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Gene Technology Based Therapies in the Brain

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Abstract

Gene therapy potentially represents one of the most important developments in modern medicine. Gene therapy, especially of cancer, has created exciting and elusive areas of therapeutic research in the past decade. In fact, the first gene therapy performed in a human was not against cancer but was performed to a 14 year old child suffering from adenosine deami-

nase (ADA) deficiency. In addition to cancer gene therapy there are many other diseases and disorders where gene therapy holds exciting and promising opportunities. These include amongst others gene therapy within the central nervous system and the cardiovascular system. Improvements of the efficiency and safety of gene therapy is the major goal of gene therapy development. After the death of Jesse Gelsinger, the first patient in whom death could be directly linked to the viral vector used for the treatment, ethical doubts were raised about the feasibility of gene therapy in humans. Therefore, the ability to direct gene transfer vectors to specific target cells is also a crucial task to be solved and will be important not only to achieve a therapeutic effect but also to limit potential adverse effects.

Keywords: Gene therapy; viral vectors; Parkinson's disease; Alzheimer's disease; brain tumours; cerebral vasospasm.

Introduction to Gene Therapy: The Past, Present and Future

Scientific understanding of the molecular basis of life increased dramatically after Oswald T. Avery's discovery in 1944 that deoxyribonucleic acid (DNA) was the "transforming principle" – the secret code of life. Then Francis Crick and James Watson described the "double helix" structure of DNA in 1953. The process, however, by which DNA replicates itself during cellular reproduction, or how DNA expresses its genetic information, was still a mystery in the late 1950s. A little less than 20 years after Oswald T. Avery's discovery, Marshall Nirenberg and his colleagues in 1962 deciphered UUU (one three-unit batch of uracil, which was a "code word" for identifying phenylalanine) as the first word in the chemical dictionary of life. Nearly 30 years after Nirenberg's breakthrough, in 1990 the first clinical study involving gene transfer was commenced (Mountain, 2000) and it contributed to the start of a whole new industrial area – biotechnology.

A four-year old girl called Ashanti de Silva became the first gene therapy patient on September 14, 1990 at the NIH Clinical Center. She had adenosine deaminase (ADA) deficiency, a genetic disease which left her defenceless against infections. White blood cells were taken from her blood, and the normal genes for making adenosine deaminase were inserted into them. Afterwards the corrected cells were re-injected back into her circulation. Unfortunately, the effects of Ashanti's gene therapy were not clearly demonstrated due to simultaneous enzyme replacement therapy with polyethylene glycol adenine deaminase (PEG-ADA), which she had to take as a back up.

Since the commencement of the first clinical trial, the field has grown rapidly. Today there are close to 1000 ongoing gene therapy clinical trials worldwide (Edelstein, 2004), most of which are targeted against cancer.

Table 1. *Most common clinical targets for gene therapy. Edelstein, 2004. Gene therapy clinical trials worldwide 1989–2004 – an overview. Copyright John Wiley & Sons Limited. Reproduced with permission*

Indications	Gene therapy clinical trials	
	Number	%
Cancer diseases	656	66,5
Monogenic diseases	93	9,4
Vascular diseases	80	8,1
Infectious diseases	65	6,6
Other diseases	29	2,9
Gene marking	52	5,3
Healthy volunteers	12	1,2
Total	987	

Table 1 lists the most common clinical targets for gene therapy (http://www.wiley.co.uk/genmed/clinical).

Unfortunately, gene therapy clinical trials experienced one drawback after another as several clinical trials failed to show efficacy (Scollay, 2001).

In September 1999, the worst case scenario for gene therapy became reality, when 18 year old Jesse Gelsinger took part in a gene therapy clinical trial at the University of Pennsylvania in Philadelphia. He suffered from a partial deficiency of ornithine transcarbamylase (OTC), a liver enzyme that is required for the removal of excessive nitrogen from amino acids and proteins. Four days after treatment, Jesse Gelsinger died because of multi-organ failure. He was the first patient in whom death could be directly linked to the viral vector used for the treatment. A little later, in April of the following year the journal Science published an article from Maria Cavazzana-Calvo *et al.* (Cavazzana-Calvo, 2000) where they reported the first definitive cure of disease by gene therapy. Three young children suffering from the fatal X-linked SCID-XI syndrome had developed a functional immune system after gene therapy treatment. After that success several more patients have been treated using the same gene therapy strategy. Some years later 2 out of 11 treated patients had developed a leukaemia-like disease obviously as a result of the use of the murine leukaemia virus (MLV) vector (Hacein-Bey-Abina, 2003). After the tragedy of Jessie Gelsinger's death the number of approved clinical trials have decreased worldwide (Fig. 1).

Nevertheless, despite these drawbacks gene therapy research and development itself has never stopped, or slowed down. As a result of that, on October the 16th 2003, China became the first country to approve the commercial production of a gene therapy. Shenzhen SiBiono GenTech (Shen-

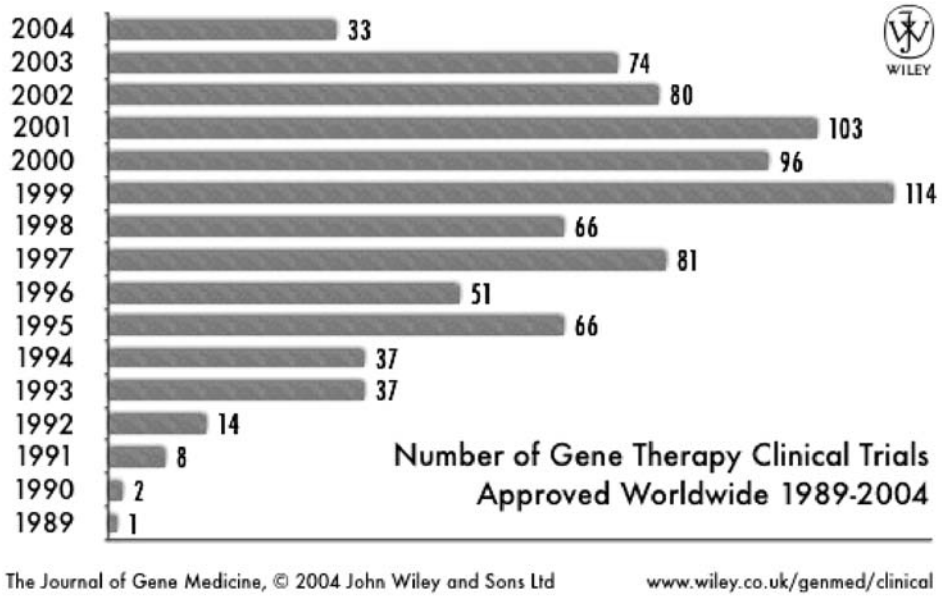

Fig. 1. Decrease in the number of approved gene therapy clinical trials after the year 1999. Edelstein, 2004. Gene therapy clinical trials worldwide 1989–2004 – an overview. Copyright John Wiley & Sons Limited. Reproduced with permission

zhen, China), obtained a drug license from the State Food and Drug Administration of China (SFDA; Beijing, China) for its recombinant Ad-p53 gene therapy (Gendicine) for head and neck squamous cell carcinoma (HNSCC). At the same time there are some very promising ongoing gene therapy clinical trials worldwide for the treatment of diseases such as tissue ischemia (Morishita, 2004), cancer (Trask, 2000, Prados, 2003, Lamont, 2000, Immonen, 2004), haemophilia A or B (Monahan, & White, 2002) and Parkinson's disease (Howard, 2003) with potential for the launching into the market. But so far the American Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale.

