of where manufacturing is taking place. In essence, this means that the person(s) responsible for assurance that the production and characterization testing have all been performed properly and have met specified criteria (quality assurance) are separate from and not direct subordinates of the person(s) responsible for conducting these tests and filing these reports (quality control).

As product development moves from Phase 1 into later phases, cGMPs also stipulate development of validated assays that must be in place by product licensure. Data regarding assay performance (specificity, sensitivity, and reliability) should be submitted to the agency as part of the validation process.

III. Development of Recommendations for the Manufacture and Characterization of Adenoviral Vectors

Many factors contribute to development of FDA recommendations and requirements for characterization of adenovirus vectors. First are the regulations found in the various applicable parts of the Code of Federal Regulations (CFR). These include the regulatory requirements that biological products administered to humans must be sterile (21 CFR 610.12 or another valid alternative testing of equal sensitivity), be free of mycoplasma (21 CFR 610.30), and meet endotoxin limits (limulus amebocyte lysate [LAL] per 21 CFR 610.9 or pyrogenicity test 21 CFR 610.13(b)). These establish minimum criteria to assure that products administered to humans are not contaminated with microbial organisms or their toxic byproducts.

Next are FDA review staff who have accumulated experience from review of many adenovirus vector and other gene therapy products. Some reviewers maintain active research programs in areas related to adenovirus biology or have participated in such research in the past. CBER reviewers have regular internal meetings to discuss relevant issues and develop consistency in oversight of adenovirus vector products. A major effort in this regard was launched March 6, 2000, with the issuance of a letter to Gene Therapy Sponsors requesting comprehensive information on product, preclinical, clinical, and QA/QC areas (see section XVI). These data have been tremendously useful and will be used to refine CBER's recommendations regarding adenovirus and other vector products. The cumulative experience of FDA reviewers is also utilized to develop guidance documents, several of which are relevant to the manufacture of adenovirus gene transfer products [4–6]

The experience of the gene therapy community has also played a key role in development of FDA recommendations in regulation of adenovirus vector products. The experience of adenovirus vector manufacturers is communicated in meetings between the manufacturer and FDA staff, at presentations at scientific meetings, and at presentations to the NIH Recombinant DNA Advisory Committee (RAC).

The NIH RAC has played an important role in the development of recommendations and it provides a public forum for discussion. Following the death of a patient in a gene therapy trial in late 1999, the RAC empanelled an ad hoc advisory group, the RAC Working Group on Adenoviral Vector Safety and Toxicity (Ad-SAT), to examine data from adenovirus gene transfer trials with the intent of formulating recommendations to improve the safety of these clinical trials. One important discussion centered on the accuracy of adenovirus vector titers in terms of both total particle and infectious particle titers [25]. Since toxic vector doses are just above doses with potential therapeutic effect, there was particular concern over lack of accuracy and comparability between titers determined for different product lots and between different clinical trials. This discussion highlighted the need for a reference standard that could be used to help standardize adenovirus vector titer measurements.

This public discussion helped stimulate a gene therapy community initiative to develop such standards. Several public meetings to develop consensus on the need for a standard, to discuss the nature of the reference material, and to discuss mechanisms for its development were held in late 2000 and early 2001 [7]. An Adenovirus Reference Materials Working Group (ARMWG) was formed under the auspices of the Williamsburg Bioprocessing Foundation (WBF), and an WBP/FDA partnership agreement was formulated that allowed participation of FDA staff in development of a reference stock of wild-type adenovirus type 5 which can be used to calibrate assays for particle number and infectivity. The role of FDA is to lend scientific and regulatory expertise in the form of recommendations to the ARMWG, which oversees the development of the reference material. Information on this initiative is available at the WBF website (www.wilbio.com) and the CBER website (www.cber.fda.gov). The information includes meeting minutes, transcripts from FDA cosponsored meetings, and explanations of the bid mechanisms by which participants volunteered donations of goods and services toward production and characterization of the reference material. This reference material will provide another mechanism for FDA to formulate recommendations for characterization of adenovirus-based gene transfer products.

FDA also seeks input from advisory committees such as the Biological Response Modifiers Advisory Committee (BRMAC) for recommendations regarding characterization of adenovirus vectors. BRMAC meetings allow FDA to obtain advice on scientific issues that impact gene transfer experiments in a public forum in which all interested parties are allowed to participate. Transcripts of these meeting are also available on the CBER website (www.cber.fda.gov). The BRMAC's advice on issues such as the amount and type of structural characterization of gene transfer vectors, discussed at two recent committee meetings, has been valuable as CBER staff develop and update policy [8, 9]

In summary, the FDA receives input and feedback from a variety of sources in formulating recommendations regarding adenovirus manufacturing and characterization. The recommendations may change with advances in technology and through accumulating experience. FDA considers the potential risks and benefits of each vector product and each proposed clinical trial when making its recommendations. This case-by-case approach, which takes into account the severity of the disease and the proposed patient population, permits some flexibility in product manufacture and characterization.

IV. Considerations in Manufacturing Adenoviral Vectors

A. Components and Characterization

While the goal of adenovirus vector manufacturing is to produce a safe, pure, and efficacious vector, the complexity of the process necessitates careful control of the entire manufacturing procedure and of the components used. Raw materials can be a source of adventitious agents or toxic impurities that negatively impact safety of the final product. At early stages of product development, certificates of analysis (CoA) for many raw materials such as buffers, and basic tissue culture components should be part of the documentation demonstrating that these reagents are pure and free of adventitious agents. These CoAs should be kept in the manufacturer's records and sample CoAs should be submitted to the agency. At later stages of product development, development of testing and acceptance criteria for some of these materials may be required of the sponsor. As an example, current techniques for adenovirus vector production require mammalian cell substrates. Raw materials include a source of serum, usually fetal bovine serum (FBS), and enzymes such as porcine trypsin for cell culture. These reagents can be contaminated with adventitious virus. Trypsin has been identified as a potential source of porcine parvovirus while FBS can harbor several adventitious viruses. Therefore, FBS and porcine trypsin should come only from sources where appropriate testing is conducted and documented in a CoA. A manufacturer of adenovirus vectors should retain all such CoAs and submit sample copies to FDA. Also, bovine serum from geographic areas known to harbor endemic bovine spongiform encephalopathy agent (BSE) is considered inappropriate for use in manufacturing a biological for use in humans.

Since adenovirus vector production relies on cells that support replication of the vector, cell banking is an important aspect of production. Cell banks are cryopreserved stockpiles consisting of very well characterized cell populations that have been shown to be free of adventitious virus, are sterile, and have the capacity to support production of the adenovirus vector. Ideally, cell banks are derived from early cell passages and assure that a reliable and consistent source of qualified cells is available for the foreseeable future production needs. Details of the necessary characterizations for cell banks are discussed below.

In similar fashion, virus banks are an important aspect of adenovirus production. Virus banks consist of frozen stocks of very well characterized molecular vector clones. Characterization includes structural, physical/biochemical, and functional assessments in addition to assessments of microbial sterility and freedom from adventitious viruses. Virus banks are derived as an early step in vector manufacturing and assure that a reliable source of infectious vector is available for foreseeable future production needs. Details of the necessary characterizations for virus banks are discussed below.

B. Protocols

The protocols used for each step of manufacture are important records which can demonstrate that the production process and the starting materials for vector production are of a quality sufficient to assure that the final product is pure and safe. Detailed descriptions of each step should be maintained and submitted to the FDA as part of an IND. Many protocols are an integral part of manufacturing and should be part of standard operating procedures (see below). Even though many protocols such as the molecular biology techniques used to assemble a vector are not repeated steps, detailed protocols for these stages are essential.

V. Process Controls

Control of the manufacturing process is obtained through testing and characterization of intermediates and final product in the production scheme. For adenovirus vectors this includes characterization of the cell substrate (master and working cell banks), the virus seed stock (master virus bank), the bulk vector preparation, and the final formulated product. Details of the testing are outlined below. The goal of process control is twofold; to ensure safe, pure, and efficacious vector products and to demonstrate that the production process is highly reproducible.

A. Standard Operating Procedures

Standard operating procedures are a mechanism to ensure that process controls and protocols for product manufacture and characterization are carried out in a reproducible and documented fashion for each stage of manufacturing and product testing. SOPs consist of detailed written documents describing each step of a process conducted in manufacturing. SOPs can also refer to many different types of processes that impact adenovirus production, such as required training of personnel, acquisition and acceptance of raw materials, procedures for shipping and handling final product, and conduct of quality oversight. For early product development, SOPs should be developed for the manufacturing and testing steps discussed below. For later stages in product development, consultation with the FDA is advisable to assure comprehensive coverage of the manufacturing process by appropriate SOPs.

B. Quality Assurance and Quality Control Programs

Quality assurance and quality control programs are considered essential steps in assuring safe and high-quality adenovirus vector products. A key concept in a QA/QC program is that there should be separation of authority between the personnel responsible for conduct of testing and manufacturing and the personnel who examine and approve the test data and final product characterization. This can be accomplished in a variety of ways. For instance, separate QA and QC departments in the same institution can be used provided that the responsible personnel not be under direct supervision of one another.

