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REVIEW ARTICLE

Gene therapy clinical trials worldwide to 2007 – an update

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Summary

To date, over 1340 gene therapy clinical trials have been completed, are ongoing or have been approved worldwide. In 1997 we set up a database to bring together global information on gene therapy clinical trials as comprehensively as possible. The data are compiled and regularly updated from official agency sources, published literature, conference presentations and posters and from information kindly provided by investigators or trial sponsors themselves.

This review updates our descriptive overview of the data in 2004 [1], presenting our analysis of the clinical trials that, to the best of our knowledge, have been or are being performed worldwide. As of July 30 2007, we have stored entries on 1309 trials in 28 countries. We have analyzed the geographical distribution of trials, the disease indications (or other reasons) for trials, the proportions to which different vector types are used, and which genes have been transferred. Details of the analyses presented, and our interactive, searchable database can be found on The Journal of Gene Medicine Gene Therapy Clinical Trials Worldwide website at: http://www.wiley.co.uk/genmed/clinical.

Introduction

Since the first human gene therapy trial in 1989 by Rosenberg *et al*. [2], over 1340 clinical trials have been completed, are ongoing or have been approved in 28 countries, using over 100 genes. Since our last review was published in 2004 [1], over 400 new gene therapy clinical trials have been initiated or published and we have obtained data on trials in four new countries.

As of July 2007, we report 1309 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 should be noted that it is not our purpose here to make judgments regarding the ethical or scientific merits or shortcomings of these trials.

Progress: moving on from one step forward, two steps back?

While gene therapy holds much promise, there have been a few unfortunate cases of serious adverse events, and these have had an inevitable effect on progress in this area. The first major adverse event of a gene therapy treatment, the death of 18-year-old Jesse Gelsinger, who suffered from ornithine transcarbamylase (OTC) deficiency, occurred on September 17, 1999 [3]. The death was attributed to a totally unexpected and devastating inflammatory

reaction to the adenovirus-based 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 its final judgment on the case in February 2005 [4] the US Department of Justice held the University of Pennsylvania (where the trial was conducted) responsible, ordered them to pay a $517 000 settlement, and placed research restrictions on the doctors who conducted the trials. This was widely seen as a major blow for the gene therapy community.

In 2000 the morale of the community was lifted 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 [5]. The excitement gave way to alarm at the end of 2002, when two of the ten children (who had both been treated with high levels of cells) developed a leukemia-like clonal lymphocyte proliferation [6] subsequently shown to be related to retrovirus vector integration near the LMO2 proto-oncogene promoter, leading to aberrant transcription and expression of LMO2 [7]. The trial was voluntarily halted while the cause of these conditions was investigated and the patients were treated for them, and the French government and the US FDA halted similar trials. An ongoing UK trial using a similar approach but with a different protocol to treat SCID-X1 was allowed to continue and went on to achieve successful results [8], as did the treatment of a SCID-X1 child in a similar trial in Australia [9]. The French trial was restarted with a revised protocol using lower doses of modified cells. In January 2005 a third child developed a proliferative condition, involving a different oncogene; this patient and one of the earlier two patients responded well to chemotherapy treatment and are in complete remission. Sadly, one of the three affected patients did not respond to the chemotherapy treatment for the leukemia-like condition and died in October 2004; however, all of the other patients in the French trial have benefited from the gene therapy, with correction of their immunodeficiency extending beyond 6 years for the first patients treated.

Since then, the UK group has gone on to achieve successful reconstitution of immune function in a child with the adenosine deaminase (ADA)-deficient form of severe combined immunodeficiency (ADA-SCID) [10].

The first two serious adverse events seen in the French trial fuelled a global debate over the future of gene therapy and led investigators and gene therapy societies in Europe and the USA to critically examine the risk/benefit ratio of gene therapy, and particularly of using retroviral vectors [11–13]. The inordinately negative media response was typified by an article that appeared in Nature [14], which prompted a joint letter from the presidents of the American Society of Gene Therapy (ASGT) and the European Society of Gene Therapy (ESGT) to the editors of that journal [15]. In response to the adverse events, the UK Gene Therapy Advisory Committee opened a discussion on the use of retroviral vectors, inviting comments from the community on the use of these vectors and the potential of self-inactivating (SIN) vectors as an alternative form of retroviral vector [16]. The general feeling of the community was voiced in a commentary from the ESGT [17], which concludes that there is no evidence as yet that SIN vectors will show an improved safety profile and that conventional gamma-retroviral vectors should continue to be used following a thorough case-by-case risk-benefit analysis.

