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Preclinical Development Strategies for Novel Gene Therapeutic Products*

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ABSTRACT

With over 220 investigational new drug applications currently active, gene therapy represents one of the fastest growing areas in biotherapeutic research. Initially conceived for replacing defective genes in diseases such as cystic fibrosis or inborn errors of metabolism with genes encoding the normal, or wild-type, gene product, gene therapy has expanded into other novel applications such as treatment of cancer or cardiovascular disease, where the risk : benefit profiles may be more acceptable in relation to the severity of the disease. Different types of vectors, including modified retroviruses, adenoviruses, adenovirus-associated viruses, and herpesviruses and plasmid DNA, are used to transfer foreign genetic material into patients' cells or tissues. Developing a toxicology program to determine the safety of these agents, therefore, requires a modified approach that encompasses the pharmacology and toxicity of both the gene product itself and the vector system used for delivery in the context of the application for the clinical trial. In general, the issues involved in designing and developing appropriate preclinical testing to determine the safety of these products are similar to those encountered for other recombinant molecules, including protein biotherapeutics. Limitations to some of the typical toxicology studies conducted for a traditional drug development program may exist for these agents, and nontraditional approaches may be required to demonstrate their safety. Many factors may affect the safety and clinical activity of these agents, including the route, frequency, and duration of exposure and the type of vector employed. Other safety considerations include quantitation of the duration and degree of expression of the vector in target and other tissues, the effects of gene expression on organ pathology and/or histology, evaluation of trafficking of gene-transduced cells or vector after injection, and interactions of the host immune system with the transduced cell population. Because of the unique concerns regarding each of these therapies, the Center for Biologics Evaluation and Research encourages sponsors to obtain toxicity data whenever possible while evaluating the pharmacologic activity of the vector in a species or animal model relevant to their clinical indication. Sponsors are encouraged to discuss preclinical study design and results with the Center during product development to facilitate early identification of safety concerns prior to entry of these novel agents into the clinical setting and to ensure an uninterrupted course of development while addressing issues required for licensure.

Keywords. Animal studies; adenovirus; plasmid DNA; vector; genetic disease

INTRODUCTION

The Food and Drug Administration (FDA) defines gene therapy as the "introduction into the human body of genes or cells containing genes foreign to the body for the purposes of prevention, treatment, diagnosis, or curing of disease" (1). This definition is fairly broad, allowing for administration of genetically modified, corrected somatic cells such as peripheral blood lymphocytes or hematopoietic stem cells and for the direct administration of corrected genes into the target tissues in patients. However, this definition does not encompass administration of genetic material intentionally designed to improve or enhance metabolic, structural, or functional processes nor does it include administration of a vector targeted to the germ cells, with the intention of genetically modifying future generations. These areas are currently under discussion by both the FDA and the Recombinant DNA Advisory Committee (RAC) as to the safety, desirability, and ethics of these approaches and are beyond the scope of this review.

Approximately 40 new clinical trials in gene therapy have been initiated each year over the past 3 yr, making this field one of the fastest growing areas of clinical research regulated by the FDA Center for Biologics Evaluation and Research (CBER) (Fig. 1). As an example of how quickly this field has moved, the very first patient to receive gene therapy was treated September 14, 1990, with autologous lymphocytes that had been transduced with a retroviral vector encoding the human adenosine deaminase (ADA) gene. As of June 1998, there are 244 gene therapy protocols either currently active or in review by the RAC for a variety of different indications (Fig. 2), with over 2,000 patients treated worldwide (4).