An important topic that is often misunderstood is the division of responsibilities between an IND sponsor and a multiuse facility contracted to do some part of product manufacturing. When these facilities are used to produce more than one gene transfer vector, they are termed multiuse facilities. Many gene-therapy vectors are produced in multiuse facilities. IND sponsors often assume that the contract lab will provide all necessary QA/QC, manufacturing, and product testing information to the FDA and do not involve themselves sufficiently in designing the testing, examining the data, and/or answering FDA questions. Although the contract lab plays an important role, the responsibility for oversight of QA/QC and reporting lies with the sponsor. The sponsor must recognize that the FDA holds them accountable for oversight of production and testing conducted by a contract organization. An additional concern with multiuse facilities is the potential for cross-contamination of one product with a product made previously or concurrently. The multiuse facility should test for cross-contamination or validate the production and purification process to rule out cross-contamination.

The entire production process, from raw materials to oversight of testing and product release, is important in assuring that adenovirus and other gene transfer vectors are as safe and consistent as possible. The next sections describe in greater detail the characterization that should be done for each of the major components or intermediates as well as the final product in adenovirus vector production. These include the cell banks, the virus bank, the bulk virus preparation, and the final vector product.

VI. Characterization of Adenoviral Vector Production Intermediates

The necessity for and specifications for each of these characterizations is assessed on a case-by-case basis and can change depending on the phase of product development and as a result of feedback from the numerous sources discussed above. Therefore, the following material is intended to give the reader an overview of FDA expectations. Consultation with CBER at the pre-IND stage is strongly recommended.

A. Master Cell Bank

Testing of the cell banks used in adenovirus vector production is of two general types; safety testing and characterization. Table I is an overview of the recommended characterizations. The safety testing is intended to demonstrate that the cell bank is free of any detectable microbial contamination including bacterial, fungal, and viral. Sterility testing is a universal requirement for biologics and is set forth in 21 CFR 610.12. Alternative sterility assays validated to be of equal sensitivity may also be used. The basic premise is to apply the product, in this case cells from the master cell bank, to several growth media and to look for outgrowth of microbial contaminants over the course of 14 days. The specification for this test is no contaminants.

Mycoplasma testing is conducted by inoculation of both cells and cell supernatants into appropriate cultures and examining for growth of

Table I
Characterization of the Cell Banks[a]

Safety	Identity
Sterility	Morphology
Mycoplasma	Isoenzyme tests
Adventitious virus	Cell-specific identity test
• *In vitro* and *in vivo* virus	
• Bovine, porcine, canine viruses (ancillary product dependent 9CFR113.47)	
• Human viruses: EBV, HBV, HCV, CMV, HIV 1&2, HTLV 1&2, AAV, B19 (other cell substrate specific)	
Tumorigenicity	

[a] The necessity for and specifications for each of these characterizations is assessed on a case-by-case basis and can change depending on the phase of product development and as a result of feedback from the numerous sources discussed above. Therefore, this list is intended to give the reader an overview of FDA oversight. However, consultation with CBER is strongly recommended before submission of an IND.

mycoplasma. This testing is described in FDA guidelines [5]. Alternative tests such as PCR could be utilized following proper demonstration of the sensitivity and comparability to the culture-based assay.

Adventitious virus tests are also intended to show that the test material is free of a variety of viruses. The *in vitro* adventitious virus test is conducted by inoculating cell cultures with the test material, in this case supernatants from the master cell bank (MCB). Following 14 days in culture, cells are tested for their ability to mediate hemadsorption or hemagglutination with red blood cells from three different species. The cell lines are chosen for their ability to support replication and detection of many different viruses. A list of viruses that can be detected is given in Table II. The *in vitro* adventitious virus assay provides a nonspecific screen for many different viruses and can sometimes be used to identify certain viruses. The *in vivo* adventitious virus test is conducted by inoculating animals from several species with supernatant from the cell bank material. The species are chosen to optimize detection of possible contaminating adventitious viruses. The *in vivo* virus test is capable of detecting an array of viruses complimentary to those detected by the *in vitro* assay. A list of viruses that can be detected is given in Table II. For both types of adventitious virus tests, the acceptable specification is no detection of virus.

In addition to these nonspecific tests, a variety of specific tests for many different viruses may be required. As the current cell lines used to support adenovirus replication are of human origin, a variety of human virus tests are included. FDA-approved test kits should be used when available. Although the cell lines used to produce adenovirus are not generally thought to support replication of several of these viruses, experimental data to preclude this possibility do not exist. In addition, if sensitive cell-line-specific identity tests are not part of the MCB characterization, it is possible that other human cell lines could be present and may serve as a reservoir for some of these

Table II[a]
***In Vitro* and *In Vivo* Adventitious Virus Testing**

In vitro adventitious virus testing	*In vivo* adventitious virus testing
Picornaviruses: e.g., poliovirus, Coxsackie B, echovirus, rhinovirus	Picornaviruses: e.g., influenza, Coxsackie A and B, poliovirus
Togavirus: e.g., rubella	Bunyavirus: e.g., LCMV, hantavirus
Paramyxovirus: e.g., parainfluenza, mumps measles, RSV	Herpesvirus: e.g., HSV-1
Orthomyxovirus: e.g., influenza	Paramyxovirus: e.g., mumps
Adenovirus	Coronavirus
Herpesvirus	Flavivirus[b]

[a] "Fields Virology," Chap. 17 [33].
[b] "Fields Virology," Chap. 31 [34].

viruses. In addition, it is surprising that some viruses not thought to replicate in cell lines such as HEK 293 (human embryonic kidney fibroblasts) have been detected in adenovirus product lots. For the above reasons, these tests are currently recommended at various steps for all adenovirus vector production. Currently, the specific virus tests include Epstein–Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), human immunodeficiency viruses I and II (HIV 1 and 2), human parvovirus B19, human T-lymphotrophic viruses 1 and 2 (HTLV 1 and 2), and adeno-associated virus (AAV). The test methods, specifications and sensitivities for these tests should be submitted as part of the proposed acceptance criteria for cell banks.

Nonhuman cell lines could also be used to produce adenovirus vectors. In such cases additional testing may be necessary. For example, if rodent cells were used, the MCB should also be tested by the appropriate antibody production test: murine antibody production (MAP), rat antibody production (RAP), or hamster antibody production (HAP) [6].

Current adenovirus production methods commonly use fetal bovine serum (FBS) and porcine trypsin for propagation of producer cell lines. The use of FBS carries two types of risks; the potential for patient exposure to BSE and to adventitious bovine viruses. The use of porcine trypsin carries risk of patient exposure to porcine parvovirus. Producer cell lines with sufficient documentation may be usable without tests for bovine or porcine viruses or BSE. When FBS is used, sufficient documentation includes the following: certificates of analysis (CoA) showing that the FBS is not from one of the countries on the USDA list of countries where BSE is found and that the FBS has been tested for bovine viruses. For porcine trypsin, sufficient documentation includes CoAs showing that the trypsin is negative for porcine parvovirus. If documentation of viral testing is unavailable, the testing will be requested as per 9 CFR 113.47. Once an MCB is tested or shown to have an accepted history of nonexposure to these agents, these tests may be omitted in subsequent stages of production if CoAs of FBS and porcine trypsin contain the proper testing and come from approved geographic locations.

Although tumorigenicity testing has often been requested, it is acknowledged that the cell line used in adenovirus production may be tumorigenic in immunodeficient mouse strains. In later stages of product development, this test may be required. For products that are in Phase 1 of clinical testing, it may be possible to omit this test if there is sufficient testing of the product for cell substrate DNA (see below).

In addition to safety testing, characterization of MCBs should include tests for identity of the cell lines. Isoenzyme analysis can show the cell line is of the correct species. For most current adenovirus producer cells, this involves testing for human isoenzymes. Morphology is also assessed to show that the cell line retains the expected shape and size. Development of a cell-specific identity test is currently recommended so that accidental contamination of the adenovirus vector producer cell line can be detected.

B. Working Cell Bank

Working cell banks are expanded cell populations derived from the MCB and are tested after a defined number of cell generations. The testing of WCBs is similar to that requested for MCBs and consists of the following safety tests: sterility, mycoplasma, and *in vitro* adventitious virus. Characterization includes morphology and isoenzyme analysis.

C. Master Virus Bank

A master virus bank (MVB) consists of a well-characterized stock of virus-based vector that serves as the inoculum for all subsequent large-scale vector production. It is sometimes referred to as a vector seed stock. Table III gives a summary of the types of characterization recommended by the FDA. Safety testing for a master virus bank is very similar to that done for a master cell bank. Thus a master virus bank is tested for sterility and mycoplasma, *in vitro* and *in vivo* adventitious virus, and specific viruses (EBV, HBV, HCV, CMV, HIV 1 and 2, AAV, B19, HTLV 1 and 2) if the cells used to produce the MVB were not fully characterized as described for the MCB. Depending on the degree of characterization of the FBS and porcine trypsin, a MVB may require testing for bovine viruses and porcine parvovirus.

Table III
Characterization of the Master Virus Bank[a]

Safety	Characterization
Sterility	Identity
Mycoplasma	• Sequence insert and flanking regions restriction map[b]
Adventitious Virus	Activity
• *In vitro* and *in vivo* virus	• Transgene specific protein expression
• Bovine, porcine, canine viruses (ancillary product-dependent 9CFR113.47)	• Other
• Human viruses: EBV, HBV, HCV, CMV, HIV 1&2, HTLV 1 & 2, AAV, B19	Titer
	• Infectious titer
• Replication-competent adenovirus	• Particle count

[a] The necessity for and specifications for each of these characterizations is assessed on a case-by-case basis and can change depending on the phase of product development and as a result of feedback from the numerous sources discussed above. Therefore, this list is intended to give the reader an overview of FDA oversight. However, consultation with CBER is strongly recommended before submission of an IND.

