

Gene therapy clinical trials worldwide 1989–2004 – an overview

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Summary

In 1989, Rosenberg *et al.* performed the first human gene therapy trial when they used a retrovirus to introduce the gene coding for resistance to neomycin into human tumor-infiltrating lymphocytes before infusing them into five patients with advanced melanoma. This study demonstrated the feasibility of using retroviral gene transduction in humans and set the stage for further studies. Since then, over 900 clinical trials have been completed, are ongoing or have been approved worldwide. These trials have been designed to establish feasibility and safety, to demonstrate the reality of expression of therapeutic protein(s) *in vivo* by the genes transferred and, in some cases, to show therapeutic benefit.

There is no single source of information that presents an overview of all the clinical trials undertaken worldwide. In 1997 we set up a database to bring all the information on clinical trials together as comprehensively and as globally as possible. The data were compiled and are regularly updated from official agency sources, the published literature, presentations at conferences and from information kindly provided by investigators or trial sponsors themselves. As of January 31, 2004, we have identified 918 trials in 24 countries. The USA accounts for two-thirds of these trials. Cancer is by far the most common disease indication, followed by inherited monogenic diseases, and cardiovascular diseases. Viral vectors have been the most frequently used vehicles for transferring genes into human cells, with retroviruses and adenoviruses representing the vast majority. Plasmid (naked) DNA and other non-viral vectors have been used in one-quarter of the trials. Over 100 distinct genes have been transferred. This article aims to provide a descriptive overview of the clinical trials that, to the best of our knowledge, have been or are being performed worldwide. Details of the data presented, including an interactive, searchable database that currently holds information on 918 trials, can be found on The Journal of Gene Medicine clinical trials website [1]. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords gene therapy; clinical trials

Introduction

In 1963, Joshua Lederberg wrote: “We might anticipate the *in vitro* culture of germ cells and such manipulations as the interchange of chromosomes and segments. The ultimate application of molecular biology would be the direct control of nucleotide sequences in human chromosomes, coupled with recognition, selection and integration of the desired genes” [2]. Just 26 years later, in 1989, the first human gene therapy clinical trial was performed by Rosenberg *et al.* [3] at the Division of Cancer Treatment of the National Cancer Institute in Bethesda. These investigators used a retrovirus to introduce the bacterial gene coding for resistance to neomycin into human tumor-infiltrating

lymphocytes. They then reinfused the modified lymphocytes into five patients with metastatic melanoma. This pioneering study established a number of important prerequisites for future clinical gene transfer studies. The demonstration that retroviral gene transduction for human gene therapy was feasible sparked hopes that the technique could be used to treat serious inherited diseases for which there is no conventional cure.

Shortly thereafter, in 1990, the first therapeutic trial was performed in two girls with a form of severe combined immune deficiency characterized by adenosine deaminase deficiency [4]. The 1990s witnessed the initiation of an ever-growing number of trials. In 1999, the occurrence of a death caused by an overwhelming reaction to an adenoviral vector [5] and, in 2002, the development of a leukemia-like illness due to retroviral insertional oncogenesis [6] had a sobering effect and slowed clinical development.

Despite these setbacks, gene therapy continues to harbor the potential for an entirely new approach to many clinical conditions for which treatment is currently either non-existent or unsatisfactory. As of January 2004, we report 918 gene therapy clinical trials around the world, and examine their distribution by year of initiation, countries where they were performed, the indications addressed, trial phase, genes transferred and vector used. It is not our purpose here to make judgments regarding the ethical or scientific merits or shortcomings of these trials.