In April 2006, a report on a Swiss-German phase I/II gene therapy clinical trial aimed at treating chronic granulomatous disease (CGD), an inherited primary immunodeficiency that affects phagocytes, brought new hope of success [18]. In this trial, following non-myeloablative pre-conditioning by busulfan treatment, retrovirally modified CD34-positive cells from the mobilized peripheral blood are infused into the patient. At the time of the report, two of the three patients showed clear benefit from this treatment, as evidenced by a lack of infections and improved quality of life, for several months to 2 years. Sadly, in April 2006 (2 years after treatment), one of these two patients died from a severe bacterial infection [19]. It appears that the patient's death was not due to a side effect of the gene therapy treatment, rather to a return of the CGD symptoms over time. The team behind the trial is currently investigating the reason for the loss of correction of the disease, which appears to be due to a loss of expression of the delivered gene, rather than to a loss of cells carrying the delivered gene.

Much excitement was caused by the report of successful immunotherapy treatment of two patients with metastatic melanoma in September 2006 [20]. The Rosenberg group was able to engineer tumor recognition in autologous lymphocytes from peripheral blood using a retrovirus encoding a T cell receptor. High, sustained levels of circulating engineered cells were retained in two of the patients up to 1 year after infusion, resulting in regression of their metastatic melanoma lesions; a dramatic improvement for patients who had only been expected to live for 3 to 6 months. Although stable engraftment of the transduced cells was seen for at least 2 months after infusion in 15 other patients, they did not respond to the treatment. It seems that it is critical to obtain an effective tumor-infiltrating lymphocyte population for the treatment to be successful, and further work is underway to improve response rates and refine the approach.

In October 2006, there were two promising announcements of success in treating Parkinson's disease by gene therapy in phase I trials in quick succession. On October 16 Ceregene Inc., and Rush University Medical Center announced that CERE-120, a gene therapy product they are developing for Parkinson's disease, was well tolerated and appeared to reduce symptoms by approximately 40% in a phase 1 study with 12 patients with advanced disease [21]. In the trial, an adeno-associated virus vector carrying the gene for neurturin (NTN) was deposited into the putamen of each patient. The company are planning

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a phase 2 randomized controlled trial involving approximately 50 patients and the Michael J. Fox Foundation for Parkinson's Research is donating $1.9 million for the follow-up study.

On October 17, Matthew J. During of New York-Presbyterian Hospital and Cornell Weill Medical Center reported successful results from a phase I gene therapy trial for Parkinson's disease to the 36th Annual Meeting of the Society for Neuroscience, held in Atlanta [22]. In the trial 12 patients had an adeno-associated virus vector encoding the glutamic acid decarboxylase (GAD) gene infused into the subthalamic nucleus on one side of their brains. At the time of the announcement, 3 years after treatment, the first patient was dramatically improved, and 9 of the other patients showed on average 37% improvement. Neurologix Inc., the company developing this therapy, was co-founded by During and Michael G. Kaplitt; they are now working towards a phase 2 randomized controlled trial.

Going beyond the stage of clinical trials, the first gene therapy product, Gendicine, an adenovirus vector carrying the p53 gene as a treatment for cancer developed by SiBiono GeneTech Co. of Shenzhen, China, was approved for clinical use by the Chinese State Food and Drug Administration in October 2003. This was followed by a license for its commercial production in spring 2004; however, this went ahead without data from a standard phase III trial, and it seems that the approval was made on the basis of tumor shrinkage, rather than extension of patient lifetime. There has been quite some concern from gene therapy researchers elsewhere in the world as to the quality of the trials performed and thereby the safety and efficacy of the treatment. To date, the phase I and phase II trial results in patients with head and neck squamous cancers have only been published in Chinese language medical journals; the only English language article on the trials is a review summarizing the data [23], the value of which has since been questioned by several groups since it appears to omit significant information when compared to the translations that they have had made of the Chinese language manuscripts [24]. Despite these concerns, some patients have flown to China to try it, and the company stated in December 2006 that they had treated more than 4000 patients with Gendicine [24].