[b] BRMAC Advisory Committee Meeting, November 16, 2000: recommended entire sequence for vectors <40 kb [8].

In addition to the above testing for cell banks, an adenovirus MVB should be tested for replication-competent adenovirus (RCA). RCAs are a common byproduct of adenovirus vector production and are currently considered a safety risk. RCAs most often arise due to molecular recombination between the vector and endogenous elements of the producer cell line genome. Some of these recombinations restore the replication competence of a normally replication-defective vector and give rise to RCA. For example, in the HEK 293 cell-line, endogenous E1 sequences are required to allow replication of the E1 defective adenovirus vectors. The vectors can undergo homologous recombination with the endogenous E1, thus restoring their replication competence. This is a stochastic and unavoidable consequence of the biology of the certain producer cells. Development of producer cells with smaller or no regions of homology between vector and endogenous sequences may reduce homologous recombination but may still support nonhomologous recombination. For most replication defective adenovirus vectors, RCA testing is performed by inoculation of the test material onto a cell line that will support replication of a RCA but not of defective vector. Supernatant from this treatment is passaged to a second cell monolayer. Development of cytopathic effects (CPEs) or lysis indicates the presence of RCA. The current recommended specification for RCA is ≤ 1 RCA in a total of 3×10^{10} virus particles.

Characterization of the adenovirus vector MVB encompasses a variety of approaches to establish the physical, biochemical, and biological properties of the vector preparation. Identity is an important parameter and demonstrates that the intended product is the actual starting material for large-scale production. Current FDA recommendations for structural characterization of adenovirus vectors include determination of the nucleotide sequence of the transgene insert and flanking regions. The remainder of the structure can be demonstrated by techniques such as restriction mapping and PCR. In cases where extensive characterization of the transgene protein is available, no sequencing is necessary and restriction mapping of the vector would be sufficient. However, at a recent meeting of the BRMAC which addressed the issue of structural characterization, it was recommended that vectors ≤ 40 kb in length should be characterized by sequencing of the entire vector genome [8]. It is likely that the FDA will adopt this recommendation in the near future. For adenovirus vectors, the MVB would be the most appropriate material for this sequence analysis.

Another important characteristic of an adenovirus vector MVB is the activity of the transgene. Although this is not a potency assay per se, this parameter suggests that the therapeutic transgene will be functional in clinical trials and thereby justifies the risks of exposure to patients. Activity assays can include demonstration that the transgene-encoded protein is expressed and demonstration that the protein is functional in some biochemical assay. Assays that determine the expression and activity of the adenovirus vector should be part of the acceptance criteria for each MVB. Methods and acceptable specifications for these assays should be part of IND submissions.

The number of adenovirus vector particles in a MVB is measured in two ways. One method is to determine the particle count. Most often this is determined by a measurement of the amount of DNA in a vector preparation which is then related to particle number by an agreed-upon conversion factor. Although this is a physical/chemical assay, the precision is affected by several factors including formulation of the vector preparation and nonviral nucleic acid content of the preparation. Cellular nucleic acids as well as differences in DNA sequence between vectors can affect the precision of this measurement, which can vary on the order of 10%.

A second measure of adenovirus vector quantity in a MVB is the infectious titer. This is an assessment of how many of the particles retain the capacity to interact with cell surface receptors and subsequently undergo internalization. This measure is an indication that the manufacturing process is gentle enough to preserve viral coat protein structure and will largely determine the ability of adenovirus preparations to infect patient cells and thereby introduce the desired genetic material. This assay is subject to much more variability than the particle number determination. In recent years, some sources of variability have been identified. The concentration and diffusion rate of adenovirus particles are two important parameters to consider [10]. Infectious titer assays utilize adherent cells sitting at the bottom of tissue culture dishes. Since adenovirus particles do not settle out of solution but instead randomly diffuse, the volume of material tested can have a profound impact on the apparent infectious titer.

A recent initiative to develop an adenovirus reference material should lead to increased accuracy in both particle and infectious particle determinations [11]. A reference material consisting of wild-type adenovirus 5 with a known particle and infectious titer will be produced and distributed. Comparisons between different adenovirus vector preparations within and between lots can be made using this reference material as an index to calibrate assays done in different places and at different times.

VII. Characterization of Adenoviral Vector Final Products

Testing of the final adenovirus vector product consists of safety testing and product characterization. Such testing involves physical/chemical and biological assessments. Table IV provides a summary of the currently recommended testing. Whereas production intermediates such as the MCB and MVB are subject to acceptance criteria, the final product characterization is subject to lot release and is recorded on a CoA with specified tests, methods, sensitivities, and results. Some of the safety testing is similar to that done for the

Table IV
Characterization of the Final Product[a]

Safety	Product characterization
Sterility	Identity
Mycoplasma	• Restriction map, structural characterization
Endotoxin	Activity
Adventitious virus[b]	• Transgene specific
• *In vitro* virus	Potency
• AAV	• Required by phase II/III
• Replication-competent adenovirus	Titer
General safety	• Particle count/infective particle ratio <30:1
• Required by time of licensure	Purity
	• Cell substrate DNA <10 ng/dose, <100–200 bp in size
	• Cell substrate protein
	• Ancillary products
	• Process residuals

[a] The necessity for and specifications for each of these characterizations is assessed on a case-by-case basis and can change depending on the phase of product development and as a result of feedback from the numerous sources discussed above. Therefore, this list is intended to give the reader an overview of FDA oversight. However, consultation with CBER is strongly recommended before submission of an IND.

[b] These tests should be done on the unpurified bulk in order to maximize sensitivity and not deplete final product.

MCB and MVB. Thus sterility and mycoplasma testing should be performed, as should testing for endotoxin levels in the final formulated product (LAL per 21 CFR 610.9 or pyrogenicity test 21 CFR 610.13(b)). Adventitious virus testing consists of the *in vitro* virus test, and tests for AAV and RCA. In general, an *in vivo* adventitious virus test is not recommended for final product. These adventitious virus tests should be performed on the unpurified bulk in order to maximize sensitivity and not deplete the final product. One other test that is required for licensed products is that of General Safety 21 CFR 610.11. The current recommendation for RCA is ≤ 1 RCA per 3×10^{10} virus particles.

Current recommendations for final product characterization are similar to those for MVB. However, identity (structural characterization) need not be done by DNA sequence analysis. Rather, other methods such as sensitive restriction mapping combined with Southern blot analysis or PCR mapping may be used to show that the final vector preparation is homogenous within the limits of the assays. The same activity assay used on the MVB can be used on the final vector preparation. Development of a potency assay that reflects the intended biological function of the vector preparation should commence as soon as possible during product development and should be in place by the end of Phase 2 or the beginning of Phase 3. Test methods, sensitivities, and specifications for lot release should be submitted as part of an IND submission.

The number of particles and the infectious titer per unit volume should be measured and reported. Currently the recommendation is that the ratio of total particles to infectious particles in the final product should be no greater than 30:1. The previous recommendation of 100:1 was developed shortly after the first adenovirus vector trials were initiated and has remained constant until recently. However, review of data received in response to the March 6, 2000, letter to gene-therapy sponsors suggests that almost all adenovirus vector lots have a ratio of less than 30:1 particles to infectious particles. Advances in understanding of infectious titer assays and the development of an adenovirus reference material will be helpful in reassessing this recommendation in the near future.

Product characterization should also include assessments of potential impurities such as production cell DNA and protein. If the cell line used for production is tumorigenic, current FDA recommendations for adenovirus vector products are that no more than 10 ng/dose of cell substrate DNA be present. In addition, the DNA that is present should be degraded to a size less than 100–200 bp in length. If these criteria are met, the need for tumorigenicity assays of the cell substrate is less pressing. The current recommendation for cell substrate protein is that the sponsor should measure and report amounts present in order to set lot release acceptance criteria by Phase III. If cell substrate proteins are present, their potential for immunogenicity should be considered.

Other potential impurities should also be assessed in analysis of the final product. These include fetal bovine serum, other tissue culture reagents, antibiotics, process residuals such as CsCl, or column chromatography materials. Other tests that may be necessary include pH of the formulated final product, assessment of particulates, volume, and appearance. The necessity and extent of these tests should be discussed with FDA.

All lot-release testing of the product should be summarized in a certificate of analysis that accompanies the vector product.

A final consideration for product characterization is vector stability. Stability testing should be conducted on the final formulated, vialed product. In early phases of product development, stability testing should also assess procedures for shipping and handling of the final product. Stability testing should be initiated during Phase 1 and should be conducted according to a plan that has been discussed with the FDA.

VIII. Preclinical Testing of Adenoviral Vectors

In the development of a new adenoviral vector for gene transfer, the preclinical pharmacology and toxicology programs are typically conducted in conjunction with the development of the product manufacturing. The overall purpose of preclinical animal and *in vitro* studies is to support the safety and rationale for use of the product in human subjects. Although not unique to gene therapy vectors in general, or more specifically, to adenoviral vector development, there are several basic goals to be achieved by preclinical testing which contribute to the design and conduct of the initial clinical trials. These include, but are not limited to, (i) identification of dose(s) which confer the desired biologic effect; (ii) definition of a safe starting dose and escalation scheme; (iii) identification of pharmacodynamic measures of biologic activity; (iv) identification of safety and toxicity parameters to monitor in the clinical trial; (v) definition of inclusion and/or exclusion criteria based on observed toxicities, and, finally, (vi) designated stopping rules for the clinical trial based on the toxicity profile observed in animals.