Regarding the variety of areas where gene therapy could be applicable, one notes that only a few are in fact directed to diseases of the central nervous system (CNS). These areas include treatment of brain tumours, e.g. glioblastoma, and degenerative conditions, e.g. Alzheimer's disease and Parkinson's disease, and ischemic brain diseases.

Potential Areas for Gene Therapy in the Brain

The "tenacious start" of gene therapy for neurological diseases is not really surprising, since gene therapy to the brain faces unique obstacles in addition to those one faces with gene therapy in general. Evaluation of the appropriate vector, route of administration, efficiency of the transgene ex-

pression, and immune response against gene transfer vectors being used are some of the problems one faces with gene therapy. In addition to those, gene therapy to the brain has to overcome obstacles such as the blood brain barrier and the limited space within the brain, which restricts the volume of gene transfer vectors that can be injected if administered locally.

Also, the lack of appropriate animal models for neurodegenerative disease such as Parkinson's disease, or Alzheimer's disease has been a problem. That again posed an obstacle that hindered gene therapy to move into the clinic. However, in recent years tremendous strides have been made in developing appropriate animal models of human neurodegenerative diseases, and along the development of these animal models the movement of gene therapy from benchside to the clinic has been justified.

Feasible areas of gene therapy in the brain include Alzheimer's disease (Mattson, 2004), ischemic brain diseases (Zadeh, & Guha, 2003), Parkinson's disease (Samii, 2004), and brain tumours (Abeloff, 2000), of which gliomas have been the subject of the largest number of gene therapy strategies (Chiocca, 2003). Also, epilepsy (Gutierrez-Delicado & Serratosa, 2004) amyotrophic lateral sclerosis (ALS), a motor neuron disease, (Weiss, 2004), lysosomal storage disease (LSD) (Futerman, & van Meer, 2004), and Huntington's disease (Hogarth, 2003) have been subject of numerous promising gene therapy strategies. For example, approaches such as the fibrinogen-galanin encoding adeno-associated viral vector gene therapy (Haberman, 2003), the gene transfer of the Neuropeptide Y (Richichi, 2004), as well as the gene transfer of the aspartoacylase (ASPA) gene (Seki, 2004, McPhee, 2005) has been used in studies against epilepsy. However, because of limited space, this review will be focusing only onto the first four diseases mentioned above. The reader is referred to the following references for more information about gene therapy in epilepsy (McCown, 2004), ALS (Boillée & Cleveland, 2004, Bruijn, 2004, Alisky & Davidson, 2000, Azzouz, 2004, Azzous, 2000, Pompl, 2003, Ascadi, 2002, Kaspar, 2003, Wang, 2002), LSD (Kaye & Sena-Esteves, 2002, Cabrera-Salazar, 2002, Eto & Ohashi, 2002), and Huntington's disease (McBride, 2003, Bemelmans, 1999, Bachoud-Levi, 2000, Bachoud-Levi, 1998, MacMillan, 1994).

Gene Therapy for Parkinson's Disease

Diseases which are commonly related to aging have received major interest worldwide. From an epidemiological perspective Parkinson's disease and Alzheimer's disease share an increasing prevalence with aging, whereas clinically they are characterized by different clinical symptoms and molecular etiology with very limited potential for cure at present (Winkler, 1998).

The pathology of Parkinson's disease reveals prominent loss of dopami-

nergic neurons, especially in the substantia nigra, usually in connection with the formation of extracellular inclusions, termed Lewy bodies. Clinical symptoms usually do not appear in adults until when about 80% of striatal dopamine and 50% of nigral neurons are lost (Samii, 2004).

Attaining focal, sustained physiologic delivery of L-Dopa or dopamine, and preventing further death of dopaminergic neurons has been the main focus of gene therapy of Parkinson's disease (Finkelstein, 2001). This has been achieved by mainly two different strategies. One strategy is to provide localized growth factors to sustain dopaminergic neurons, preventing them from undergoing apoptosis. The neuroprotective effect of growth factors has been demonstrated in several studies (Eberhardt & Schulz 2004). Among these growth factors, glial cell line-derived neurotrophic factor (GDNF) is one of the most promising candidates for gene therapy of Parkinson's disease. Studies using intracerebral injections of the recombinant GDNF protein have shown that GDNF can provide almost complete protection of nigral dopamine neurons against 6-hydroxydopamine (6-OHDA) – or MPTP-induced damage in rodents and non-human primates, promote axonal sprouting and regrowth of lesioned dopamine neurons, and stimulate dopamine turnover and function in neurons spared by the lesion (Björklund, 1997; Gash, 1998; Kordower, 2000).

Another approach to sustain physiological delivery of L-Dopa, or dopamin is replacing/supplementing critical enzymes in the dopaminergic pathway. The three most relevant enzymes for dopamin production are 1) tyrosin hydroxylase, the rate limiting enzyme in the synthesis of dopamin, 2) GTP cyclohydrase (GCH), to generate more tetrahydrobiopterin (a essential cofactor for TH) and 3) aromatic amino acid decarboxylase (AADC), an enzyme that converts L-Dopa to dopamine. It became clear, that these enzymes represent potential targets for gene therapy and therefore they have also been subjected to a lot of research (During, 1994, Lampela, 2002, Sun, 2003; Eberling, 2003; Sanchez Pernaut, 2001; Shen, 2000). Also the delivery of the gene encoding for glutamate decarboxylase (GAD) has been subjected to research (Luo, 2002). Currently, there are two ongoing gene therapy clinical trials regarding Parkinson's disease. One uses the approach of subthalamic GAD gene transfer; the other uses the approach of intrastriatal gene transfer of ADDC (www.gemcris.od.nih.gov).

Gene Therapy for Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of dementia (Palmer, 2002). Dementia is a collective name for progressive degenerative brain syndromes which affect memory, thinking, behaviour and emotion. The precise mechanisms that lead to this disease are not fully understood and many genetic, cellular and molecular irregularities are implicated. Central

to the disease, however, is the altered proteolytic processing of the amyloid precursor protein (APP) resulting in the production and aggregation of neurotoxic forms of amyloid β-peptide (Aβ) (Mattson, M. P. 2004). In addition, neuropathological examination of AD brains reveals neuronal and synaptic loss and neurofibrillary tangles. The progression of AD is slow, starting with mild memory problems and ending with severe intellectual impairment. It is the cognitive areas of the brain that are the first to be affected from this disease leading, amongst other things, to memory loss and behavioural abnormalities. It then spreads to the parts of the brain that control movement. Eventually, the loss of brain function becomes so severe that it can be the primary cause of death (Brown, 2003).

Several specific neurotransmitter systems are regularly and substantially altered in AD brains. One of the most prominent systems affected in the course of AD are the cholinergic neurons of the nucleus basalis magnocellularis (NBM) (Winkler, 1998). Several gene therapy approaches have been documented to be promising in experimental animal models. In this regard, greatest interest as a potential gene therapy approach, and also subject of two ongoing clinical trials (www.gemcris.od.nih.gov), is the use of nerve growth factor (NGF) as a neuroprotective molecule (Tuszynski, 2002, Wu, 2004, Winkler, 1998, Tuszynski, 1998). NGF has been shown to be able to prevent the death of cholinergic neurons after axotomy, and that it was also able to reverse spontaneous age-related morphological and behavioural decline in rat (Kromer, 1987, Fisher, 1987). In addition to the use of NGF for the treatment of AD, there are studies about the feasibility of neprilysin (NEP) gene transfer for the treatment of Alzheimer's disease. Marr and colleagues (Marr, 2004) demonstrated that injection of NEP expressing lentiviruses into the hippocampus of transgenic mice led to an approximate 50% reduction in the number of amyloid plaques.