Sources of data

The data reported in this article have been compiled from information provided by regulatory agencies, or obtained from the published literature, presentations at meetings and personal contacts with sponsors and investigators. Policy on the public availability of data held by regulatory agencies varies widely from country to country from total transparency in the United States to varying degrees of confidentiality in European and Asian countries. In the US the National Institutes of Health (NIH) compiles a database of all ongoing or completed gene therapy clinical trials and is our primary source of information for trials performed in that country [7]. In the United Kingdom, The Gene Therapy Advisory Committee (GTAC), a division of the Department of Health, maintains a website that provides a summary table of UK gene therapy research [8]. Further information was kindly provided by GTAC and is gratefully acknowledged. The Belgian Biosafety Server, managed by the Service of Biosafety and Biotechnology (SBB), provides very comprehensive and up-to-date information about gene therapy clinical trials in Belgium [9]. In Germany, the Zentrum Klinische Studien at the University Hospital in Freiburg (funded by the German Ministry of Education and Research) has recently set up an interactive database of trials being conducted in Germany in cooperation with the “Commission of Somatic Gene Transfer” (KSG) and

the “German Society for Gene Therapy” (DG-GT) [10]. The Zentrum Klinische Studien has shared their data with us and we gratefully acknowledge their collaboration. In Australia, The Gene and Related Therapies Research Advisory Panel (GTRAP), established under the Research Committee of the National Health and Medical Research Council (NHMRC), provides freely available but limited information on their website [11]. GTRAP has established a gene therapy trial register. The register is held in strict confidence by the NHMRC and was not available to us.

In other countries that have regulatory bodies for gene therapy, the information is generally deemed confidential and the level of information we were able to obtain ranged from “significant” in Switzerland, to nil in France. Most other countries do not have dedicated bodies for gene therapy, which is usually the responsibility of the Ministry of Health, or National Drug Agencies. Attempts to contact these agencies have proved frustratingly unsuccessful, and most of the information on trials in these countries was obtained via personal contacts or through literature searches.

Number of trials 1989–2004

The first therapeutic human gene therapy clinical trial was approved in 1990 and involved two children suffering from a form of severe combined immunodeficiency (SCID) resulting from adenosine deaminase (ADA) deficiency [4]. From then until 1999, the number of trials initiated climbed rapidly (Figure 1). During this period, some voices expressed concern regarding the potential dangers of the procedure and critics pointed to the fact that gene therapy had proved of little therapeutic benefit thus far. In 1999, the number of trials peaked with 113 trials approved. Following severe adverse events in 1999 and 2002 [5,6] (see below), the momentum slowed as several regulatory agencies put a temporary hold on new or ongoing trials. In 2003, only 53 new trials were approved worldwide, the lowest number since 1996.

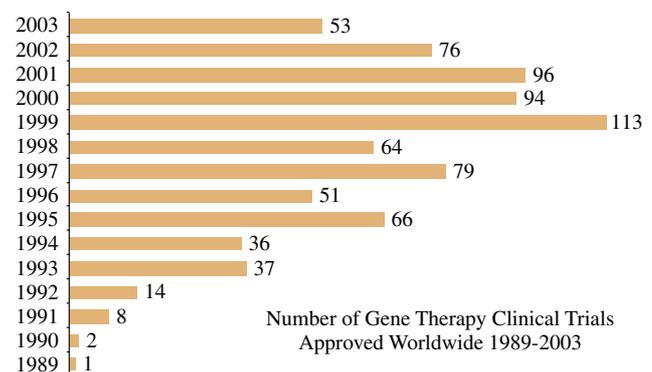


Figure 1. New trials approved by year 1989–2003

The hopes and the setbacks

In 1999, the first major adverse effect [5] was to have a dampening effect on recruitment of patients into gene therapy clinical trials. That year, 18-year-old Jesse Gelsinger, suffering from ornithine transcarbamylase (OTC) deficiency, died on September 17 while participating in a gene therapy trial. The vector used for this trial was based on human adenovirus type 5, deleted in E1 and E4, and contained human ornithine transcarbamylase (OTC) cDNA. It was infused into the right hepatic artery. The death was attributed to a totally unexpected and devastating inflammatory reaction to the adenoviral vector. In January 2000, the US Food and Drug Administration (FDA) put a hold on the trial and several other trials were also halted.