Although the initial clinical trials were focused mainly on correction of the monogenic diseases such as cystic fibrosis or enzymatic defects such as ADA deficiency in leukocytes, clinical research in gene therapy has expanded to include such applications as (a) increasing tumor antigenicity through introduction of a foreign HLA haplotype, (b) conferring resistance to chemotherapeutic agents through transfection of target cells with multidrug resistance genes, and (c) the introduction of wild-type tumor suppressor genes in cancer. Modeling these diseases in the preclinical setting and designing toxicity studies to evaluate the gene therapeutic approach have been challenging to traditional toxicology testing programs. Each therapeutic approach, including the way the vector is administered and the contribution of the under-

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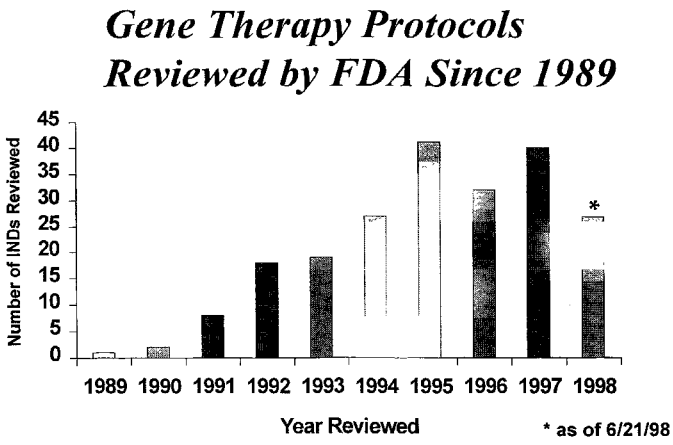

FIG. 1.—Number of gene therapy protocols reviewed by FDA since 1989.

Gene Therapy Protocols Reviewed by the RAC and/or FDA Through 1998*

Indication or Disease State	Submissions
cancer	147
bone marrow marking	30
AIDS	23
inborn errors of metabolism	18
cystic fibrosis	16
coronary/periph vascular disease	7
other (RA, GVHD, non-therapeutic)	6

* as of 6/21/98

FIG. 2.—Gene therapy protocols by indication, reviewed by the RAC through May 12, 1998.

lying disease to both the safety and effectiveness of gene transfer, must be evaluated individually in determining the relative risk of any given vector. Additionally, the incorporation of molecular biology techniques as part of the studies to evaluate the safety and efficacy of this class of therapeutic agents has led to a new understanding of the mechanisms by which gene expression and host responses can affect both the toxicities and the biologic effects of gene therapies.

The FDA recognizes that novel issues exist in designing and interpreting preclinical studies for gene therapeutic agents, and has produced several documents to assist sponsors in developing their preclinical programs (1–3). The initial document (1) published in 1991 has been updated and revised (3) and represents the FDA's current thinking on the development and regulation of somatic cell therapy products. It contains current information regarding regulatory concerns for production, quality control testing, and administration of recombinant vectors for gene therapy and strategies for preclinical testing of both cellular therapies and vectors.

Although viral or plasmid DNA preparations used as preventive vaccines are not covered by this document, there is some overlap in the issues governing both gene therapy and genetic vaccines. A separate document regarding the use of plasmid DNA products to prevent infectious diseases was recently published (4) and should be consulted for information on the issues and areas of concern specific to these products.

Guidance for Preclinical Studies

Preclinical studies for biotechnologically derived products are performed to define the pharmacologic and toxicologic effects predictive of the human response both prior to initiation of clinical trials and throughout drug development. The pattern of preclinical evaluation of gene therapies parallels that of the more conventional biologic agents in many respects. Initially, the pharmacologic activity of a proposed therapy is evaluated, either *in vitro* or *in vivo,* to determine whether the feasibility and efficiency of the gene transfer and the biologic activity in correcting the genetic defect or conferring the desired response is observed (e.g., multidrug resistance in hematopoietic stem cells). When available, animal models that mimic the human disease, either through genetic or pharmacologic mechanisms, may be used as "proof of concept" to demonstrate that transfer of the gene is actually able to correct the genetic defect, ameliorate or slow down progression of the disease, or alleviate some of its symptoms. Based on the responses observed, a decision is made to further evaluate the candidate therapy for safety with the intention of entering it into clinical trials or to terminate development of potentially unsuccessful products.