Gene Therapy for Vascular Brain Diseases

There are several potentially feasible applications of gene therapy for the treatment of vascular brain diseases. One application is the prevention of vasospasm after subarachnoid hemorrhage (SAH). Another application is the stimulation of growth of collateral blood vessels in the area of ischemia, and third, stabilization of atherosclerotic plaques, inhibition of thrombosis, and prevention of restenosis after angioplasty of the carotid and posterior circulation arteries (Toyoda, 2003).

Vasospasm after SAH typically occurs slowly several days after subarachnoid hemorrhage (Dietrich, 2000), and therefore the timing for gene therapy seems feasible, since maximal expression of the transgene occurs usually a few days after gene transduction with viral vectors. In addition, the risk of vasospasm after SAH is transient (Lüders, 2000). Thus, even

with current available vectors, which provide transient transduction, gene therapy for the prevention of vasospasm after subarachnoid hemorrhage may be achievable. Gene therapy to prevent vasospasm after SAH has been done, for instance, using endothelial NOS (eNOS) gene transfer. Endothelial NOS improved NO-mediated relaxation *in vitro* after experimental SAH (Onoue, 1998), but did not demonstrate a therapeutical effect *in vivo* after intracisternal injection of adenovirus containing the gene for eNOS in dogs (Stoodley, 2000), even though an increase of cerebral blood flow could be demonstrated in rats after intracisternal injections of replication-defective adenovirus containing the gene for eNOS (Lüders, 2000). Compared to eNOS, injection of adenovirus containing the gene for human extracellular superoxide dismutase (ECSOD) into the cisterna magna 30 minutes after induction of experimental SAH, reduced cerebral vasospasm after subarachnoid hemorrhage in rabbits (Watanabe, 2003).

Preservation of cerebral circulation and prevention of cerebral infarction could be achieved by stimulation of growth of collateral blood vessels. A variety of growth factors have been reported to induce angiogenesis in different experimental animal models (Ylä-Herttuala & Martin, 2000) and have shown to be therapeutically effective, many of them being used in clinical trials of gene therapy. Growth factors that induce angiogenesis include vascular endothelial growth factors (VEGFs), basic fibroblast growth factor (bFGF) (Ylä-Herttuala & Alitalo, 2003), and hepatocyte growth factor (HGF) (Morishita, 2004). For angiogenesis in the brain, Yukawa (Yukawa, 2000) demonstrated that adenoviral gene delivery of bFGF into the cerebrospinal fluid (CSF) induced angiogenesis in the bilateral paraventricular region in rat brains.

Regarding ischemic stroke, the initial damage after stroke is not a feasible target for gene therapy. That's because the therapeutic window is (at most) only a few hours after onset of ischemic stroke. At the same time the expression of the transgene requires hours to days, depending on the vector used. For that reason gene therapy strategies can be used only for prevention of succeeding damages in the ischemic penumbra. Shimamura *et al.* (Shimamura, 2004) demonstrated that gene transfer of HGF into the brain resulted in attenuation of brain ischemic injury even if HGF was transduced 24 hours after the ischemic event. Also, the expression of genes such as Bcl-2 (Yenari, 2003), the 72-kD inducible heat shock protein (HSP72) (Hoehn, 2001), or the cyclooxygenase-1 (COX-1) (Lin, 2002) have also been demonstrated to reduce ischemic injury.

Some strategies focus on inhibition of genes that are expressed and believed to be harmful after ischemic stroke, such as the interleukin-1 receptor (Yang, 1997). In addition, genes such as interleukin-10, transforming growth factor-β1 (TGF-β1), glial cell line-derived neurotropic factor (GDNF) (Shirakura, 2004), and nerve growth factor (NGF) (Shirakura,

2004), have been targets for gene therapy (Shimamura, 2004). Fibroblastic growth factor-2 (FGF-2) has been shown to decrease brain injury after cerebral ischemia when administered systemically (Bethel, 1997). As a result to that Shigeru *et al.* developed a systemic gene therapy using macrophages infiltrating the infarct to deliver and express FGF-2 (Shigeru, *et al.* 2004).

So far none of the above mentioned strategies have reached clinical trials.

Gene Therapy for Brain Tumours

Regarding brain tumours, malignant gliomas have been the primary target for gene therapy. The average life expectancy of a patient diagnosed with glioblastoma multiforme is 10 months after diagnoses (Ammirati, 1987). Several therapeutic approaches to treat cancer are limited in their success because of the lack of specificity of the drugs used for therapy. The therapeutic index of several cytotoxic drugs is very narrow, which limits the possibility to reach effective tissue concentrations. In addition, some of the difficulties encountered include inaccessibility to resective surgery because of the anatomical location of the tumour and because of infiltration of tumour cells into surrounding tissues. For that reason gene therapy of brain tumour has been one of the most exciting and elusive areas of therapeutic research in the past decade. It potentially represents one of the most important developments in the treatment of brain tumours. However, gene delivery to brain tumours is a formidable obstacle. Transduction rates $> 5\%$ of the tumour mass are difficult to achieve (Puumalainen, 1998a), even in experimental tumours. For that reason the therapeutic effect must not be limited only to transduced cells, but it must be able to exert a therapeutic effect on neighbouring, non-transduced, cells as well (bystander effect).

One of the most studied gene therapy strategies in the treatment of malignant gliomas is the combination of thymidine kinase and Ganciclovir. This approach has also been called 'suicide' gene therapy as the non-toxic pro-drug is converted in transduced cells into a toxic molecule, which can kill tumour cells (Fecci, 2002, Puumalainen, 1998b, Smitt, 2003, Sandmair, 2000a, Sandmair, 2000b). Currently, there are 7 ongoing clinical trials regarding thymidine kinase ganciclovir therapy (www.gemcris.od.nih.gov) either alone or in combination with other gene therapy strategies. Even though promising results regarding HSV-tk/ganciclovir therapy have been obtained in human trials using adenoviral vectors (Immonen, 2004) there have been also failures regarding therapeutic efficacy of HSV-tk/ganciclovir therapy when a retroviral vector was used for gene transfer (Rainov, 2000, Shand, 1999).

In addition to thymidine kinase ganciclovir treatment there are three other well characterized pro-drug activating systems that have been used

in experimental animal models in the treatment of gliomas. These are the Escherichia coli cytosine deaminase/5-fluorocytosin (CD/5-FC) (Miller, 2002), the rat cytochrome P450 2B1/cyclophosphamide (CPA) (Manome, 1996, Ichikawa, 2001) and the Escherichia coli reductase/CB1954 system (Friedlos, 1998, Weedon, 2000, Palmer, 2004). (Connors, 1995)

Other gene therapy strategies are triggering apoptosis in tumour cells via tumour suppressor genes (such as p53, Fas, ras, TNF-α and caspases) (Shimoura & Hamada, 2003), inhibition of angiogenesis (Puduvalli, 2004, Kirsch, 2000, Tanaka, 1998), augmentation of extracellular matrix protein expression (Lakka, 2003, Mohanam, 2002), modulation of the immune system (Friese, 2003, Yamanaka, 2003, Witham, 2003, and Yang, 2004), eradication of the tumour via oncolytic viruses (Rainov & Ren, 2003, Gromeier & Wimmer, 2001, Lou, 2004), and the use of small interfering RNA (siRNA) (Uchida, 2004), ribozymes (Ge, 1995), and antisense oligonucleotides (Gondi, 2004, Datta, 2004). Also, the generation of fusion proteins that are expressed on the surface of cell membranes and capable of binding a specific ligand could be used for the treatment of brain tumours. We recently constructed and demonstrated the functionality of two different avidin-fusion proteins *in vitro* and *in vivo* using viral vector expression systems in rat brain (Lehtolainen, *et al.* 2002, and Lehtolainen, *et al.* 2003).