In 2000, new hopes were raised with the first report from France of successful treatment of children suffering from a rare form of X-linked severe combined immunodeficiency (SCID-X1) characterized by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the γ_c cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors [12]. The excitement gave way to alarm at the end of 2002, when two of the ten children treated developed a leukemia-like condition [6], subsequently shown to be related to retrovirus vector integration near the LMO2 proto-oncogene promoter, leading to aberrant transcription and expression of LMO2 [13].

These unexpected events fuelled debate over the future of gene therapy and led investigators and gene therapy societies in Europe and the United States to critically examine the risk/benefit ratio [14–16]. The current view can best be summarized by a joint statement of the American Society of Gene Therapy (ASGT) and the European Society of Gene Therapy (ESGT) [17] in their response to an inordinately negative article that appeared in Nature [18]. The two Societies stated: “*The field of gene therapy is working to develop new and better methods to treat a variety of severe disorders, including genetic diseases such as hemophilia and SCID, and also cancer and AIDS. The clear-cut therapeutic benefits seen in recent clinical trials of gene therapy for XSCID and ADA-deficient SCID warrant judicious consideration of the benefits and risks of this approach compared to imperfect alternatives, such as haplo-identical hematopoietic stem cell transplantation.*”

Countries participating in gene therapy trials

Gene therapy clinical trials have been performed in 24 countries, on the five continents (Figure 2). Two-thirds of all the trials performed have been carried out in the United States. Europe accounts for 27% of the total, with a small number of trials in eastern European countries. Within Europe, the United Kingdom accounts for 11% of the world total with close to 100 trials, Germany 6%,

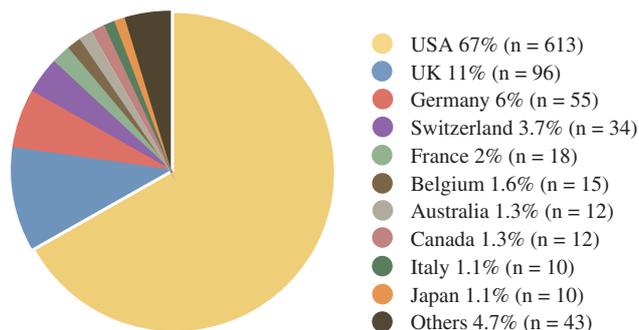


Figure 2. Geographical distribution of completed or ongoing clinical trials in gene therapy

Switzerland 3.7%, France 2% (though this last figure is known to be an underestimate due to the paucity of information available), Belgium 1.6% and Italy 1.1%. Twelve trials have been reported from Australia, 12 from Canada and 10 from Japan. The other countries where gene therapy trials have been performed are the Netherlands (6 trials); Spain, China, Finland and Poland (3 trials each); Austria, Israel, Singapore, New Zealand and Sweden (2 trials each); one trial each in the Czech Republic, Egypt, Mexico and South Korea. Eleven of the trials involve collaboration between two or more countries.

Diseases targeted for gene therapy

The aim of gene therapy is to introduce a defined DNA sequence into specific cells of a patient either to replace a defective gene, or to impart a new function to the cell in order to induce it to secrete a protein that has a putative therapeutic function. This is a vast agenda and signifies that almost any disease may, at least in theory, be a candidate for gene therapy. In practice, 85% of gene therapy clinical trials have addressed cancer, vascular disease and inherited monogenic diseases. The first two because of their enormous prevalence, impact and potentially fatal outcomes, the latter because the concept of replacing a well-defined defective gene with its correctly functioning counterpart has an obvious appeal and rationale. Table 1 and Figure 3 show the diseases in which gene therapy has been approved so far.