So, at the present time, the field is certainly making good progress, with promising results in a range of diseases; no further serious adverse events; and a sense that the tide of public opinion is turning. However, it is to be hoped that all gene therapy researchers and investors around the globe have taken on board the lessons that must be learned from the unfortunate cases discussed above.

Sources of data

The data reported in this article have been compiled as much as possible from information provided by regulatory agencies. Where this is not possible, data is 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 USA to varying degrees of confidentiality in European and Asian countries. In the USA the NIH Recombinant DNA Advisory Committee (RAC) [25] compiles a database of all ongoing or completed gene therapy clinical trials and is our primary source of information for trials performed there. In the UK, 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 [26] and kindly provide us with further information that 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 [27]. In Germany, the Zentrum Klinische Studien at the University Hospital in Freiburg (funded by the German Ministry of Education and Research) has set up an interactive database of trials being conducted in Germany (DeReG) [28] in cooperation with the Commission of Somatic Genetransfer (KSG) and the German Society for Gene Therapy (DG-GT). 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 [29]. The GTRAP has established a gene therapy trial register, but it is held in strict confidence by the NHMRC and is not available to us. In Switzerland, the Swiss Expert Committee for Biosafety maintains a Swiss gene therapy clinical trial register, albeit with limited information [30]. In China, the State Food and Drug Administration (SFDA) maintains a clinical trial database not specific to gene therapy, which is available only in Chinese. Although we have managed to identify gene therapy trials from the database [31], we are still in the process of translating information and more data about Chinese gene therapy trials should be available in the forthcoming updates of our database. The Dutch Ministry of Housing, Spatial Planning and the Environment's (VROM) office for licensing of genetically modified organisms (GMOs) maintain a database in Dutch [32], from which we are in the process of gathering and translating information to incorporate into our website.

The European Agency for the Evaluation of Medicinal Products (EMEA) is compiling a database of European Community clinical trials (EudraCT) [33], which includes gene therapy clinical trials. It contains trial sponsor-submitted data on all trials initiated in the EU from 1 May 2004 onwards but unfortunately the data is confidential.

In other countries that have regulatory bodies for gene therapy, the information is generally deemed confidential and the level of information we are able to obtain is variable. Countries such as France, Canada and Norway

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all keep their data confidential, but we have been given limited information on the Canadian trials. 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 is obtained via personal contacts or through literature searches.

Number of trials 1989–2007

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 [34]. 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 116 trials approved. Following the serious adverse events in 1999 and 2002 [3,6] the momentum slowed as several regulatory agencies put a temporary hold on new or ongoing trials. In 2003, only 81 new trials were approved worldwide, the lowest number since 1998. Negative press coverage has undoubtedly dampened the enthusiasm for gene therapy and restriction from regulatory bodies while the adverse events were investigated will have slowed the growth in numbers of trials approved. However, recent successes are encouraging, and the number of trials per year has increased in 2004 (95), 2005 (98) and 2006 (97).

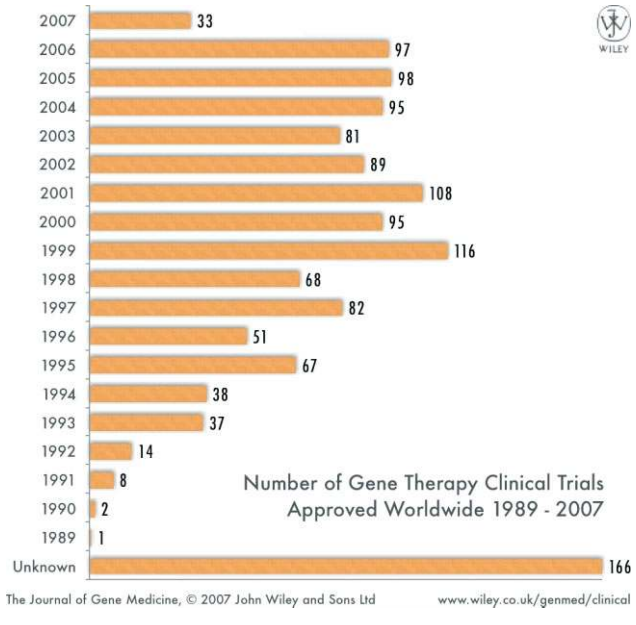

Figure 1. New trials approved by year 1989–2007

Countries participating in gene therapy trials

Gene therapy clinical trials have been performed in 28 countries, with representatives from all five continents (Figure 2). Data on trials from four new countries have been added since our last review, these being Norway, Denmark, Russia and Taiwan.