In vivo studies have demonstrated that avidin-fusion protein expressed in rat malignant glioma cells were capable of binding biotinylated molecules administered either locally to the brain or systemically into the right carotid artery. Systemically administered biotinylated ligands targeted with high specificity to the intracerebral tumours of rats that were expressing the fusion protein. This again could be achieved by local gene transfer of the target tissue with the fusion protein, followed by *i.v.* administration of a biotinylated drug (Lehtolainen, 2003). These results suggest, that local gene transfer of the fusion protein to target tumour may offer a novel tool for the delivery of biotinylated molecules *in vitro* and *in vivo* for therapeutic and imaging purposes, offering a possibility for an enhanced local effect and a decreased systemic exposure to toxic therapeutic compounds or the imaging agents.

Challenges of Gene Therapy in the Brain

As mentioned earlier, gene therapy to the brain faces unique difficulties in addition to the general issues one faces with gene therapy. A major limiting factor is the delivery of the gene to the brain. The brain is surrounded by the blood brain barrier (BBB), which most gene expression vectors do not naturally cross. The BBB is a capillary barrier that results from a continuous layer of endothelial cells bound together with tight junctions. The endothelial barrier excludes molecules from the brain based on electric

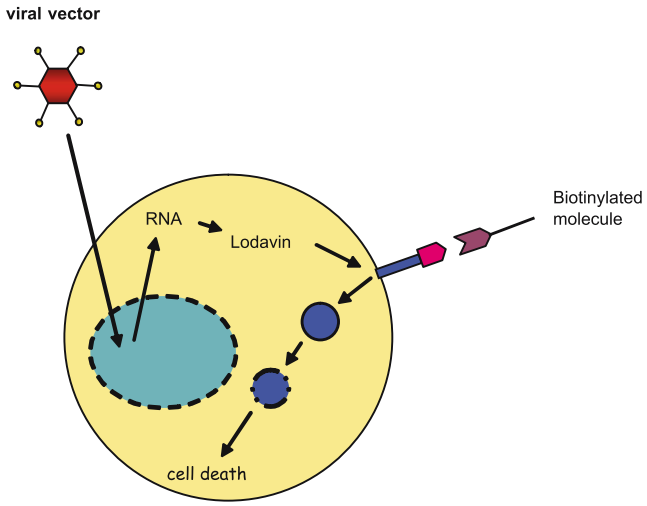

Fig. 2. The principle of biotin binding fusion proteins expressed on the cell surface of the target cell. The target cell/tissue is transduced with an appropriate gene transfer vector containing the gene for Avidin-fusion protein. The cell synthesizes the protein and transports it to the cell membrane. After binding of a biotinylated molecule the Avidin-fusion protein is endocytosed into the cytoplasm of the cell with the biotinylated molecule. The biotinylated molecule is released inside the cytoplasm and the Av-fusion protein is transported back to the cell surface, ready to bind another biotinylated molecule

charge, lipid solubility and molecular weight. Special transport systems are available at brain capillaries for glucose, amino acids, amines, purines, nucleosides, and organic acids. All other materials must cross between endothelial cells (paracellular route) or across cytoplasm (transcellular route) to move from the capillary blood into the tissue (Haluska & Anthony, 2004). Molecules of greater than >500 kDa do not pass in general the BBB. In result to that, under normal conditions large biological molecules such as antibodies and complexes such as viruses have no or very little access across the BBB (Neuwelt, *et al.* 1995, Pardridge, 2002). However, there are viruses that naturally do cross the BBB. One example of these is the Semliki Forest Virus (Fazakerley, 2004).

There are three main strategies of gene expression vector delivery into the brain that have been studied in animal models: 1) Stereotactic inoculation of the gene expression vector into the brain (Qureshi, 2000), 2) intrathecal or intraventricular administration of the gene expression vector (Shimamura, 2003), and 3) intravascular application of the gene expression vector (Rainov, 1999). Of those three methods the stereotactic inoculation

of gene expression vector by burrhole is the most commonly used strategy. So far, only the stereotactic inoculation or the craniotomy based inoculation of gene expression vector, including injection into the wall of the tumour cavity, and the intrathecal injection methods, have reached clinical trials.

The intracerebral injection of gene expression vectors is the simplest approach for local gene therapy (e.g. for gene therapy of brain tumours) and an easy way of solving the problems caused by the BBB. In addition, it has the advantage of targeting the vector mechanically into the treatment area. However, there still remain some obstacles to overcome. For example, direct intraparenchymal injections are limited by the small volumes that can be injected into focal and extracellular areas. Also, diffusion of the gene transfer vector is very low. The gene expression vectors do not significantly penetrate into brain parenchyma, which means that the transduced area may be restricted to only a few micrometers. (Puumalainen, 1998b, Rainov & Kramm, 2001, Hsich, 2002) However, a recently developed method to improve the tissue distribution of macromolecules, such as viruses, or liposomes to the brain is the bulk flow convection-enhanced infusion that maintains a pressure gradient during interstitial infusion (Bobo, 1994, Saito, 2004, Nguyen, *et al.* 2003).

The delivery of genes into the brain via the transvascular route has been attempted through BBB disruption using a intracarotid infusion of hyperosmolar solutions and vasoactive compounds. One of the earliest techniques and the first to be used in humans was the injection of a sugar solution into arteries of the neck (Neuwelt, 1980, Greg, 2002). The idea of using hyperosmolar solution is that the resulting high sugar concentration in the capillaries sucks water out of the endothelial cells, shrinking them and opening gaps between cells. The disadvantage of this approach, however, is that it requires arterial access, and the disruption of the BBB may lead to chronic neuropathological changes in the brain. Blood proteins such as albumin are toxic to the brain cells and BBB disruption allows blood components to enter the brain (Schlachetzki, *et al.* 2004). Another strategy to deliver genes through the BBB is the use of certain endogenous transport systems within the BBB. The capillary endothelium, which forms the BBB, expresses receptor-mediated transcytosis systems for certain endogenous peptides, such as insulin and transferrin, (Pardridge, 2002a+b). This strategy has been mainly used in the context of non-viral gene transfer vectors, e.g. with liposomes. Liposomes covered with peptides or antibodies that bind to a specific transcytotic receptor on the endothelium of the BBB are also often referred to as "Trojan horses". These "Trojan horses" have been mainly developed and used for cancer treatment (Pardridge, 2002a+b).

In addition to the problems related to the BBB there is a second major

impediment that remains to be overcome when using viral gene transfer vectors: the immune system. Immune-mediated vector toxicity has been reported with a broad range of viral vectors, including herpes simplex viruses (Wood, 1994, Bowers, 2003, Wakimoto, 2003), adenoviruses and adeno-associated viruses (Lowenstein & Castro, 2003, Joos & Chirmule, 2003, Sun, 2003, Byrnes, 1995, Kajiwara, 2000), and retroviruses (Rainov, 2000b). It has been shown that in animals intravascular injection of viral vectors induces the release of cytokines, interleukins, activates macrophages, induces T-cell and B-cell responses, induces viral neutralizing antibodies, and induces the activation of the endothelium (Lowenstein, 2004). The majority of immunological studies regarding viral vectors have been done with adenoviruses and HSV-1. For example, Wood *et al.* (Wood, 1994) described a strong inflammatory response, characterized by diffuse up-regulation of major histocompatibility complex class I antigens and the activation of microglia after stereotactic injection of a defective HSV-1 vector into rat brain. In general, an immune response can be generated against both, the virion and the proteins expressed by the viral genome.