A. Pharmacologic Activity

Initially, the pharmacologic activity of a proposed vector system is evaluated either *in vitro* or *in vivo*, as demonstration of "proof of concept." These studies are designed to determine the feasibility and efficiency of the gene transfer, and whether the biologic activity in correcting the genetic defect or conferring that the desired response is observed (e.g., multidrug resistance in hematopoietic stem cells). When available, animal models which mimic the human disease, either through genetic or pharmacologic mechanisms may be used as "proof of principle," to demonstrate that transfer of the gene is actually able to correct the genetic defect, ameliorate or slow progression of the disease, or alleviate some of its clinical signs or symptoms. Based on the responses observed in the preclinical pharmacology program, a decision is made by the investigators to either further evaluate the candidate vector for safety with the intention of entering it into the clinic or to terminate the development of potentially unsuccessful products.

Preclinical pharmacology data are provided both to CBER in support of an IND application and to the NIH RAC in support of use of adenoviral vectors for gene transfer in several different clinical indications. Of the data which have been publicly reviewed and discussed, biologic activity of adenoviral vectors have been evaluated in murine tumor models and murine:human tumor xenografts, transgenic mouse models of human disease (e.g., ornithine transcarbamylase deficiency), human cell xenografts in immunodeficient rats and/or mice, and in pharmacologically induced disease states in rodents, monkeys, and dogs. Advantages of using adenoviral vectors are their ability to transduce a variety of different, nondividing cell types, high levels of gene expression for relatively short durations of time, and a large enough capacity to carry relatively large, transgene sequences.

IX. Toxicology Testing

A. Scope of Toxicity Testing

The next step in the preclinical program for a candidate gene transfer vector is the toxicology testing. Prior to initial entry of a new drug or biologic agent into humans, the basis for the determination of *in vivo* safety is the preclinical testing performed in animals. Toxicology studies to demonstrate safety of gene transfer vectors, including adenovirus, are intended to answer specific questions regarding the acceptable risk:benefit ratio to the patient, and provide an indication of what expected toxicities may occur on introduction of the product into humans.

Traditional drug development programs, evaluating the safety of small molecule or protein therapeutics typically conduct toxicology testing in normal animals, using a well-defined paradigm to establish the acute, subchronic, and cumulative toxicities of an agent prior to its first use in man. At least two animal species are used for the initial demonstration of safety; typically, testing is done both in rodents (i.e., mice, rats, or hamsters) and one nonrodent species (i.e., dog, pig, or nonhuman primate). The advantages of this approach are that a wide range of doses may be investigated to give high multiples of the expected human exposure, the metabolism and disposition profiles in the different species may be established as a basis for comparison for the clinical dosing, and the background incidence of any specific, adverse findings may be well-documented in that particular strain of animal being tested. The use of more than one species in traditional drug evaluation programs is encouraged to increase the chance of detecting any toxicity expected for the clinical trial.

Traditional toxicology programs, however, frequently are of little value in the determination of safety of gene transfer agents. For many of the vectors in development, the issues of species-specificity of the transgene product under study, as well as limitations in the doses that are feasible to administer and the interaction of the agent with its specific receptor must be taken into account in designing the safety program. In gene transfer research, demonstration of safety must also take into account toxicities due to both expression of the transgene, or the ultimate therapeutic agent, as well as any adverse effects associated with the vector, or delivery system used to introduce the foreign gene. Additionally, any underlying pathology associated with the disease being investigated may either exacerbate or confound any toxicity related to the gene transfer system. These points must be considered in designing a preclinical program to evaluate the safety and efficacy of a gene transfer agent.

The FDA recognizes that novel issues exist in designing and interpreting preclinical studies for gene transfer vectors, and has provided several guidance documents to assist investigators in developing their preclinical programs. CBER's recently published guidance document provides a framework for the design of preclinical safety programs in gene therapy, based on the available data from both *in vitro* and *in vivo* efficacy models, as well any specific concerns for the clinical population planned for study [4]. The CBER document follows the guidance set forth by the International Conference on Harmonization S6 document, "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (ICH S6). Although the ICH guidance does not directly address toxicology study design for gene transfer agents, many of the principles of this document apply [12]. In general, toxicity study design for gene transfer agents follows many of the principles set forth by ICH S6 regarding dose and species selection, route of administration, and study timing. Each of these points is addressed separately in the context of gene transfer, below.

To understand the safety of gene transfer vectors, the design of preclinical studies should take into consideration the following points: (i) the class of vector to be administered, (ii) the animal species, gender, age, and physiologic state most relevant for the clinical indication and product class, and (iii) the intended doses, route of administration, and treatment regimens planned for the clinical trial. With many of the gene transfer vectors, these considerations will be dependent, as the route of administration or the maximal feasible dose for the preclinical study may be influenced by the species selected for testing, and vice versa.

B. Species Selection

The recent death of a patient while participating in a clinical trial of adenovirus-mediated gene transfer, as well as the finding that data in Rhesus monkeys using the same class of vectors and route of administration predicted many of the toxicities observed in this subject have highlighted the importance of preclinical data, and the relevance of the animal model in determining a safety profile for these agents. CBER's recommendations for selection of species for safety evaluation of adenoviral vectors have generally followed the guidance set forth by the ICH S6 document, taking into account the limitations of the animal model being tested. Preclinical pharmacologic and safety testing of vectors for gene transfer should employ the most appropriate, pharmacologically relevant animal model available. In contrast to traditional drug development programs, for many biologic products including gene transfer vectors, safety evaluation and toxicology testing in a single, relevant species is permissible prior to Phase 1 studies in the clinic.

A relevant animal species would be one in which the biological response to the therapy would be expected to mimic the human response. Relevant animal species for safety evaluation may also be selected based on the clinical population intended for study and/or intended route of administration, or by the species-specificity of the transgene product. In some cases, the interaction of the transgene product with its specific receptor occurs only in humans and nonhuman primates, necessitating toxicology testing in monkeys. In many cases, however, the toxicities observed are independent of the transgene product (e.g., inflammatory reactions in response to adenovirus capsid proteins) and may be tested in rodent species or other small, nonrodent laboratory species. In other cases, specific information regarding the safety of a gene transfer approach may be obtained only in an animal model of the disease, in which the underlying disease pathology can influence significantly the safety of the intervention. When evaluating the pharmacologic activity of a vector in an animal model of the clinical indication, it is recommended that safety data be gathered at the same time, in order to assess the contribution of disease-related changes in physiology or underlying pathology to the response to the vector.

C. Route of Administration

Most gene transfer studies, both in humans and in animals, are expected to involve either single administrations or a small number of repeat administrations over a short duration of time. CBER recommends that both the route of administration and the dosing schedule in animal studies mimic those intended for the clinical trial as closely as possible. However, there are issues specific to the gene transfer that need to be incorporated into the study design, for example, the persistence of gene expression following transduction of the target organ, which will impact upon the duration of the toxicity study. Another example would be the physical characteristics of the agent being studied (i.e., vector aggregation at high concentrations). The dose and the route of administration for the preclinical safety studies of cellular and gene therapies should mimic those intended for the clinical trial as closely as possible. It is understood, however, that some dosing techniques and/or regimens intended for the clinical trial may be difficult to achieve in a small animal species, such as a rodent. In these cases, a method of administration similar to that planned for use in the clinic is advised. For example, intrapulmonary instillation of adenoviral vectors by intranasal administration in Cotton rats or mice is an acceptable approach in lieu of direct intrapulmonary administration through a bronchoscope.

D. Selection of Dose

Current recommendations for dose selection for safety testing are based on those demonstrated in efficacy models to provide gene transfer sufficient for pharmacologic effect, as well as inclusion of doses with a likelihood of demonstrating toxicity. Dose selection should be based on preliminary activity data from studies both *in vitro* and *in vivo*. For the determination of safety, a no-observable adverse effect level dose (NOAEL), an overtly toxic dose, and several intermediate doses should be evaluated, to determine not only the dose relationship of the toxicities to the amount of vector administered and/or transgene expression, but also to evaluate the shape and steepness of the dose–response curve. Preclinical safety studies should include one dose equivalent to, and at least one dose escalation level exceeding, those proposed for the clinical trial. The multiples of the human dose required to determine adequate safety margins may vary with each class of vector employed and the relevance of the animal model to humans.

Allometric scaling of doses based on either body weight or total body surface area as appropriate facilitates comparisons across species and allows determination (retrospectively) of whether an animal model was predictive of toxicities observed in the clinic. For example, adenoviral vectors used in cystic fibrosis demonstrated very similar toxicities after direct instillation into the lungs of Cotton rats, mice, hamsters, Rhesus monkeys, and baboons (Table V). These toxicities included dose-related, perivascular, and peribronchiolar inflammation, mononuclear inflammatory cell infiltrates, pulmonary edema, and interstitial pneumonia. When the NOAEL doses were calculated for each species after scaling by total body surface area, with the exception of Rhesus monkeys, it was discovered that these values were remarkably similar between the different species. Additionally, when scaled by total body surface area, the NOAEL doses in mice, Cotton rats, hamsters, and baboons for direct instillation of adenovirus into the lungs were approximately equivalent to the human dose of 2×10^9 IU, or 1.2×10^9 IU/m^2, which was the first dose in humans at which toxicity was observed, when scaled by body surface areas.