On the whole, the distribution of trials has not changed significantly since 2004. The USA accounts for 64.2% of trials, slightly less than 3 years ago (67%). Europe as a whole represents 26.6% of trials (27% in 2004) and Asia 2.7% of all trials.

Within Europe, the UK accounts for 11.1% of the world total with 150 trials, Germany 5.5% (74 trials), Switzerland 3.1% (42 trials), France 1.5% (20 trials) (though we know that this last figure is an underestimate due to the paucity of information available), Belgium 1.4% (19 trials) and Italy 1.1% (15 trials). Eastern Europe is starting to have an impact on the gene therapy community with six trials in Poland (3 in 2004), one in the Czech Republic (unchanged) and one in Russia (previously 0). We know that there are more trials taking place in Russia but we have been unable to obtain further data.

Seventeen trials have been reported from Australia, 16 from Japan and 54 from Canada. It should be noted that we have not been given complete information on all of the Canadian trials to date, so only 17 of the 54 are presently represented by an entry in our database, such that we presently hold entries on 1309 of the 1346 trials known to us.

The other countries where gene therapy trials have been performed are The Netherlands (13 trials), China (8), Israel (6), Spain, Norway and South Korea (4 trials each), Finland (3), Austria, Singapore, New Zealand, Denmark and Sweden (2 trials each), and one trial each in Egypt, Mexico and Taiwan. Twelve of the trials are reported as 'multi-countries' although a large proportion of the trials initiated in one country have centers in several other countries.

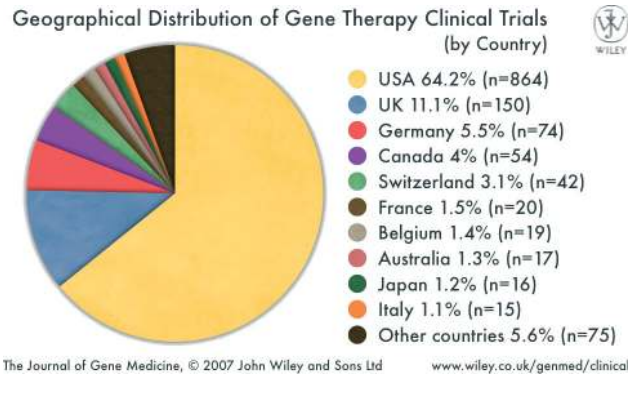

Figure 2. Geographical distribution of completed or ongoing clinical trials in gene therapy

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Diseases targeted by gene therapy

The vast majority (83.9%) of gene therapy clinical trials to date have addressed cancer, cardiovascular disease and inherited monogenic diseases (Figure 3); 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. Interestingly, trials targeting cardiovascular disease have outnumbered trials for monogenic disease since 2004, although the greatest successes of gene therapy to date have been achieved in the latter group. As Table 1 shows, the range of indications for which gene therapy trials have been approved so far has widened since our last review.

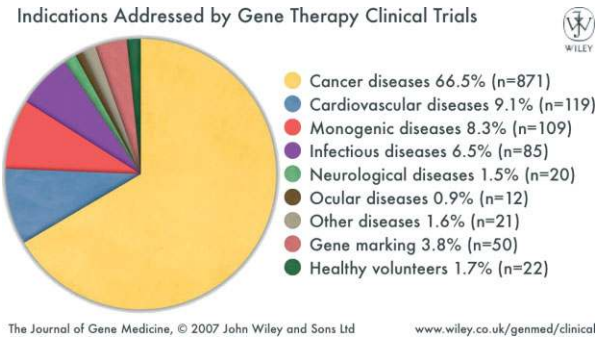

Figure 3. Clinical targets for gene therapy

Cancer

Thus far, most of the clinical trials in gene therapy have been aimed at the treatment of cancer (66.5% of all gene