Toxicology studies to demonstrate the safety of cell and gene therapies are intended answer specific questions regarding the acceptable risk : benefit ratio to the patients and generally incorporate novel technologies and often newly developed methodology to obtain these answers. To understand the safety of gene therapies, the design of preclinical studies should take into consideration the following points: (a) the population of cells to be administered or the class of vector to be used, (b) the animal species, gender, age, and physiologic state most relevant for the clinical indication and product class, and (c) the intended doses, route of administration, and treatment regimens planned for the clinical trial. With many of the gene therapy vectors, these considerations will be interactive because the route of administration or the maximal feasible dose for the preclinical study may influence or be influenced by the species selected for testing.

Preclinical pharmacologic and safety testing of cellular and gene therapies should employ the most appropriate, pharmacologically relevant animal model available. A relevant animal species would be one in which the biologic response to the therapy would be expected to mimic the human response. Other issues affecting the choice of species for testing, such as species specificity of the transduced gene, permissiveness for infection by viral vectors, and comparative physiology, should also be considered in the design of these studies. Animal models mimicking the clinical disease may be useful in obtaining sufficient

Cross-Species Comparison of NOAEL Doses of Adenoviral Vectors for Cystic Fibrosis

Species	Apparent NOAEL	NOAEL (IU/m^2)
C57 BL/6	2.6 x 10^7 IU/mouse	2.4 x 10^9 IU/m^2
hamster	3.6 x 10^7 IU/hamster	1.7 x 10^9 IU/m^2
cotton rat	1-5 x 10^7 IU/rat	0.4-1.9 x 10^9 IU/m^2
Rhesus monkey	2 x 10^7 IU/monkey*	4.6 x 10^7 IU/m^2
baboon	7 x 10^8 IU/monkey	1.8 x 10^9 IU/m^2
human toxic dose	2 x 10^9 IU/patient	1.2 x 10^9 IU/m^2

***NOAEL not available; lowest dose tested with minimum pathology**

FIG. 3.—Cross-species comparison of no observable adverse effect level (NOAEL) doses of adenoviral vectors after direct instillation into the lungs.

safety and efficacy data prior to entry of these agents into clinical trials and should be considered for use when available. When evaluating the activity of a vector in an animal model of the clinical indication, safety data should be gathered at the same time to assess the contribution of disease-related changes in physiology or underlying pathology to the response to the vector.

Selection of the dose and route of administration for the preclinical safety studies of cellular and gene therapies should mimic that intended for the clinical trial as closely as possible. However, an exact match 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 trial is advised. For example, intrapulmonary instillation of adenoviral vectors by intranasal administration in cotton rats or mice is an acceptable alternative to direct intrapulmonary administration through a bronchoscope. 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 (NOAEL) dose, an overtly toxic dose, and several intermediate doses should be evaluated to determine both the relationship of toxicity to the amount of vector administered and the shape and steepness of the dose–response curve. Preclinical safety evaluations should include 1 dose equivalent to and at least 1 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 body weight or total body surface area as appropriate facilitates comparisons across species and allows determination (retrospectively) of whether an animal model is 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 (Fig. 3). When the NOAEL doses were calculated for each species after scaling by total body surface area, the obtained values were remarkably similar among the different species. In fact, the NOAEL doses in the animals for direct instillation of adenovirus into the lungs were approximately equivalent to the first dose in humans at which toxicity was observed when scaled by body surface areas. This finding allowed for a redesign of the clinical approach to gene therapy for cystic fibrosis. To date, patients have been treated using even higher doses of adenovirus without the toxicities observed in the initial clinical trial.

In cases where gene therapy vectors may be in limited supply or for cellular therapies or 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 to allow determination of a margin of safety for use of the vector in the clinical trial and to gauge an acceptable dose-escalation scheme.

One novel 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 should be incorporated into the toxicology testing. The dose levels selected should follow those used in the toxicity studies and should include either vehicle-treated 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 these investigations 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.