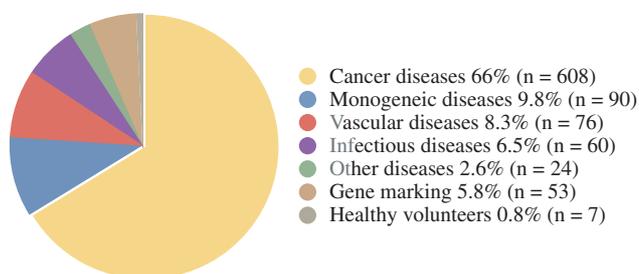


Figure 3. Clinical targets for gene therapy

Table 1. Conditions in which human gene transfer has been approved

Monogenic disorders	Cancer
Cystic fibrosis	Gynaecological: breast, ovary, cervix
SCID	Nervous system: glioblastoma, leptomeningeal carcinomatosis, glioma, astrocytoma, neuroblastoma
Haemophilia A and B	Gastro-intestinal: colon, colorectal, liver metastases, post-hepatitis liver cancer
Hurler syndrome	Genito urinary: prostate, renal
Hunter syndrome	Skin: melanoma
Huntington's chorea	Head and neck
Duchenne Muscular Dystrophy	Lung: adenocarcinoma, small cell, non small cell
Canavan disease	Mesothelioma
Chronic granulomatous disease	Haematological: leukaemia, lymphoma, multiple Myeloma
Familial hypercholesterolaemia	Sarcoma
Gaucher disease	Germ cell tumors
Fanconi's anaemia	
Purine nucleoside phosphorylase deficiency	
Ornithine transcarbamylase deficiency	
Leukocyte adherence deficiency	
Gyrate atrophy	
Fabry disease	
Amyotrophic lateral sclerosis	Other diseases
Junctional epidermolysis bullosa	Inflammatory bowel disease
	Rheumatoid arthritis
Vascular disease	Chronic renal disease
Peripheral arterial disease	Carpal tunnel syndrome
Coronary heart disease	Alzheimer's disease
Venous ulcers	Fractures
Vascular complications of diabetes	Diabetic neuropathy
	Parkinson's disease
Infectious disease	Erectile dysfunction
HIV/AIDS	Superficial corneal opacity
Tetanus	Retinitis pigmentosa
CMV infection	Glaucoma
Adenovirus infection	

Inherited monogenic diseases

The replacement of a defective gene with its functioning counterpart in order to restore normal function and thereby cure the patient is the most evident application of gene therapy. The ultimate aim is the correction of the disorder by the stable transfer of the gene into dividing cells (stem cells) to ensure the permanence of the correction. We have identified 90 trials for inherited monogenic disorders, one-third of which were performed in cystic fibrosis, the most common inherited genetic disease in Europe and the United States. The average life expectancy of patients with cystic fibrosis is less than 40 years, hence the interest in this disease as a prime target for gene therapy.

The second most common inherited group of diseases targeted has been the severe combined immunodeficiency syndromes, representing about 20% of the trials for monogenic diseases. This is the only group of diseases in which gene therapy has shown any lasting and clinically meaningful therapeutic benefit [12,19].

Twenty or so other monogenic diseases have been treated (Table 1) and most of the trials have shown transient expression of the gene transferred with detectable protein in some cases, but no obvious therapeutic benefit.

Cancer

Thus far, most of the clinical trials in gene therapy have been aimed at the treatment of various types of

cancer (66% of all gene therapy trials). Several different strategies have been used in an array of different tumor types.

Inserting tumor suppressor genes

Many tumors lack functional tumor suppressor genes. These genes code for proteins that can arrest tumor growth, and promote apoptosis of the tumor cells. The tumor suppressor gene most frequently tested in gene therapy trials has been the gene coding for p53. The p53 protein is able to arrest the cell cycle following DNA damage and is also involved in apoptosis. Efficient delivery and expression of the wild-type p53 gene has been shown to cause regression of established human tumors, prevent the growth of human cancer cells in culture or render malignant cells from human biopsies non-tumorigenic in nude mice. Some clinical trials using the p53 gene have been combined with standard therapeutic modalities such as chemotherapy and radiotherapy.