Table 1. Conditions for which human gene transfer trials have been approved

Monogenic disorders
Cystic fibrosis
Severe combined immunodeficiency (SCID)
Alpha-1 antitrypsin deficiency
Haemophilia A and B
Hurler syndrome
Hunter syndrome
Huntington's chorea
Duchenne muscular dystrophy
Becker muscular dystrophy
Canavan disease
Chronic granulomatous disease (CGD)
Familial hypercholesterolaemia
Gaucher disease
Fanconi's anaemia
Purine nucleoside phosphorylase deficiency
Ornithine transcarbamylase deficiency
Leukocyte adherence deficiency
Gyrate atrophy
Fabry disease
Familial amyotrophic lateral sclerosis
Junctional epidermolysis bullosa
Wiskott-Aldrich syndrome
Lipoprotein lipase deficiency
Late infantile neuronal ceroid lipofuscinosis
RPE65 mutation (retinal disease)
Mucopolysaccharidosis

Cardiovascular disease
Peripheral vascular disease
Intermittent claudication
Critical limb ischaemia
Myocardial ischaemia
Coronary artery stenosis
Stable and unstable angina
Venous ulcers
Vascular complications of diabetes
Pulmonary hypertension
Heart failure

Infectious disease
HIV/AIDS
Tetanus
Epstein-Barr virus
Cytomegalovirus infection
Adenovirus infection
Japanese encephalitis
Hepatitis C
Hepatitis B
Influenza

Cancer
Gynaecological
- breast, ovary, cervix
Nervous system
- glioblastoma, leptomeningeal carcinomatosis, glioma, astrocytoma, neuroblastoma
Gastrointestinal
- colon, colorectal, liver metastases, post-hepatitis liver cancer, pancreas
Genitourinary
- prostate, renal
Skin
- melanoma
Head and neck
- nasopharyngeal carcinoma
Lung
- adenocarcinoma, small cell, non small cell
Mesothelioma
Haematological
- leukaemia, lymphoma, multiple myeloma
Sarcoma
Germ cell

Neurological diseases
Alzheimer's disease
Carpal tunnel syndrome
Cubital tunnel syndrome
Diabetic neuropathy
Epilepsy
Multiple sclerosis
Myasthenia gravis
Parkinson's disease
Peripheral neuropathy

Ocular diseases
Age-related macular degeneration
Diabetic macular edema
Glaucoma
Retinitis pigmentosa
Superficial corneal opacity

Other diseases
Inflammatory bowel disease
Rheumatoid arthritis
Chronic renal disease
Fractures
Erectile disfunction
Anaemia of end stage renal disease
Parotid salivary hypofunction
Type I diabetes
Detrusor overactivity
Graft versus host disease

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therapy trials). Many different cancers have been targeted throughout the years, including lung, gynaecological, skin, urological, neurological and gastrointestinal tumors, as well as haematological malignancies and paediatric tumors. A range of different strategies has been applied to cancer gene therapy, from inserting tumor-suppressor genes, to immunotherapy, to gene-directed enzyme pro-drug therapy (GDEPT).

Efficient delivery and expression of the wild-type p53 tumor-suppressor gene has been shown to cause regression of established human tumors, to prevent the growth of human cancer cells in culture, or to 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 of cancer aims to control or eradicate tumors by intensifying the normally weak humoral and/or cellular reactions to tumor antigens in tumor-bearing hosts. A number of different strategies have been employed, 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.

The GDEPT approach consists of the targeted introduction or expression of genes that encode enzymes (often termed 'suicide genes') capable of converting pro-drugs into cytotoxic drugs. Non-toxic prodrugs can thus be administered in high doses with no untoward effects and converted *in situ* into the cytotoxic drug where needed (i.e. in the tumor and its immediate environment). This strategy enables better utilization of conventional chemotherapy. Most commonly, herpes simplex virus (HSV) thymidine kinase is used to convert the non-toxic pro-drug ganciclovir into the cytotoxic triphosphate ganciclovir.