The extent of inflammatory and immune response to other viral vectors such as with alphaviruses, and adeno-associated viruses injected into the brain remains to be elucidated in more detail.

Targeting of the gene transfer vectors to target cells and avoidance of the transduction of unwanted non-target cells is a general problem in gene transfer based therapies. Regarding brain gene therapy several approaches have been tackled in order to target gene transfer vectors to neuronal/cancer cells. These include amongst other things the use of a) tissue specific promoters such as the human PDGF-beta, the neuron specific enolase or the glial fibrillary acidic protein promoter (Liu, 2004, Jakobsson, 2003), b) antibody based targeting (e.g. liposomes) or re-targeting (e.g. adenovirus) of the gene transfer vectors (Zhang, 2004, Miller, 1998), or c) conditionally replicating viruses (Gomez-Manzano, 2004, Markert, 2000). Figure 3 gives an example of how adenoviruses for example can be modified in order to make them cell or tissue type specific.

Gene Transfer Vectors

There are two main types of gene delivery vectors: viral and non-viral vectors. Retroviruses/lentiviruses, recombinant herpes simplex virus, adenoviruses, and adeno-associated viruses are the most common viral vectors that have been used for the delivery of genes into the CNS. More recently, there have been studies also about the possible use of Baculoviruses (Lehtolainen, 2002, Tani, 2003) Semliki Forest viruses (SFV) (Lundstrom, 2001), Sindbis virus (Ehrengruber, 2002) and recombinant Simian virus-40 (SV40) (Cordelier, 2003) for gene therapy in the brain.

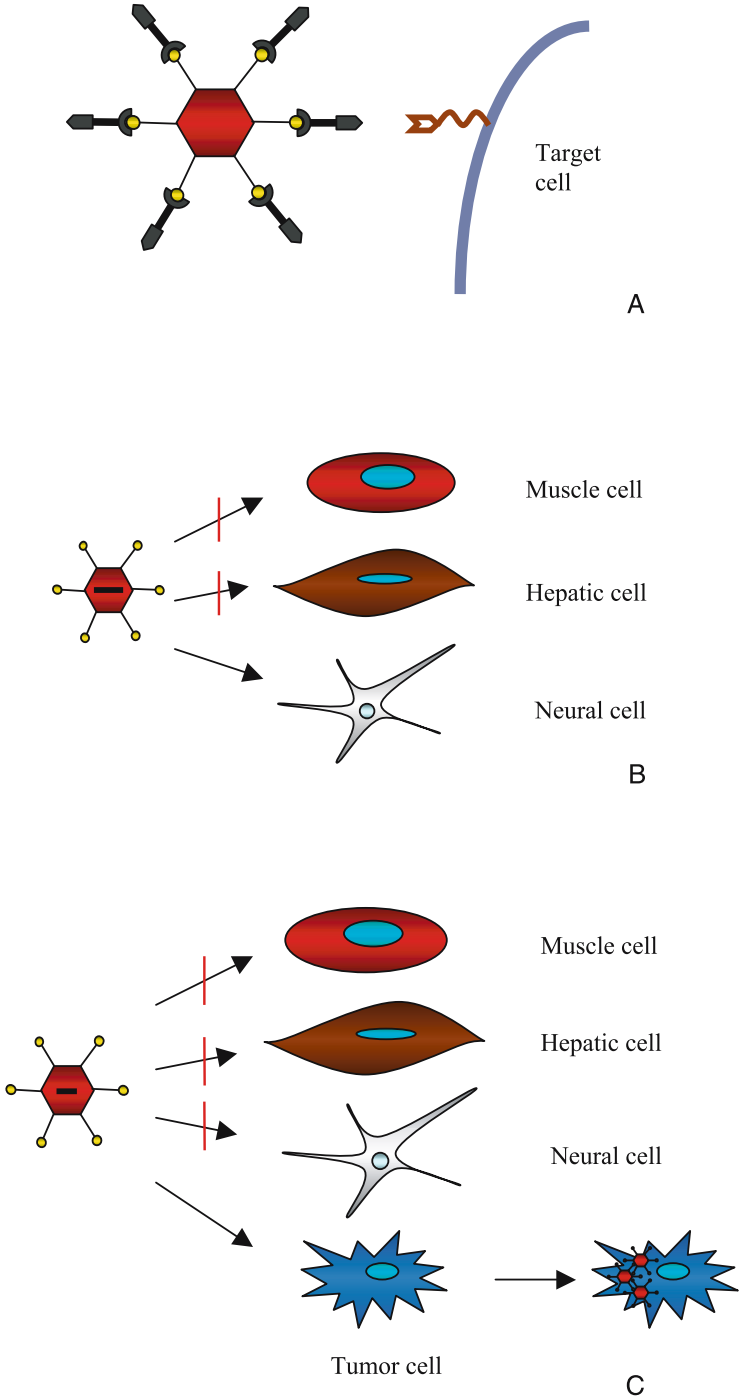

Fig. 3. Targeting of adenoviruses can be achieved by (A) re-directing the vector capsid to new cellular receptors using molecular adaptors, such bi-specific antibodies, through (B) placing the transgene under the control of a cell type-specific promoter, or through (C) genetically modifying them into conditionally replicating viruses

Viral Gene Transfer Vectors

Retroviruses

DNA can be introduced into cells using retrovirus vectors. Retroviruses allow stable integration of expressed genes. The retroviridae are a large group of viruses associated with many diseases ranging from completely benign infections to fatal conditions such as HIV and tumours caused by oncogenic viruses (Coffin, 1990). Brain tumours are theoretically suitable for retrovirus-mediated gene transfer, since retroviruses only infect proliferating cells, while normal, generally non-dividing brain tissue remains intact (Miller, 1990). However, the failure of a phase III trial where 248 patients with newly diagnosed, previously untreated glioblastoma multiforme were treated by retrovirus-mediated transduction of glioblastoma cells with the HSV-tk gene and subsequent systemic treatment with ganciclovir, was a drawback for the retroviral vector. Especially, since the failure of that trial was mainly attributed to poor rate of delivery of the HSV-tk gene (Rainov, 2000a).

Recently, retroviral vectors based on lentiviruses (such as the human immunodeficiency virus) have been developed that are capable of transducing also non-dividing cells in a long lasting manner (Naldini, 1996, Kirik & Bjorklund, 2003, Jakobsson, 2003). Since retroviruses are integrating vectors one concern has been the possibility of random integration of foreign DNA into target cells, carrying the potential risk of insertional mutagenesis, the perturbation of other genes involved in growth control, or inactivation of tumour suppressor genes (Temin, 1990). However, especially in cancer gene therapy the risk of insertional mutagenesis is only of minimal concern. Despite some safety and ethical concerns about the use of lentiviruses, they seem to be feasible gene transfer vectors to the brain (Van den Haute, 2003, Marr, 2003, Watson & Wolfe, 2003, Koponen, 2003, Regulier, 2002).