Table V
Allometric Scaling of Adenovirus Dose in Animals and Man

Species	Apparent NOAEL	NOAEL (pfu/m^2 surface area)
C57Bl/6 mouse	2.6×10^7 pfu/mouse	2.4×10^9 pfu/m^2
Hamster	3.6×10^7 pfu/hamster	1.7×10^9 pfu/m^2
Cotton rat	5×10^7 pfu/rat	1.9×10^9 pfu/m^2
Rhesus monkey	2×10^7 pfu/monkey[a]	8.2×10^7 pfu/m^2
Baboon	7×10^8 pfu/monkey	1.8×10^9 pfu/m^2
Human	2×10^7 pfu/patient	1.2×10^7 pfu/m^2[b]

Note. Cotton rats, mice, and hamsters were administered increasing doses of adenoviral vectors encoding the human CFTR gene by intranasal instillation. Baboons, Rhesus monkeys, and humans were treated with adenoviral vectors encoding CFTR via bronchoscopic instillation into an isolated lobe of the lung. Animals were sacrificed 3 to 5 days after vector administration, and histologic sections of the lung were examined microscopically for evidence of inflammation [15]. The human data were obtained via chest radiograms and CT scans of a patient in a phase 1 clinical trial [13].

[a] NOAEL not available; lowest dose tested with minimum pathology

[b] Toxic dose in humans, 2×10^9 IU, or 1.2×10^9 IU/m^2.

This finding allowed for a redesign of the clinical approach to gene therapy for cystic fibrosis, using smaller volumes for instillation of vector and a more targeted approach to deliver the adenovirus to the larger airway epithelial surfaces. To date, cystic fibrosis patients have been treated using two to three logs higher doses of adenovirus with this newer approach without the toxicities observed in the initial clinical trial [13].

In cases where gene transfer vectors may be in limited supply, or for vectors with inherently low toxicity, a maximum feasible dose may be administered as the highest level tested in the preclinical studies. In all studies, and especially when using animal models of the clinical indication, appropriate controls, such as naive or vehicle-treated animals should be included. This should allow determination of an adequate margin of safety for use of the vector in the clinical trial, as well as an acceptable dose-escalation scheme.

X. Biodistribution

One issue with direct administration of genetically modified cells or viral or other vectors is that the injected material may not stay where it is initially introduced. Therefore, localization studies designed to determine the distribution of the vector, or the trafficking of genetically modified cells after administration to the proposed site are incorporated into the toxicology testing. These studies have two purposes: (i) first, to identify potential distribution of the vector to sites other than the intended target site, where presence of the vector and/or aberrant expression of the transgene may lead to toxicity; and (ii) to evaluate potential distribution of vector to gonadal tissues and/or transfection of germ cells. In a discussion by the NIH RAC about the risk of potential, inadvertent gene transfer to germ cells, it was concluded that the risk of vertical transmission of the foreign gene was very small. A discussion by the RAC and several expert panelists in gene transfer or reproductive biology recommended that unless there were significant safety issues associated with either the vector or the transgene product, preclinical biodistribution studies in animals were not always required prior to initial Phase 1 trials. In addition, the panel concluded that in cases such as adenoviral vectors, where a large body of literature exists regarding their distribution and potential for toxicity, minor changes in the vector (e.g., substitution of a different transgene with no potential toxicity associated with it) did not require further preclinical distribution studies prior to initiating clinical trials [14]. Biodistribution studies, in which the disposition of the vector is detected after administration by the intended clinical route not only provide data regarding the potential for gonadal uptake and inadvertent germ-line gene transfer, but can also identify any target organs in which aberrant vector distribution or gene expression may be detrimental. CBER's current recommendation is that biodistribution studies of gene transfer agents are not always required prior to Phase 1 clinical trials; however, these studies should be incorporated into the drug development plan so that data are available prior to commencing large-scale, pivotal studies in the clinic [14].

Dose levels selected for biodistribution studies should follow those used in the toxicity studies and include either vehicle or untreated control animals, and the route of administration should be relevant to that employed in the clinical trial. Transfer of the gene to normal, surrounding, and distal tissues as well as to the target site should be evaluated using the most sensitive detection methods possible and should include evaluation of gene persistence. When aberrant or unexpected localization is observed, studies should be conducted to determine whether the gene is expressed and whether its presence is associated with adverse effects. Additional groups of animals may be treated intravenously, as a "worst-case" scenario in cases where widespread vector dissemination may be expected to cause toxicities in organs other than the target site [15].

A. Good Laboratory Practices

Preclinical studies in support of use of gene transfer vectors including adenovirus, in clinical studies should be conducted in compliance with the regulations for Good Laboratory Practices (GLPs) as set forth in 21 CFR, part 58. Compliance with these regulations is intended to assure the quality and integrity of the animal safety data used in support of human research studies, as well as marketing approval.

There is often some confusion as to what types of studies need to be conducted under the GLP regulations. Preclinical pharmacology, "proof-of-concept," and efficacy studies in animals, as well as *in vitro* pharmacology studies are not expected to be conducted in full compliance with GLP. However, *in vitro* and animal toxicology studies, including single- and repeat-dose toxicity testing, reproductive toxicity and carcinogenicity studies, and, for gene transfer research, biodistribution studies are expected to follow the guidelines set forth by the regulation. Although studies for gene transfer vectors in early stages of clinical development need not be in full compliance with the GLP regulations (i.e., quality assurance audits, validation of test and other methodology may be omitted in early studies), CBER expects that any pivotal toxicology studies submitted to an IND or licensing application will be conducted under the auspices of GLP.

XI. Introduction to Clinical Testing

The goal of clinical testing is to provide information about the product's safety and effectiveness and, ultimately, allow new products to come to the marketplace. As discussed in the introduction to the preclinical section, the principles described below are neither unique to gene transfer vectors in general, nor to adenoviral vectors in particular.

A. Phases of Clinical Development

Premarket clinical testing proceeds in a stepwise fashion, often referred to as Phases 1, 2, and 3 of clinical development, although the phases are not always discrete. Phase 4 studies are those performed after marketing. Each phase of product clinical testing has its series of goals or objectives.

The primary goals of Phase 1 testing are to learn about the product's safety and pharmacokinetic profile and to identify a safe dose or doses for further study. Phase 1 studies involve small numbers of study participants who are closely monitored for the development of drug effects. A common Phase 1 design is a single dose, rising dose, cohort study. Escalation to the next dose cohort occurs after sufficient safety assessment of the proceeding cohort. The starting dose and dose escalation scheme employed depend on the data gleaned from product and preclinical testing, and other clinical data, if available (e.g., closely related products or same product studied in different populations). Dose escalation usually proceeds until a defined endpoint, such as a maximal tolerated dose, or an optimal biologic dose, is reached.

Phase 1 studies for some drugs may be conducted in healthy volunteers. This approach is common when anticipated side-effects of the product are expected to be minimal and transient and the target population (those with the disease or condition of interest) have high background rates of adverse events, making it difficult to tease out the safety profile of the product. However, for many classes of drugs and biologicals, including adenovirus gene transfer products, the potential short- and long-term adverse effects (see section XIII) generally makes their risks unacceptable for testing in healthy volunteers.

The next phases of clinical testing, Phases 2 and 3, build upon the information generated from the prior studies. The goal of Phase 2 testing is to gain preliminary evidence of the product's activity in the disease or condition of interest and to begin to characterize that activity. Phase 2 is the ideal time to optimize the dose and/or dosing regimen, the patient population, the response parameters that are most likely to reflect clinical benefit, as well to build upon the safety database. Phase 2 trials often are randomized, controlled, and conducted in multicenters.

Phase 3 of clinical testing includes clinical studies to establish the product's effectiveness. The number of efficacy trials, trial design(s), and size of the safety database necessary to determine net clinical benefit depend on a number of factors, including but not limited to the class of product under development, the condition or disease being studied, and the availability of other therapies.

Phase 4 of clinical testing are studies conducted after market approval. Their purpose is to address questions that arose during the premarketing investigations, or to evaluate the product in other related setttings, such as the elderly, or people with more advanced stages of the disease. The design of a postmarketing study (such as a randomized controlled clinical trial or a registry) depends on the questions to be addressed.

XII. Good Clinical Practices

Good Clinical Practices (GCPs) are a set of principles and procedures intended to preserve and protect the rights and confidentiality of human research subjects and to assure, to the extent possible, that the clinical research generates valid scientific data. The origins of a code of conduct to protect human subjects in clinical research date back to the Nuremberg war trials and the Declaration of Helsinki. In 1996, the FDA, under the auspices of the International Conference on Harmonization (ICH), published the guidance document entitled: "E6 Good Clinical Practice (GCP) Consolidated Guideline." Basic principles of GCP will be discussed below; the reader is referred to the CBER website http://www.fda.gov/cber/guidelines.htm for the full document [16].

A. Responsibilities of a Sponsor and Investigators

The sponsor oversees the IND and communicates with the FDA. As set forth in regulations at 21 CFR 312, subpart D, and in the ICH GCP guidelines, the oversight function includes selecting study investigators, reporting safety information to the FDA, and providing accurate and timely information to all investigators. In some cases, a sponsor may transfer all or some of its obligations to a contract research organization (CRO), although the sponsor retains ultimate responsibility for the IND.