Cardiovascular gene therapy

Cardiovascular gene therapy has grown from 8.3% of all trials in 2004 to 9.1% in 2007, becoming the second most popular application for gene therapy. 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 have addressed therapeutic angiogenesis 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 fibroblast growth factor (FGF) family and the vascular endothelial growth factor (VEGF) family have been widely applied, and a small number of trials have used platelet-derived growth factor (PDGF) to treat foot ulcers resulting from the microvascular disease of diabetes. The induction of hypoxia-inducible factor (HIF) as a trigger to stimulate angiogenesis has been used in eleven trials.

Inherited monogenic diseases

The ultimate aim in treating monogenic diseases by gene therapy is the correction of the disorder by the stable transfer of the functioning gene into dividing cells (stem cells) to ensure the permanence of the correction. We have identified 109 trials for inherited monogenic disorders, one-third of which targeted cystic fibrosis, the most common inherited genetic disease in Europe and the USA. 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 group of inherited diseases targeted has been the severe combined immunodeficiency syndromes, representing about 20% of the trials for monogenic diseases. This is a group of diseases in which gene therapy has shown lasting and clinically meaningful therapeutic benefit [5,8–10]. Another monogenic immunodeficiency, chronic granulomatous disease, has also been the target of a successful trial [18].

Around 20 other monogenic diseases have been treated (see Table 1) and most of the trials have shown transient expression of the gene transferred, with detectable protein in some cases, but as yet no obvious therapeutic benefit.

Other indications

A total of 85 trials (6.5% of the total) have been performed for infectious diseases. Human immunodeficiency virus (HIV) infection is the major target in this category but trials aimed at tetanus, cytomegalovirus (CMV) infection, and adenovirus infection have been conducted.

Neurological diseases have also been targeted by gene therapy, with 20 registered phase I and II trials aimed at a variety of diseases such as multiple sclerosis, myasthenia gravis, neurological complications of diabetes, Alzheimer's disease, and recently Parkinson's disease.

Ocular pathologies have also been tackled, with 12 trials to date focused on conditions including retinitis pigmentosa, glaucoma and age-related macular degeneration.

A small number of essentially phase I trials have addressed various other diseases including inflammatory bowel disease, rheumatoid arthritis, chronic renal disease, and fractures.

Genes transferred into humans

Over 220 different genes have been introduced into cells in human gene therapy trials; Figure 4 summarizes the

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main gene types used. It is impossible to discuss each gene in detail here, but, as would be expected, the gene types transferred most frequently match the most common group of diseases treated: in around 60% of the trials the genes transferred are either genes coding for antigens used to stimulate an immune response, cytokine genes, tumor-suppressor genes or suicide genes, all of which are primarily used to combat cancer. Growth factors were transferred in 8.2% of trials, almost all of these being aimed at cardiovascular diseases. Deficiency genes were used in 7.9% of the trials, and genes for receptors (most commonly used for cancer gene therapy) in 5.1%. Marker genes were transferred in 4.1% of trials, whereas 3.7% of trials used replication inhibitors, to target HIV infection.

In 2.1% of trials oncolytic viruses were transferred (rather than genes), these being aimed at destroying cancer cells, and 1.7% of trials involved the transfer of antisense or short interfering RNA, with the aim of blocking the expression of a chosen gene.

Vectors used in gene therapy

Figure 5 shows the proportionate application of different vectors/delivery techniques in gene therapy trials to date. Although non-viral approaches have become more common, viral vectors remain by far the most popular approach, having been used in about two-thirds of the trials performed to date.