Herpes Simplex Virus-1 (HSV-1)

HSV's have some characteristics that render them particularly suitable for use as neuronal vectors. They are neurotropic, and hence, infect neurons efficiently. In addition, they can accommodate large inserts, since approximately half of its genome is composed of non-essential genes that can be replaced with heterologous genes. Another interesting feature of HSV is that it can be transported retrogradely in neurons and transferred across synapses. (Simonato, 2000) Replication defective HSV-1 vectors are produced by deleting all, or a combination, of the five immediate-early genes (ICP0, ICO4, ICO22, ICP27 and ICP47) (Thomas, 2003), which are required for lytic infection and expression of all other viral proteins. Vec-

tors derived from HSV-1 still remain capable to infect a wide range of cell types – including neurons in the CNS.

A number of attenuated strains have been developed. Thymidine kinase/Ganciclovir therapy for brain tumour has been successfully applied with HSV-tk viruses transfecting the tumour to express the thymidine kinase gene (Todo, 2000, Moriuchi, 1998, Kramm, 1996). Disadvantages with the use of HSV-1 vectors are lytic infections and potential neurotoxicity (Zlokovic & Apuzzo, 1997). Because HSV-1 do not have a long lasting gene expression, their use is mainly restricted to brain tumours.

Adenoviruses

Adenoviruses are large, double stranded DNA viruses which can carry large fragments of foreign DNA. Adenoviruses exist extrachromosomally within the cell although the DNA migrate into the cell nuclei. Adenoviruses have a known tropism for pulmonary and intestinal epithelial cells; they are not neurotoxic and are linked to only minor diseases in human. Adenoviruses have a broad host and cell range. They are capable of high-efficiency gene delivery into a variety of organs, including lung, skeletal muscle, heart, liver, blood vessels and the central nervous system (Sandmair, 2000b). For these reasons recombinant adenoviral vectors have been extensively used in experimental models, as well as in clinical protocols. Because of their broad host and cell range adenoviruses have been modified by different means to make them specific to certain cell types (Fig. 3). One approach involves genetically modifying the fibre knob, through which attachment of the virus to the cell receptor and entry into the cell occurs. A second approach is immunological modification of the adenovirus tropism using bi-specific molecules that on one side bind to the fibre knob or the penton base of the adenovirus, and on the other side bind to the cell surface receptor, different from the viral receptor (Wickham, 2003).

Also, specificity of adenoviruses has been achieved using tissue specific promoters, such as the human synapsin 1 gene promoter for neuron specific transgene expression (Kugler, 2003), or more recently, using conditionally replicating adenoviruses (Steinwaerder, 2001). The use of genes such as the HSV-thymidine kinase gene that specifically targets dividing/tumour cells has also been studied extensively (Moolten, 1994).

Adeno-Associated Virus (AAV's)

So far, eight distinct AAV serotypes have been identified which infect different cell types with different efficiency (Thomas, 2003). However, most recombinant AAV vectors have been derived from AAV2 and most of the

in vivo studies have been performed using AAV2 vectors containing the strong cytomegalovirus immediate-early (CMV) promoter (Tenenbaum, 2004). AAV vectors have been shown to transduce a broad range of neural cells. Transduction efficiency, however, varies markedly from one region to another.

AAV vectors are integrating vectors. Wild-type AAV integrates exclusively into a single site on human chromosome 19, whereas it appears that AAV recombinants integrate much less efficiently and more randomly (Balague, 1997). AAV vectors have been shown to give sustained transgene expression upon in vivo administration. Expression of homologous genes has been detected two years after injection in mice and several months after injection in dogs, primates, and man. (Mountain 2000) AAV vectors can transfer genes efficiently to both quiescent and proliferating cells. The main disadvantages of AAV vectors are the small insert size they can accommodate and the use of helper viruses in the manufacturing process, which caused problems, such as low titre, contamination and costly purification procedures. However, progress has been made in manufacturing process, which allows high-titre production without helper viruses. (Ferrari, 1997, Snyder & Flotte 2002, During, 2003)

Currently, there are two ongoing brain gene therapy clinical trials where AAV vectors are used for the treatment of Parkinson's disease (www.gemcris.od.nih.gov).

Non-Viral Vectors

Viral vectors have been shown to be efficient gene transfer tools. Nevertheless, drawbacks, such as the bloodstream's rapid clearance of viral vectors (when injected systemically), their immunogenic and inflammatory potential, together with certain safety concerns, urged the development of new synthetic gene delivery vectors. (Poly)cationic carriers and cationic lipids have been studied extensively as alternatives for viral vectors (da Cruz, 2004, Anderson, 2003, Lesage, 2002, Goldman, 1997). The (poly)cationic carriers possess groups which are protonated at physiological pH. The electrostatic attraction between the cationic charged polymer and the negatively charged DNA results in a particular complex – the polyplex, which is the transduction reagent. As with (poly)cationic carriers, cationic lipids posses additionally a hydrophobic group, which ensures that the cationic lipids assemble into bi-layer vesicles on dispersion in aqueous media (Brown, 2001). A great advantage of non-viral vectors is that they can be produced more easily than viral vectors. However, compared to viral gene transfer vectors these non-viral vectors are facing different types of problems, such as binding to plasma proteins or blood cells, which can lead to aggregates and clogging of capillaries (Ogris & Wagner, 2002).

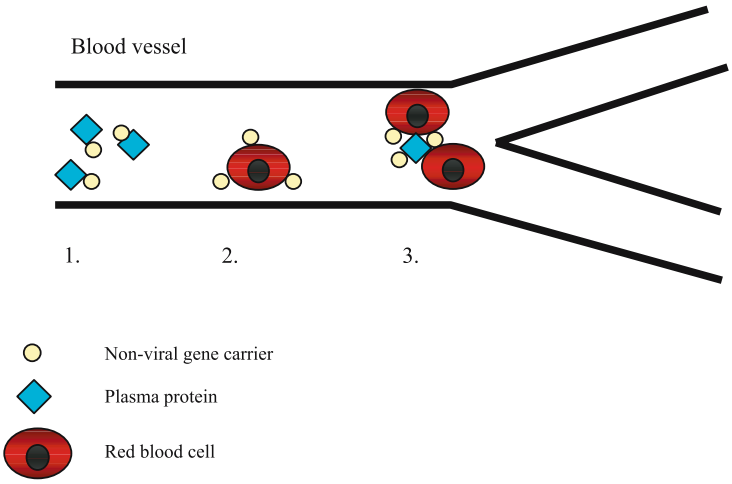

Fig. 4. Obstacles for positively charged non-viral gene transfer vectors within the blood circulation. *(1)* They can bind to plasma proteins or *(2)* blood cells, and *(3)* aggregates can clog capillaries. In addition, one of the major drawbacks of non-viral vectors is their low transducing efficiency in vivo (Ogris, 2002)

Ethics

Gene therapy raises many questions among the society. They raise concern about their safety in humans and their offspring, their environment safety, and their impact on the status within the society; Is it going to be a treatment modality only accessible for a certain group of people (people with a high social status) or is it going to be accessible for everyone? Opinions and points of views about gene therapy vary from one extreme to another. Cultural as well as religious points of views have strong impact on these standpoints.

Several questions have to be asked in order to justify gene therapy in humans; Questions such as which are the diseases where gene therapy is ethically acceptable? It appears that gene therapy is more tolerated for life-threatening diseases (e.g. diseases like cancer or AIDS) than e.g. in the correction of a learning disorders (Rabino, 2003). Also, somatic gene therapy appears to be more tolerated than germline gene therapy. Several questions have to be asked in order to justify gene therapy in humans, like which are the diseases where gene therapy is ethically acceptable? Where the use of gene therapy in the treatment of a genetic disease (e.g. cancer) might be ethically justified, how about when dealing with genetic 'disorders'? Would it be ethically acceptable to practise gene therapy on people with Down's syndrome? What is the justification of using gene therapy in those people?