Clinical investigators also have specific obligations, delineated in 21 CFR 312, subpart D, and in the ICH GCP guidelines. Investigators are responsible for selecting study participants based on eligibility requirements of the protocol and for obtaining the protocol-specified evaluations. The investigator is responsible for the welfare of the study subjects at his/her clinical site. This includes collecting safety data and reporting safety information to the IND sponsor. The investigator also must account for all investigational medical product, maintain accurate records, provide annual updates to the Institutional Review Board (IRB), and obtain consent from all study participants.

Where the sponsor and investigator are distinct, their separate roles, with the former overseeing the latter, incorporate the checks and balances that minimize bias and maximize patient safety and trial validity. These checks and balances may be lacking when the investigator is also the sponsor, and additional external oversight is advisable. Individual physicians who assume the role of sponsor, investigator, or sponsor/investigator should be familiar with guidances and federal regulations that set out the respective duties of the sponsor and the investigator.

B. Adverse Event Reporting

Adverse event collection and reporting is a fundamental aspect of drug development and of human subjects protection. The clinical investigator is the individual who identifies, evaluates, and documents adverse events experienced by study participants at his or her site and who is responsible for updating the IND sponsor and the IRB as appropriate, as set forth in federal regulations (at 21 CFR 312.64).

The sponsor is responsible for submitting safety information to FDA. The timing and reporting format will depend on the nature of the adverse event. The sponsor must report to FDA in writing all serious and unexpected adverse event information associated with the use of the investigational product within 15 calendar days of receipt of the information. Any unexpected life-threatening or fatal event associated with the use of the investigational product must be reported by telephone (or facsimile) within 7 calendar days of receipt of the information (as per 21 CFR 312.32). The telephone and written reports constitute expedited reports. Although causality assessment is integral to expedited reporting, a determination that a given investigational product caused or was associated with an adverse event in the course of a clinical study is not always possible. The most reliable way to assess the contribution of a test article to an adverse event is by comparing adverse event rates and severity in treatment and control groups. Randomized controlled trials, however, are infrequent in early phases of clinical testing. Although one cannot always be certain that there is a relationship between the administration of the study product and the adverse event, the level of suspicion required for reporting is quite low. Except if there is no reasonable possibility that the product caused or contributed to an unexpected serious adverse event, that event must be reported to the FDA according to specified time frames.

The sponsor is also required to submit to the IND an annual report that includes a summary of the most frequent and the most serious adverse events (21 CFR 312). The ICH guideline entitled "E3: Structure and Content of Clinical Study Reports" describes the manner in which safety data for individual studies should be organized and presented to regulatory authorities in marketing applications [17]. A marketing application includes an integrated summary of the entire safety experience for the product. FDA, as part of the ICH process, is developing a guideline entitled "The Common Technical Document for the Registration of Pharmaceuticals for Human Use" that addresses, among other items, formatting of integrated safety data [18]. Once marketed, a passive surveillance system allows for the continued collection and reporting of safety information [19]. For some products, such as ones that pose unique long-term risks, a more active type of postmarketing follow-up will be required.

C. Consent and Vulnerable Populations

In general, prospective participants cannot be enrolled into a trial without their consent. Elements of the consent form and the consent process are set forth in 21 CFR part 50. Before consenting, study participants must be informed of known and potential toxicities that may occur from participation in a trial of an investigational product, even if the likelihood of toxicity is remote. The IRB at each institution participating in a study must review and approve the consent form and the clinical research protocol before the study can be initiated at that institution. The composition and duties of the IRB are described in the ICH GCP guidelines and in 21 CFR part 56.

For some of the disorders that are targets of gene therapy, such as inborn errors of metabolism, the affected population will be pediatric subjects. Mechanisms exist to strengthen the human subject protections for study participants who may be particularly vulnerable, such as children, who cannot give valid consent [20]. When a child is to be enrolled in a research study, the parent or legal guardian consents (gives permission) for the child to be in the study. The FDA, as part of the ICH process, has published a guidance document that addresses clinical trials in children, including ethical issues [20].

In rare circumstances where it is not possible to obtain a participant's consent because of the nature of his or her illness or injury, and in which obtaining consent from a legally acceptable representative (e.g., next of kin) is not feasible, the FDA may permit the clinical trial to proceed with a waiver of consent, as set forth in 21 CFR 50.24.

D. Monitoring and Auditing

Monitoring and auditing are fundamental aspects of GCP. Although their purposes are similar (to assure appropriate trial conduct and data validity), the approaches differ. As stated in the ICH GCP document, monitoring is "the act of overseeing the progress of the clinical trial and ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, GCP, and applicable regulatory requirements." Medical monitors, usually employees of the sponsor, perform on-site (and, if indicated, off-site) evaluations of trial-related activities. The extent and frequency of monitoring should be appropriate for the length, complexity, and other particulars of the trial. Among the functions of the monitor is identification of deviations in protocol conduct so that the sponsor may take appropriate corrective steps, e.g., retraining investigators, closing out certain sites, etc.

Auditing is defined in the ICH CGP document as "the systematic and independent examination of trial-related activities and documents." The audit is usually conducted at the conclusion of the trial. The sponsor may hire auditors who document findings in a written report to the sponsor. FDA field inspectors also conduct independent study audits. Traditionally, the purpose of the FDA audits has been to verify the data submitted to the FDA in support of a marketing application. However, the FDA and the sponsor may conduct "for cause" or directed audits at any stage of clinical investigation if there is reason to suspect a problem with trial conduct or data integrity.

The FDA has performed directed inspections at a few gene therapy clinical sites since 1999. The agency also audited approximately 70 gene transfer clinical sites selected at random to assess whether systemic problems with the conduct of such clinical studies existed. Inspectional findings will be discussed in more detail in section XVI.

An additional measure of human subject protection is use of a Data Monitoring Committee (DMC) to evaluate accumulating data from a clinical trial [22]. Generally, the sponsor establishes the DMC, including selecting the members and devising the charter. The DMC members should be independent of the sponsor and clinical investigators. The role of the DMC varies according to the charter and the nature of the study. The DMC is usually empowered to recommend study modifications to enhance safety of participants; in some cases, a DMC may recommend that a study be stopped if data indicate a major safety concern. Of note, DMCs review data submitted to them but do not visit sites to directly ensure that the data are accurate, the protocol is followed, consent is documented, etc. Thus, a DMC does not perform the functions of or obviate the need for study monitors. The FDA is in the process of developing guidance on DMCs.

XIII. Clinical Safety of Adenoviral Vector Products

Most of the completed and ongoing adenoviral vector clinical trials are early, uncontrolled trials. The absence of an internal control group limits the ability to draw definitive conclusions about the contribution of the adenovirus vector product to an adverse event. Despite this caveat regarding causality assessments, administration of replication defective adenovirus is associated with an acute cellular and cytokine mediated inflammatory response. Individuals have experienced systemic reactions such as fever, chills, hypotension, and laboratory findings consistent with disseminated intravascular coagulation, including thrombocytopenia. An overwhelming systemic inflammatory response, to which has been attributed, at least in part, the death of a volunteer in a trial of ornithine transcarbamylase (OTC) deficiency who received intrahepatic artery injection of a high dose of adenovirus-containing product, has not been observed in other clinical trials, including those that employ systemic administration of similar doses of adenovirus vector. See also discussion in section XVI.

The route of administration appears to play a key role in determining the type of and occurrence of adverse events. Toxicities have been particularly prominent in organs that are the sites of adenovirus injection, including the lung, brain, and liver [13, 23, 24]. In addition to route of administration, other variables associated with the clinical trial may influence the nature, frequency, and severity of an adverse event. Such factors include the adenovirus construct, transgene, dose, and frequency of product administration, and host factors such as the underlying disease, other comorbidities, and use of concomitant medications. A committee of experts convened to discuss adenovirus safety in December 1999 questioned the role of the transgene in the toxicity profile and suggested employing null adenovirus vectors as controls when possible to tease out the relative toxicities of the transgene from the vector [25].

Preexisting antibody to adenovirus and/or the development of an antibody response following administration of an adenovirus-containing product may play a role in product safety, although a clear relationship has not been established [24]. The limited data available have not suggested a correlation between high baseline levels of neutralizing antibody and adenovirus toxicity (or activity). Moreover, in a study that involved repeat administration of an adenovirus-containing product, participants developed large spikes in serum levels of neutralizing antibody after the initial dose. However, the toxicity profiles of the first and subsequent doses were similar, again suggesting a lack of correlation. It is important that clinical investigators continue to characterize the immune status of study participants at baseline and following adenovirus vector administration, and attempt to correlate adverse events with levels of or changes in antibody titer. Ultimately, such information could be utilized in patient selection criteria or in clinical monitoring to enhance safety and effectiveness.

The long-term safety of gene transfer is under active discussion. Concerns about late adverse sequelae such as new malignancies occurring years or decades following administration of replication-competent, integrating viruses resulted in FDA guidance regarding testing for replication-competent retrovirus (RCR) in product and patient's serum and for lifelong clinical monitoring [26]. These recommendations are currently limited to retroviral vector INDs. Although adenovirus can become replication competent, the FDA had not previously recommended that patients exposed to this class of product be followed long term. Long-term follow-up of gene therapy products was discussed at recent meetings of the Biologics Response Modifier's Advisory Committee [8, 9, 9a]. FDA will revise the recommendations for long term follow up of recipients of gene transfer products including adenovirus-containing products, pending additional public discussions.