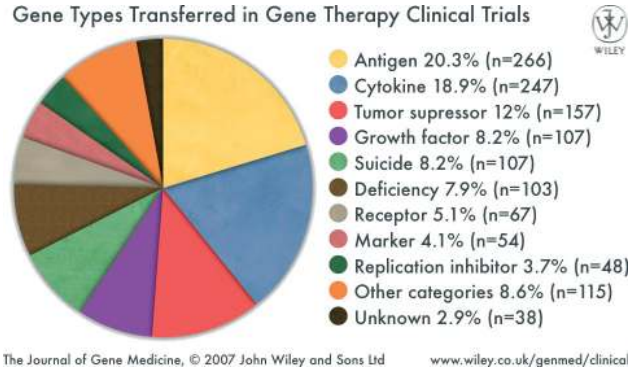

Figure 4. Genes transferred in gene therapy clinical trials

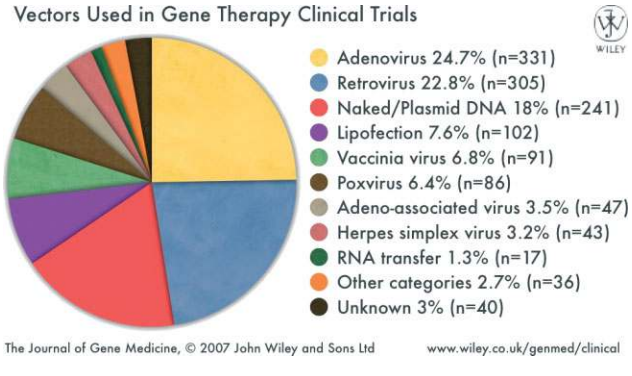

Figure 5. Gene therapy vectors used in clinical trials

Viral vectors

One of the consequences of the serious adverse events in the French SCID trial is a decrease in the proportion of trials using retroviruses. Retroviral vectors were the first vectors used in gene therapy but are now used in only 22.8% of the trials (28% in 2004). They target dividing cells with a high degree of efficiency and provide 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. Research prompted by the serious adverse events in the French SCID trial has shown that the insertion pattern of these viruses is not random, and their preference for the first introns of genes and transcriptional start sites is a cause for concern [35].

Self inactivating (SIN) retroviral vectors, also called Q vectors, are engineered so that transcription of the target gene can only be driven by an internal promoter once the expression cassette is integrated into the genome. The self-inactivation of the retroviral vector minimizes the risk that replication-competent retrovirus (RCR) will emerge. It also reduces the likelihood that cellular coding sequences located adjacent to the vector integration site will be aberrantly expressed, either due to the promoter activity of the 3′ long terminal repeat (LTR) or through an enhancer effect. These vectors should therefore avoid problems of incidental activation of endogenous oncogenes.

Adenoviruses are now the most commonly used vector (24.7% 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 required for certain clinical applications. The main advantages of adenoviral vectors are their high efficiency of transduction and high 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, however, 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 [3].

Other viruses have been less widely used and include vaccinia virus (6.8% of trials), poxvirus (6.4%), adeno-associated virus (3.5%), and herpes simplex virus (3.2%). The use of these vectors has increased significantly as alternatives to retroviruses are being explored.

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 popularity of naked DNA has increased (18% of all trials, 14% in 2004), possibly due to the concerns with regard to use of retrovirus. Naked DNA is the most popular non-viral system used in clinical trials, followed by lipofection, which involves cationic lipid/DNA complexes (used in 7.6% of all trials).

There have been 31 trials that used two vectors, 26 used poxvirus and vaccinia virus, 3 used adenovirus and retrovirus, and 2 used naked DNA and adenovirus.

Clinical trial phases

As was the case in our last review, the vast majority of gene therapy clinical trials performed to date are still phase I or I/II (Figure 6). The two categories combined represent 80.9% of all gene therapy trials; 15.7% are phase II trials, and phase II/III and III trials represent only 3.4%. There are slightly more trials in phase II, II/III and III than in 2004 (19.1% in 2007 compared to 15% in 2004) possibly indicating that gene therapy is slowly moving closer to clinical applications.

Future prospects

A new gene therapy approach that has sparked much interest is RNA interference (RNAi). A form of transcriptional inhibition initially observed in plants, RNAi was first identified as being caused by complementary double-stranded RNA (dsRNA) in nematode worms by Fire *et al.* in 1998 [36]. That the group's discovery of the mechanism behind RNAi was acknowledged by the award of the Nobel prize in Physiology and Medicine in 2006 to Andrew Fire and Craig Mello demonstrates the importance of being able to block the expression of a chosen gene using this mechanism as a technique for exploring gene function, and it is hoped, in treating disease. In humans, short interfering RNAs (siRNAs) are used to avoid the problem of long double-stranded RNA molecules inducing the interferon response in some cell types [37] and there is much ongoing work on how to deliver siRNAs and on chemical modifications to improve their stability and efficacy.