In addition to issues raised above there are also technical issues concerning the justification of gene therapy in humans. For example, what are the technical details of the DNA and vector to be used? The technical aspects involved, risks endeavoured by the patient, and the fear of human genetic engineering are some of the major reasons why human gene therapy experiments have long been delayed.

The use of viral gene transfer vectors, such as lentiviruses raises scepticism about the safety of these vectors. Non-viral vectors are not yet efficient enough, but have gained better acceptance in the society. It looks like gene therapy of brain tumours will be ethically acceptable whereas the use of genetically modified stem cells may be a much more difficult topic. However, the normal principles of good clinical research apply in the conduct of the ethical evaluation of gene therapy protocols as well. The integrity and free will of a patient should be respected, all available information for the informed consent should be given, and the safety of an individual must be the first concern of the treatment protocol.

Concluding Remarks

There is extensive research going on in the field of gene therapy and especially malignant glioma, which has been subject of an increasing interest as a possible target for it. AdHSV-tk/ganciclovir gene therapy is one of the research lines with some promising results in early clinical trials that need to be confirmed in larger patient series (Immonen, 2004). However, there still remain obstacles that have to be overcome, especially when talking about gene therapy into the brain. Gene transfer vectors have to be able to cross the BBB. The induction of immune response for some vectors must be avoided, and the production of viral vectors in large scale has to be optimized. Nevertheless, there is no doubt that none of these problems mentioned above are problems that can not be resolved.

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References

1. Abeloff MD *et al* (2000) Clinical oncology, 2nd edn. Churchill, Livinstone
2. Alisky JA, Davidson BL (2000) Gene therapy for amyotrophic lateral sclerosis and other motor neuron diseases. Hum Gene Ther 11(17): 2315–2330
3. Ammirati M *et al* (1987) Effect of the extent of surgical resection on survival and quality of life in patients with supratentorial glioblastomas and anaplastic astrocytomas. Neurosurgery 21(2): 201–206

4. Anderson DM *et al* (2003) Stability of mRNA/cationic lipid lipoplex in human and rat cerebrospinal fluid: methods and evidence for non-viral mRNA gene delivery into the central nervous system. Hum Gene Ther 14(3): 191–202
5. Ascadi G *et al* (2002) Increased survival and function of SOD 1 mice after glial-cell derived neurotrophic factor gene therapy. Hum Gene Ther 13(9): 1047–1059
6. Azzous M *et al* (2000) Increased motoneuron survival and improved neuromuscular function in transgenic ALS mice after intraspinal injection of adeno-associated virus encoding Bcl-2. Hum Mol Genet 9(5): 803–811
7. Azzous M *et al* (2004) VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 429: 413–417
8. Bachoud-Levi AC *et al* (2000) Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. Hum Gene Ther 11(12): 1723–1729
9. Bachoud-Levi AC *et al* (1998) Prospective for cell and gene therapy in Huntington's disease. Prog Brain Res 117: 511–524
10. Bemelmans AP *et al* (1999) Brain-derived neurotrophic factors-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, demonstrated by adenoviral gene transfer. Hum Gene Ther 10(18): 2987–2997
11. Bethel A *et al* (1997) Intravenous basic fibroblast growth factor-2 decreases brain injury resulting from focal ischemia in cats. Stroke 28: 609–615
12. Bobo RH *et al* (1994) Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci 91: 2076–2080
13. Boillée S and Cleveland DW (2004) Gene therapy for ALS delivers. Trends Neurosci 27(5): 235–238
14. Bowers WJ *et al* (2003) Immune responses to replication-defective HSV-1 type vectors within the CNS: implication for gene therapy. Gene Ther 10(11): 941–945
15. Brown M (2003) Gene therapy success for Alzheimer's. Drug Discov Today 8(11): 474–5
16. Brown MD *et al* (2001) Gene delivery with synthetic (non-viral) carriers. Int J Pharm 229: 1–21
17. Bruijn LI *et al* (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci 27: 723–749
18. Byrnes AP *et al* (1995) Adenovirus gene transfer causes inflammation in the brain. Neuroscience 66(4): 1015–1024
19. Cabrera-Salazar MA *et al* (2002) Gene therapy for the lysosomal storage disorders. Curr Opin Mol Ther 4(4): 349–358
20. Cavazzana-Calvo M *et al* (2000) Gene therapy of a human severe combined immunodeficiency (SCID)-XI disease. Science 288: 669–672
21. Chiocca EA (2003) Gene therapy: a primer for neurosurgens. Neurosurgery 53(2): 364–373
22. Coffin M (1990) Retroviridae and their replication. In: Fields BN, Knipe DM (eds) Virology. Raven Press Ltd, New York, p 1437–1500

23. Connors TA (1995) The choice of prodrugs for gene directed enzyme prodrug therapy of cancer. Gene Ther 2: 702–709
24. Cordelier P *et al* (2003) Inhibition of HIV-1 in the central nervous system by IFN-alpha2 delivered by an SV40 vector. J Interferon Cytokin Res 14(16): 1525–1533
25. Da Cruz MT *et al* (2004) Improving lipoplex-mediated gene transfer into C6 glioma cells and primary neurons. Exp Neurol 187(1): 65–75
26. Datta K *et al* (2004) Senzitazing glioma cells to cisplatin by abrogating the p53 response with antisense oligonucleotides. Cancer Gene Ther 11(8): 525–531
27. Dietrich HH and Dacey RG (2000) Molecular keys to the problems of cerebral vasospasm. Neurosurgery 46(3): 517–29
28. Durin MJ *et al* (2003) Development and optimization of adeno-associated virus vector transfer into the central nervous system. Methods Mol Med 76: 221–236
29. During MJ *et al* (1994) Long-term behavioral recovery in parkinsonian rats by an HSV vector expressing tyrosine hydroxylase. Science 266: 1399–403
30. Eberhardt O, Schulz JB (2004) Gene therapy in Parkinson's disease. Cell Tissue Res 63(4): 758–769
31. Eberling JL *et al* (2003) In vivo PET imaging of gene expression in parkinsonian monkeys. Mol Ther 8(6): 873–5
32. Edelstein ML *et al* (2004) Gene therapy clinical trials worldwide 1989–2004 – an overview. J Gene Med 6(6): 597–602
33. Ehrengruber MU (2002) Alphaviral vectors for gene transfer into neurons. Mol Neurobiol 26: 183–201
34. Eto Y, Ohashi T (2002) Novel treatment for neuronopathic lysosomal storage diseases – cell therapy/gene therapy. Curr Mol Med 2(1): 83–89
35. Fazakerley JK (2004) Semliki forest virus infection of laboratory mice: a model to study the pathogenesis of viral encephalitis. Arch Virol Suppl 18: 179–190
36. Fecci PE, Gromeier M, Sampson JH (2000) Viruses in the treatment of brain tumors. Neuroimaging Clin N Am 12(4): 553–570
37. Ferrari FK *et al* (1997) New developments in the generation of Ad-free, high titer rAAV gene theraby vectors. Nat Med 3(11): 1295–1297
38. Finkelstein R, Baughman RW, and Steele FR (2001) Harvesting the neural gene therapy fruit. Mol Ther 3(1): 3–7
39. Fisher W (1987) Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. Nature 329: 65–68
40. Friedlos F *et al* (1998) Gene directed enzyme prodrug therapy: quantitative bystander cytotoxicity and DNA damage induced by CB 1954 in cells expressing bacterial nitroreductase. Gene Ther 5(1): 105–112
41. Friese MA *et al* (2003) MICA/NKG2D-mediated immunogene therapy of experimental gliomas. Cancer Res 63(24): 8996–9006
42. Futerman AH, van Meer G (2004) The cell biology of lysosomal storage disorders. Nat Rev Mol Cell Biol 5(7): 554–565
43. Gash DM, Gerhardt GA, Hoffer BJ (1998) Effects of glial cell line-derived