XIV. Bioactivity of Adenoviral Vector Products

A goal of Phase 2 testing is to determine if the adenovirus containing product is bioactive and, if so, to determine whether the observed activity findings, together with the safety profile, warrant further clinical testing. Bioactivity measures may be laboratory findings, clinical outcomes, or a combination of the two. One measure of bioactivity for gene therapy products is detection of gene transfer and gene expression. This may not be possible where assays for the transgene are not yet developed or are insensitive to low levels of expression. Documentation of clinical or surrogate outcomes and/or alternative assessments (e.g., pharmacodynamic measurements), and correlations, if any, to levels of gene expression, are highly desirable in early product development. The extent to which the generation of such data will be feasible depends on, among other factors, the nature of the product, the clinical population in the study, and the state of the science regarding assays to detect the transgene.

The majority of the clinical investigations with adenoviral vectors to date target patients with cancer. In the oncology setting, studies that are in Phase 2 of development are usually designed to capture data on tumor responses (complete and partial response rates). The demonstration that the adenovirus gene therapy product results in a certain level of tumor response, and the characterization of those responses (rates of complete and partial responses, duration of response, etc.), along with an acceptable safety profile, will usually be sufficient evidence of activity to warrant efficacy trials.

Early studies of cystic fibrosis (CF) involved topical administration of the adenovirus product containing the cystic fibrosis transmembrane regulator (CFTR) protein gene to the nasal epithelium. Measures of product activity included gene transfer/gene expression and assessment of the potential difference across the nasal epithelium. Topical administration resulted in only low levels of gene transfer and limited pharmacodynamic affects. Gene transfer via aerosolized delivery systems appeared to be marginally improved over topical administration. Given the limited product bioactivity that has been seen, clinical development of adenovirus containing products for CF has largely been abandoned.

An evolving area of clinical research is use of adenoviral vector products that contain genes intended to promote vascular growth. Patients enrolled generally have vascular disease. Studies are ongoing in both cardiac and peripheral vascular disease settings. The activity measures can include laboratory measures such as myocardial perfusion, and measures of gene expression.

XV. Clinical Efficacy of Adenoviral Vector Products

FDA grants market approval for products that are shown to be safe and effective. The efficacy standard, applicable to all FDA-regulated products, as stated in section 505(d) of the Food, Drug, and Cosmetic Act, is *substantial evidence*, defined as "evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could be fairly and responsibly concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof." The following paragraphs address the issues of the quality and quantity of clinical investigations that can provide "substantial evidence."

A. Choice of Control

An "adequate and well controlled" investigation is one whose design and execution produces valid scientific data. Clinical investigations intended to show efficacy must be controlled so that the effect(s) of the intervention can be distinguished from other influences, such as spontaneous change, placebo effect, or biased observation. In Phase 2 testing, controlled trials are helpful in teasing out adverse events and in assessing the magnitude of the effect relative to the control group. Such information will be useful for sample size calculations for the efficacy trial(s).

The choice of control (e.g., historical, active, placebo, etc.) depends on the clinical setting. The agency has approved products for market based on studies with various types of control groups. Each type of control has its advantages and limitations. The reader is referred to the ICH guidance entitled "E10 Choice of Control in Clinical Trials" for an extensive discussion on this topic [27].

A control for an adenoviral-containing gene transfer product could be the adenovirus vector without the transgene (i.e., containing a null vector) as discussed previously. Such a null vector control could help delineate safety and efficacy of the vector separately from the insert, as well as show that both vector and insert contribute to product effectiveness. A null vector control, if deemed appropriate, could be incorporated earlier in product development (rather than during Phase 3) as it might be beneficial to determine early on the contribution of and need for the transgene.

Adenovirus products are currently in Phase 3 testing in patients with malignancies. Most are designed as "add-on" trials, i.e., chemotherapy + gene product vs chemotherapy + placebo (or no additional treatment if a placebo is not feasible). If a trial is not blinded, such as would be the case if the control arm could not receive a placebo, it will be important to utilize objective outcome measures and to control use of concomitant therapies. If measures are not objective, blinded third party assessors may be useful.

B. Endpoint Selection

Trials intended to provide substantial evidence of efficacy must be "adequate" in addition to "well-controlled." They must be conducted according to GCPs (as discussed in section XII) to maximize human subject protection and data validity. They must also be designed with appropriate, relevant endpoints that either reflect clinical outcomes or are acceptable surrogate endpoints.

Surrogate endpoints are laboratory or other measurements not directly indicating clinical benefit but that are expected to correlate with or predict clinical benefit. Surrogate endpoints are usually easier to measure than clinical endpoints and occur earlier in the course of the disease, allowing for shorter, smaller, and, thus, less expensive studies. Their major disadvantage is the uncertainty surrounding whether and to what extent the surrogate reflects the true clinical benefit. Thus, if FDA bases important regulatory decisions regarding product licensure on a surrogate and the medical community bases practice decisions on data generated from trials using surrogates, it is critical that the surrogate be valid for the particular treatment and disease. Once a surrogate is validated for one treatment and disease using a particular product, the extent to which that validation applies to other products in the same class and across product classes could become important, particularly as one might define a product class in the context of adenoviral-containing products. In earlier phases of clinical testing, use of surrogate endpoints may serve useful and potentially less problematic roles. For instance, during product development, a surrogate may be used to assess dose–response and thus provide the rationale for dose selection for later trials, or they may be used as initial proof-of-concept to base decisions about further clinical development. Several excellent papers provide more in-depth discussions about surrogates and validation of surrogates [28, 29].

Where the disease is serious or life threatening and without acceptable alternatives treatments, it may be possible to establish efficacy and receive FDA approval based on trials employing a surrogate endpoint that is not yet validated but reasonably likely to predict clinical benefit. If a product is marketed based on an effect on such a surrogate endpoint, Phase 4 studies are required to verify the clinical benefit. These provisions are set forth in 21 CFR 601.40, subpart E. Oncology and AIDS are two areas where this provision has been used with some frequency.

The number of adequate and well-controlled trials that will be necessary to make a determination of substantial evidence of effectiveness has been discussed in FDA guidance [30]. Sponsors should meet with the agency at the end of Phase 2 and discuss and reach agreements about critical product development issues, such as the number and types of clinical trials and the size of a safety database considered necessary to file a marketing application.

XVI. How the Role of FDA Regulators Has Changed Since September 1999

In mid-September 1999, a participant in a clinical study of an adenoviral vector product for Ornithine transcarbamylase (OTC) deficiency became profoundly compromised and ultimately died 4 days after receiving the experimental product by intrahepatic artery infusion. This event was the first death in a clinical gene transfer trial that was clearly directly attributable to the administration of a vector and resulted in a number of regulatory actions, as well as a commitment by the FDA to increase sponsor outreach programs to address issues related to the safety of all gene transfer vectors, including adenovirus. These efforts have included (i) safety symposia held in conjunction with the Office of Biotechnology Activities (OBA) at the National Institutes of Health (NIH); (ii) the FDA's issuance of a letter on March 6, 2000, to all gene therapy sponsors, requesting that they provide information regarding the oversight of their programs, including the manufacturing, animal data, and any ongoing or future clinical trials; (iii) targeted inspections of clinical sites for compliance with gene transfer protocols conducted at their site; and (iv) increased sponsor education and training in issues specific to gene transfer, as well as the conduct of clinical trials, in general.

A. Safety Symposia in Conjunction with OBA

Following the death of the study participant discussed above, the OTC trial was immediately placed on clinical hold, and the FDA initiated a search of its database to identify all protocols involving adenoviral vectors used for therapeutic intent. A total of 12 protocols were identified which used adenovirus administered by either systemic or intrahepatic artery infusion, or by direct injection into the liver. The sponsors of these protocols were informed of the death of the patient in the OTC trial and were asked to provide an assessment of the safety and toxicity of their adenovirus clinical studies, including the maximal dose of vector administered to date. After review of the information provided by these sponsors, one other clinical trial, using adenovirus encoding a tumor suppressor gene and administered by the same route of injection but at a higher dose than the OTC vector, was placed on clinical hold pending receipt and review of the safety data for that specific trial.

The NIH OBA issued a call for investigators to submit safety information from all adenoviral vector clinical and preclinical studies. On December 8 and 9, 1999, OBA held an open, public symposium whose purpose was to examine the available scientific, technical, and clinical data regarding adenoviral vectors in gene transfer, to identify specific safety issues that were unique to adenovirus, and to make recommendations to the gene transfer community where additional clinical or preclinical data should be required. Investigators from both industry and academic settings presented information regarding the biology, pathophysiology, and toxicities associated with adenovirus infection, both by the natural route of infection as well as by the different approaches used in the gene transfer research studies. Both preclinical study results and data from human subjects in adenovirus-vectored trials for cystic fibrosis, oncology, and metabolic disorders were discussed, with the majority of the clinical data coming from studies in the oncologic setting [25].

In general, comparison of the data across the different settings revealed that the toxicities associated with adenovirus, whether in animals or in human subjects were very similar, and consisted mainly of local, dose-related, and dose-limiting inflammatory responses and immune cell activation. These findings were consistent, whether the virus was administered by bronchoscopic instillation to the lungs, by direct injection into a localized tumor, or by systemic administration. Patients treated with adenovirus vectors at very high doses were found to exhibit some signs of clinical toxicity similar to those observed in the patient at University of Pennsylvania; however, there was no other incident of death attributable to the vector, even in the study where doses higher than that used in the OTC trial were administered by intrahepatic arterial infusion.