Immunotherapy

The basic precept underlying immunotherapy of cancer is that tumors possess antigens which elicit weak humoral and/or cellular reactions in tumor-bearing hosts [20,21]. By intensifying the anti-tumor immune responses, tumors could be controlled or eradicated. Intensifying the immune response has been attempted by a number of different strategies including vaccination with tumor cells engineered to express immunostimulatory molecules, vaccination with recombinant viral vectors

encoding tumor antigens, vaccination with dendritic cells expressing tumor antigens or tumor-derived RNA, naked DNA vaccines, and intra-tumoral injection of vectors encoding cytokines or major histocompatibility molecules.

Gene-directed enzyme prodrug therapy

Gene-directed enzyme prodrug therapy (GDEPT) consists of introducing genes that encode enzymes capable of converting prodrugs to cytotoxic drugs. Non-toxic prodrugs can thus be administered in high doses with no untoward effects and converted *in situ* to the cytotoxic drug where it is needed (i.e. in the tumor and its immediate environment). This strategy consists of using gene therapy to better utilize conventional chemotherapy. Several GDEPT systems have been used in clinical trials. The most commonly used system is HSV-thymidine kinase to convert the non-toxic prodrug ganciclovir into the cytotoxic triphosphate ganciclovir.

Cardiovascular gene therapy

The third major group of diseases targeted by gene therapy has been the group of cardiovascular diseases. The expectation is that gene therapy will provide a new avenue for therapeutic angiogenesis, myocardial protection, regeneration and repair, prevention of restenosis following angioplasty, prevention of bypass graft failure, and risk-factor management.

The vast majority of cardiovascular gene therapy trials to date (67 out of a total of 76) have addressed therapeutic angiogenesis, i.e. the stimulation of new blood vessel formation from existing vessels to increase blood flow to ischemic regions. Two dominant categories of ischemic diseases have been tested in approximately equal numbers, namely myocardial ischemia due to coronary artery disease and lower limb ischemia due to peripheral artery disease. The underlying principle is to increase the local concentration of growth factors by introducing genes that code for these factors. Two families of genes have been widely studied: the fibroblast growth factor (FGF) family and the vascular endothelial growth factor (VEGF) family. A small number of trials have used platelet-derived growth factor (PDGF) to treat foot ulcers resulting from the microvascular disease of diabetes.

Other indications

Sixty trials (6.6% of the total) have been performed for infectious diseases. HIV infection is the major target in this category, but trials aimed at tetanus, CMV infection, and adenovirus infection have also been conducted. A small number of essentially phase I trials have addressed various diseases including inflammatory bowel disease, rheumatoid arthritis, chronic renal disease, carpal tunnel syndrome, Alzheimer's disease, fractures, diabetic neuropathy, Parkinson's disease, superficial corneal opacity, retinitis pigmentosa and glaucoma.

Clinical trial phases

Figure 4 illustrates that the vast majority of gene therapy clinical trials performed thus far are still phase I or I/II. The two categories combined represent 84% of all gene therapy trials; 13% are phase II trials; and phase II/III and III trials represent only 2.6%.

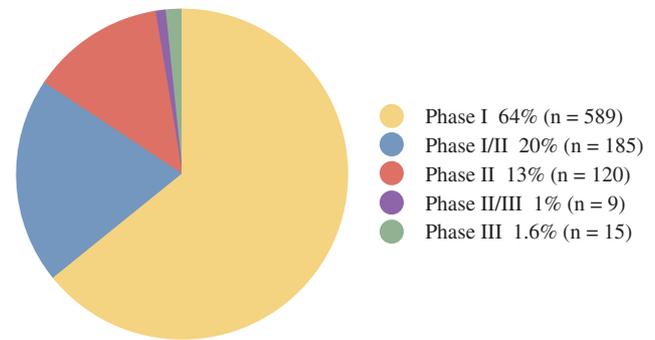


Figure 4. Gene therapy clinical trials according to phase

Genes transferred into humans

Over 100 different genes have been introduced into cells in human gene therapy trials. It is impossible here to discuss these in detail. Around 60% of the genes transferred are either cytokine genes, genes coding for antigens to modify target cells, tumor suppressor genes or suicide genes. Deficiency genes make up 7.8% of the genes, drug resistance genes 6.1%; 3.4% of genes were genes coding for receptors, whereas 2.9% coded for replication inhibitors. Figure 5 summarizes the gene types used.