For all clinical studies in which the vector is to be directly administered to patients, the risk of vector transfer to germ cells should always be evaluated in the preclinical toxicology program. Samples of testicular or ovarian tissue from treated animals should be analyzed for vector sequences using the most sensitive techniques and methodology available. If a positive signal is detected in the gonads, further studies are recommended to determine if the sequences are present in germ cells or in stromal tissues and to define the potential for transmission of the gene sequences to progeny.

Preclinical toxicology and pharmacology studies are expected whenever a novel vector system (new molecular entity) is planned for first introduction in a clinical trial or when a change in the route or schedule of administration for a vector currently in clinical use is proposed. If a vector or cellular therapy has the potential to induce an autoimmune type of host response, which may not be evaluable in the preclinical efficacy model (e.g., SCID

mice), then additional preclinical studies to address this specific issue should be performed. Proposed use of a vector for a clinical trial that has previously been associated with adverse findings in other clinical indications or by different treatment schedules would also require full preclinical safety and pharmacologic testing.

Abbreviated toxicity testing may be acceptable in some situations. For example, if a new gene therapy vector is comparable to other agents for which there is extensive previous clinical experience or in the case of a vector in which the only change is the insertion of a different expression cassette that is not expected to influence the toxicity or the dissemination of the vector, less extensive preclinical testing may suffice. Safety studies may also be minimized when a strong preclinical efficacy model that incorporates specific questions regarding the safety of the gene therapy approach into the study design is used. Bridging studies, comparing the pharmacologic activity and transfection efficiency of 2 related vector preparations, may also supplant the need for *in vivo* toxicology testing.

There are also times at which specific *in vivo* safety studies of cellular or gene therapies may not be needed at all. For example, previous human experience with a similar product, e.g., peripheral blood lymphocytes transduced with the identical retroviral vector as used in the ADA trials but encoding a different enzyme, may be used in support of the safety of this approach and thus may obviate the need for additional toxicity studies.

SUMMARY

Preclinical studies in support of novel gene and cellular therapies should be designed to answer questions specific to the class of vector or type of cells transduced, the intended route of administration, and the clinical indication and to provide an estimation of the risk for the clinical trial. The studies to determine safety are selected based on the body of information available and the specific issues to be addressed and should employ the best available technology and methods. Selection of species should be relevant to both the product and the clinical indication, but nonhuman primates are not *a priori* a necessity. Safety data may also be obtained from well-designed efficacy studies that address specific questions related to safety as part of the proof of concept. Whenever possible, safety data for cellular and gene therapies may also be obtained from studies in animal models of the human disease to determine the contribution of the underlying disease pathology or physiologic changes to the toxicity of the therapeutic approach.

There is no single right or wrong way to conduct preclinical evaluations of cellular and gene therapies. Sponsors are strongly encouraged to discuss preclinical study design for gene therapeutic agents with representatives of CBER prior to performing animal studies and during product development, and to publish their data for further advancement of the field. Through this interaction, CBER's goal is to facilitate early identification of safety concerns prior to entry of these novel agents into the clinic setting and to ensure an uninterrupted course of development while addressing issues required for licensure.

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REFERENCES

1. Food and Drug Administration, Center for Biologics Evaluation and Research (1991). Points to consider in human somatic cell therapy and gene therapy. Available at: http://www.fda.gov/cber.
2. Food and Drug Administration, Center for Biologics Evaluation and Research (December 1996). Points to consider on plasmid DNA vaccines for preventive infectious disease indications. *Fed. Register* 61: 68269. Available at: http://www.fda.gov/cber.
3. Food and Drug Administration, Center for Biologics Evaluation and Research (March 1998). Guidance for industry: Guidance for human somatic cell therapy and gene therapy. Available at: http://www.fda.gov/cber.
4. National Institutes of Health, Office of Recombinant DNA Activities (May 1998). Quarterly report on human gene transfer protocols. Available at: http://www.nih.gov/od/orda/protocol.htm.