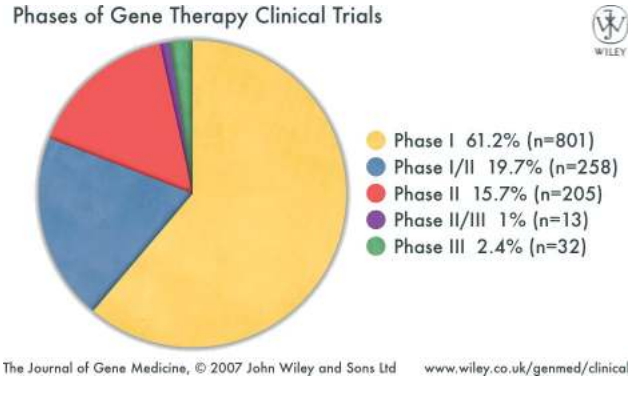

Figure 6. Gene therapy clinical trials according to phase

There is much excitement about the potential of RNAi and the technique has moved remarkably rapidly towards applications; we presently have entries on 11 siRNA trials. The pharmaceutical industry is showing quite some interest in this technology, as evidenced by the recent activity around Sirna Therapeutics, a biotechnology company developing RNAi-based therapies for diseases including age-related macular degeneration (AMD), hepatitis C, asthma and Huntington's disease. The company completed a phase I clinical trial for AMD in 2005 and is moving on to phase II trials. Sirna agreed an exclusive multi-year strategic alliance with GlaxoSmithKline in April 2006 for the development of siRNA compounds for the treatment of respiratory diseases [38], and was acquired by Merck in December 2006 [39].

Another company attracting attention from large pharmaceutical firms in this area is Mirus Bio Corporation, which has expertise in nucleic acid chemistry and delivery. Their lead product is a treatment for muscular dystrophy, which is being developed collaboratively with Transgene S.A. of Strasbourg, France. During 2006, the company was granted European and US patents for 'intravascular delivery of non-viral nucleic acid', covering administration of RNAi-inducing molecules via hydrodynamic intravascular injection [40,41]. In January 2007, the company entered into a 2-year agreement with Pfizer Inc., to investigate and optimize gene silencing methods in animal models, with the aim of targeting and suppressing the expression of genes of interest to Pfizer [42]. This interest and investment from the large pharmaceutical companies will likely serve to further increase the profile of this approach and the drive toward therapeutic products.

Another technology that has appeal in this field is site-specific genome integration, as this could avoid the problems associated with random integration, while offering stable gene transfer. In October 2005, Chen and Woo achieved site-specific genome integration of murine phenylalanine hydroxylase cDNA in the livers of phenylketonuric (PKU) mice using a bacteriophage integrase system [43]. The treatment significantly decreased the severity of the hyperphenylalaninemic phenotype in the mice, and, after three applications, stable serum phenylalanine levels within the normal range were achieved. The system uses the phiBT1 phage integrase, which catalyzes recombination between unique sequences in phage and bacterial genomes, resulting in site-specific integration. The authors identified eight pseudo-phage attachment (attP) sites in the genome of mouse 3T3 cells, all of which were located in intergenic regions. They have also recently identified several pseudo-attP sites in the intergenic regions of human 293 cells. This system represents an effective site-specific genome integration

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system in mammalian cells and should be of great use in gene therapy.

Concluding remarks

The gene therapy field has experienced intense criticism and skepticism in recent years. It should however be remembered that most commonly only severely affected patients can be enrolled in gene therapy clinical trials, and that often these patients have already tried several other treatments that have failed, or have a disease for which no alternative treatment exists. It is against this background of intractable conditions that gene therapy treatments are tested, often providing the last chance for a patient. Although there have been a small number of serious adverse events, there is a larger number of patients whose quality of life has been dramatically improved by having received gene therapy. While the unfortunate occurrence of the serious adverse events has slowed progress and dampened the ardor of some, it has also prompted others to undertake more detailed investigation into the behavior of viral vectors and more careful testing of all approaches, and we can be hopeful that as gene therapy technology is improved and refined the field will make greater steps forward.

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