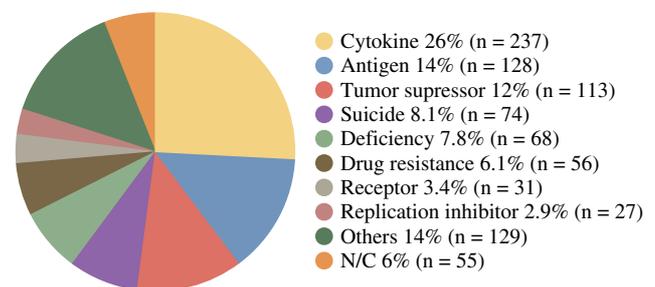


Figure 5. Distribution of gene therapy clinical trials by gene. N/C = not communicated

Vectors used in gene therapy

Viral vectors

Viral vectors have been used in about 70% of the trials performed to date (Figure 6).

Retroviral vectors

Retroviral vectors derived from murine retroviruses were the first vectors used in gene therapy and remain the

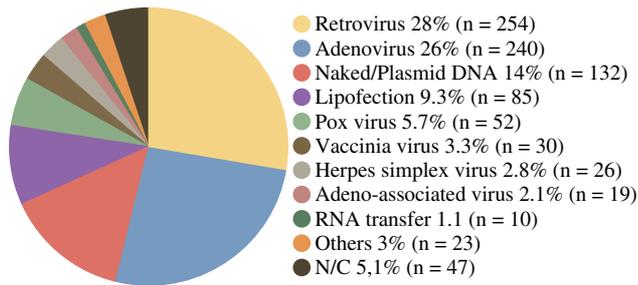


Figure 6. Gene therapy vectors used in clinical trials. N/C = not communicated

most commonly used vector today (28%). They have a relatively limited size capacity to carry therapeutic genes. They target dividing cells with a high degree of efficiency. They also lead to stable gene transfer as they integrate into the chromosomes of the target cell. The main drawback of the use of retroviruses is related to this latter property. Indeed, in the French SCID trial (see above), it has now been shown that the previously hypothetical risk of insertional mutagenesis due to the random integration of the retroviral genome into the host genome is a real risk.

Adenoviruses

Adenoviruses are the second most commonly used vector (26% of all trials). There are 42 serotypes of adenovirus known to infect humans. The ones used in gene therapy are typically based on serotype 5, with the majority of the E1a and E1b regions deleted to prevent virus replication. Adenoviruses can carry a larger DNA load than retroviruses but their capacity is still too small to accommodate the genes for all clinical applications one would like to test. The main advantages of adenoviral vectors are their high efficiency of transduction and level of gene expression, though this is transient and declines fairly rapidly. They also have the advantage of being able to infect non-dividing cells.

There are also important safety issues regarding adenoviral vectors, the main one being the possibility of provoking a severe immune and inflammatory response, as was tragically exemplified in the case of a death in a trial for OTC deficiency in 1999 (see above).

Other viruses have been less widely used and include pox viruses (5.7% of trials), vaccinia viruses (3.3%), herpes simplex viruses (2.8%) and adeno-associated viruses (2.1%).

Non-viral vectors

The limitations of viral vectors, in particular their relatively small capacity for therapeutic DNA, and safety concerns have prompted the development of synthetic vectors not based on viral systems.

The simplest non-viral gene delivery system uses 'naked' DNA, which when injected directly into certain tissues,

particularly muscle, produces significant levels of gene expression, though lower than those achieved with viral vectors. The ease of this system has made it the most popular non-viral system used in clinical trials (14% of all trials), followed by lipofection (which involves cationic lipid/DNA complexes) used in 9.3% of all trials.

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