Based on the results presented and the discussion at this symposium, a working group on adenovirus safety and toxicity (Ad-SAT) was convened by OBA, composed of clinicians and scientists from FDA, industry and academia. The recommendations from this group were presented at the close of the safety symposium, and included the need for additional information regarding adenovirus vector standardization, biodistribution in human subjects as well as in preclinical studies, and the construction of a database which would include both preclinical data which could predict expected toxicities for the clinic, as well as data from human subjects which would allow comparison of the safety across a number of different settings. The findings and recommendations of the Ad-SAT and RAC were recently published [30a].

OBA and FDA have also cosponsored three additional safety symposia on clinical trials for gene transfer since the December 1999 meeting. These have included discussions of safety issues involved in development of helper-dependent adenoviral vectors and in clinical programs of gene transfer for cardiovascular disease, as well a recent discussion of the potential tumorigenicity of adeno-associated viral vectors in mouse models of human β-glucuronidase deficiency.

B. Results of FDA's Directed Inspections

In the weeks following the death of the patient in the OTC study, the FDA conducted a directed inspection of the clinical site and the Institutional Review Board at the University of Pennsylvania, as well as an inspection of the animal experiments conducted in support of the clinical program. All three inspections found deviations and deficiencies, including inadequate clinical monitoring and oversight of the clinical trial, inadequate reporting of adverse events, and failure to follow clinical and preclinical study protocols. As a result of these directed inspections, the FDA placed the remainder of the clinical studies under the same sponsorship on clinical hold and issued warning letters to the sponsor, to all of the clinical investigators involved in the OTC trial, and to the director of the preclinical laboratory facility. The FDA also issued a Notice of Initiation of Disqualification Proceedings and Opportunity to Explain (NIDPOE) Letter to the principal investigator. Redacted versions of these letters are available at the CBER websites [31, 32].

A second clinical inspection of a different site, using a different class of vector for gene transfer in cardiac and peripheral vascular disease also found numerous discrepancies in the conduct of the clinical trials and compliance with the regulations governing investigational new agents [31]. As a result of these two inspections, the FDA determined that a more systematic review of procedures to ensure compliance with regulations was warranted. This was accomplished by two specific activities. In March of 2000, the FDA issued a letter to all Gene Therapy IND or Master File sponsors requesting information on the gene transfer product characterization, a review of the preclinical safety studies to ensure any findings that met the criteria requiring an expedited report as per 21 CFR 312.32-33 were submitted, and a summary of the procedures to ensure adequate monitoring and adequate oversight. A copy of the March 6, 2000, letter is available at http://www.fda.gov/cber/genetherapy/gtpubs.htm.

In April 2000, the FDA initiated a series of inspections of clinical sites conducting trials in gene transfer research. At the time, CBER had 211 active gene transfer IND submissions; a random sample of 30 INDs was taken and the principal investigators and clinical sites were identified. From these 30 INDs, 70 sites were identified for inspection to determine their level of compliance with the current regulations. A summary of the results of the March 6 letter and the additional site inspections is provided below.

C. Description of the March 6, 2000, Letter and Summary of Responses

The March 6, 2000, letter was sent to approximately 150 sponsors holding slightly less than 300 total active INDs or master files. Items 1–5 of the letter were questions regarding product testing and characterization data, test methods, specifications, information regarding other products produced in the facility, and quality control procedures. The goals were to: (i) ensure that all gene therapy products currently in clinical trials are adequately tested by contemporary standards, (ii) determine where testing requirements need to be made more stringent or relaxed, (iii) gather information to aid in development of additional guidance, (iv) gain information concerning product characterization and manufacturing processes and arrangements in order to move these products forward toward licensure, and (v) develop a mechanism to ensure that IND annual reports routinely contain updates of this information. In general, sponsors of adenovirus gene transfer trials have been in compliance with FDA recommendations and expectations regarding adenovirus vector product characterization. In addition, review of the adenovirus vector lot information led to recent changes in recommendations regarding vector infectious particle and total particle measurements as well as a change in the recommendation regarding RCA.

In addition to requests for information on manufacturing practices, the March 6 letter also asked sponsors to provide a summary of the monitoring program for each clinical study conducted under their IND and documentation of their oversight function. The intent was to confirm or bring sponsors into compliance with GCP as required under 21 CFR 312, subpart D, and as described in the ICH GCP guidance. FDA review of the descriptions of the clinical monitoring programs found that the monitoring programs in general incorporated many of the activities and procedures in accordance with the ICH GCP guidance and the requirements listed 21 CFR 312, subpart D. However, some areas of deficiencies were noted, including but not limited to lack of procedures to correct or remove noncompliant investigators, ensuring reporting of protocol modifications to FDA, and ensuring safety reports are filed to the IND in a timely fashion.

The last question in the March 6 letter was intended to remind sponsors that certain findings from animal experiments, i.e., severe toxicities and/or deaths on study, also rise to the level of an expedited report. It asked the sponsors to verify that such data, if relevant, either had already been submitted as required under regulation, or, if not previously submitted, that the data be promptly submitted to the IND or master file. In general, most sponsors indicated they were already in compliance with reporting requirements for such data.

D. Results of Additional Inspections

The sites inspected were chosen at random. Specific questions regarding the background information on the product and the clinical study were developed by the inspection team, and focused on the conduct of the protocol, the reporting of adverse events, blinding of study medication where applicable, and whether the clinical end points were met. CBER field inspectors conducted the inspections between April and August 2000.

In general, these inspections found that most sponsors, both commercial and academic, as well as clinical investigators, were in compliance with the regulations. Of the 70 sites inspected, 11 had no current, active clinical trials or had never initiated their proposed studies, and 23 (33%) required no further action from FDA. Approximately half of the sites had objectionable conditions or practices identified by the inspection team; however, in 33 cases (47% of total sites), only voluntary action to correct the deficiencies was called for. Only three sites were identified where official regulatory action (*i.e.*, warning letters) was required. The most common deficiencies in all of these cases were: (i) failure to follow the protocol; (ii) an inadequate consent form; (iii) lack of supporting data for case report form entries and/or discrepancies between the source documents and the case report forms; (iv) inadequate drug accountability records; and (v) the failure to notify the Institutional Review Board(s) of protocol changes, adverse events, or deaths.

In summary, the targeted inspections in gene transfer research clinical trials demonstrated, with a few exceptions, that studies were being conducted according to appropriate regulation and guidance. Where deviations were noted, they appeared to be similar to those found in routine inspections of Phase 3 studies of more traditional, biologic agents. The FDA will continue to conduct inspections of clinical, preclinical, and/or manufacturing sites involved in gene transfer research on "for cause" as well as routine bases as part of our role in protecting the safety of patients enrolled in these trials.

E. Sponsor Outreach and Education

CBER had routinely been involved in educational and training activities aimed at sponsors and investigators who are involved in gene transfer research. However, following the death of the patient in the OTC deficiency study, the agency recognized the need to inform potential sponsors of not only the issues specific to the conduct of gene transfer studies, but also on the issues involved in the design of a clinical program and the elements of GCP. Education sessions have taken place at various venues, including the Drug Information Association (DIA) annual meetings and a special satellite broadcast cosponsored by DIA and the FDA; the annual meetings of the Society of Toxicology, the American College of Toxicology, the International Society for Genetic Anticancer Agents, meetings of the Pharmaceutical Research and Manufacturer's Association, meetings of the RAC, and the annual American Society of Gene Therapy (ASGT) meetings. FDA will continue to participate in training courses held by ASGT, as well as other professional and scientific societies.

XVI. Summary

Adenovirus vectors are complex biologics. The FDA's recommendations and expectations for product manufacture and characterization, preclinical, and clinical testing incorporate the tremendous experience gained in the nearly 10 years since the first adenovirus gene transfer experiment, as well as from the experience with the entire field of gene transfer research. The FDA is cognizant of the need for flexibility in its recommendations and will consider many factors, including the intended target population, the seriousness of the disease under study, the potential benefits and risks from the investigational product, when advising sponsors about their adenovirus development program.

The agency will update and reassess recommendations for adenovirus vector production and testing based on the growing experience and on feedback from a variety of sources. The information in the above sections is intended to educate the reader about FDA processes and expectations and should be utilized in conjunction with consultation from FDA staff.

The FDA encourages new investigators to consult with FDA staff prior to submission of an IND. The formal process for FDA consultation is a pre-IND meeting. Sponsors may request information about the IND process in general through CBER's Office of Communication, Training, and Manufacturers Assistance (OCTMA) at 301-827-2000. A sponsor for a gene transfer product who is interested in meeting with the Agency should submit a written request (i.e., letter or fax) to the Director, Division of Application Review and Policy, Office of Therapeutics Research and Review, Center for Biologics Evaluation and Research. Requests for meetings should be submitted in triplicate to the following address: Center for Biologics Evaluation and Research, Attn: Office of Therapeutics Research and Review, HFM-99, Room 200N, 1401 Rockville Pike, Rockville, MD 20852-1448. Prior to submitting a written request for a meeting by fax, the sponsor should contact the Division of Application Review and Policy to determine to whom the fax should be directed and to arrange for confirmation of receipt of the fax.

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