neurotrophic factor on the nigrostriatal dopamine system in rodents and nonhuman primates. Adv Pharmacol 42: 911–915
44. Ge L *et al* (1995) Gene therapeutic approaches to primary and metastatic brain tumors: II. Ribozyme-mediated suppression of CD44 expression. J Neurooncol 26(3): 251–257
45. Goldman CK *et al* (1997) In vitro and in vivo gene delivery mediated by a synthetic polycationic amino polymer. Nat Biotech 15(5): 462–466
46. Gomez-Manzano C *et al* (2004) A novel E1A–E1B mutant adenovirus induces glioma regression in vivo. Oncogene 23(10): 1821–1828
47. Gondi CS *et al* (2004) Adenovirus-mediated expression of antisense urokinase plasminogen activator receptor and antisense cathepsin B inhibits tumor growth, invasion, and angiogenesis in gliomas. Cancer Res 64: 4069–4077
48. Greg M (2002) Breaking down barriers. Science 297(5587): 1116–1118
49. Gromeier M, Wimmer E (2001) Viruses for the treatment of malignant glioma. Curr Opin Mol Ther 3(5): 503–508
50. Gutierrez-Delicado E, Serratosa JM (2004) Genetics of epilepsies. Curr Opin Neurol 17(2): 147–153
51. Haberman RP *et al* (2003) Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. Nat Med 9(8): 1076–1080
52. Hacein-Bey-Abina S *et al* (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348(3): 255–6
53. Hagell P *et al* (2002) Dyskinesias following neural transplantation in Parkinsons disease. Nat Neurosci 5: 627–628
54. Haluska M, Anthony ML (2004) Osmotic blood-brain barrier modification for the treatment of malignant brain tumors. CJON 8(3): 263–267
55. Hoehn B *et al* (2001) Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. J Cereb Blood Flow Metab 21(11): 1303–1309
56. Hogarth P (2003) Huntington's disease: a decade beyond gene discovery. Curr Neurol Neurosci Rep 3(4): 279–284
57. Howard K (2003) First Parkinson gene therapy trial launches. Nat.Biotech 21(10): 1117–8
58. Hsich G, Sena-Esteves M, Breakefield XO (2002) Critical issues in gene therapy for neurologic disease. Hum Gene Ther 13(5): 579–604
59. Ichikawa T *et al* (2001) Intraneoplastic polymer-based delivery of cyclophosphamide for intratumoral bioconversion by a replicating oncolytic viral vector. Cancer Res 61(3): 864–868
60. Immonen A *et al* (2004) AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomized, controlled study. Mol Ther 10(5): 967–72
61. Jakobsson J *et al* (2003) Targeted transgene expression in rat brain using lentiviral vectors. J Neurosci Res 73(6): 876–885
62. Jakobsson J *et al* (2003) Targeted transgene expression in rat using lentiviral vectors. J Neurosci Res 73(6): 876–885

63. Joos K, Chirmule N (2003) Immunity to adenovirus and adeno-associated viral vectors: implications for gene therapy. Gene Ther 10(11): 955–963
64. Kajiwara K *et al* (2000) Humoral immune response to adenovirus vectors in the brain. J Neuroimmunol 103(3): 253–265
65. Kaspar BK *et al* (2003) Retrograde viral delivery of IGF-1 prolongs survival in a mouse model. Science 301: 839–842
66. Kaye EM, Sena-Esteves M (2002) Gene therapy for the central nervous system in the lysosomal storage disorders. Neurol Clin 20(3): 879–901
67. Kirik D, Bjorklund A (2003) Modeling CNS neurodegeneration by over-expression of disease causing proteins using viral vectors. Trends Neurosci 26(7): 386–392
68. Kirsch M *et al* (2000) Anti-angiogenic treatment strategies for malignant brain tumors. J Neurooncol 50: 149–163
69. Koponen JK *et al* (2003) Doxycycline-regulated lentiviral vector system with a novel reverse transactivator rtTA2S-M2 shows a tight control of gene expression in vitro and in vivo. Gene Ther 10(6): 459–466
70. Kordower J *et al* (2000) Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. Science 290: 767–773
71. Kramm CM *et al* (1996) Long-term survival in a rodent model of disseminated brain tumors by combined intrathecal delivery of herpes vectors and ganciclovir treatment. Hum Gene Ther 7(16): 1989–1994
72. Kromer LF (1987) Nerve growth factor treatment after brain injury prevents neuronal death. Science 235(4785): 214–216
73. Kugler S *et al* (2003) Human synapsin 1 gene promoter confers highly neuron-specific long-term transgene expression from an adenoviral vector in the adult rat brain depending on the transduced area. Gene Ther 10(4): 337–347
74. Lakka SS *et al* (2003) Synergistic down-regulation of urokinase plasminogen activator receptor and matrix metalloproteinase-9 in SNB19 glioblastoma cells efficiently inhibits glioma cell invasion, angiogenesis, and tumor growth. Cancer Res 63(10): 2454–2461
75. Lamont JP *et al* (2000) A prospective phase II trial of ONYX-015 adenovirus and chemotherapy in recurrent squamous cell carcinoma of the head and neck (the Baylor experience). Ann Surg Oncol 7(8): 588–592
76. Lampela P *et al* (2002) The use of low-molecular-weight PEIs as gene carriers in the monkey fibroblastoma and rabbit smooth muscle cell culture. J Gene Med 4(2): 205–214
77. Lehtolainen P *et al* (2002) Baculovirus exhibits restricted cell type specificity in rat brain: a comparison of baculovirus- and adenovirus mediated intracerebral gene transfer in vivo. Gene Ther 9(24): 1693–1699
78. Lehtolainen P *et al* (2002) Clining and characterization of Scavidin, a fusion protein for the targeted delivery of biotinylated molecules. J Biol Chem 277(10): 8545–8550
79. Lehtolainen P *et al* (2003) Targeting of biotinylated compounds to its target

tissue using a low-density lipoprotein receptor-avidin fusion protein. Gene Ther 10(25): 2090–2097
80. Lesage D *et al* (2002) Evaluation and optimization on DNA delivery into gliosarcoma 9L cells by a cholesterol-based cationic liposome. Biochim Biophys Acta 1564(2): 393–402
81. Lin H *et al* (2002) Cyclooxygenase-1 and bicistronic cyclooxygenase-1/prostacyclin synthase gene transfer protects against ischemic cerebral infarction. Circulation 105(16): 1962–1969
82. Liu BH *et al* (2004) CMV enhancer/human PDGF-beta promoter for neuron specific transgene expression. Gene Ther 11(1): 52–60
83. Lou E (2004) Oncolytic viral therapy and immunotherapy of malignant brain tumors: two potential new approaches of translational research. Ann Med 36(1): 2–8
84. Lowenstein PR, Castro MG (2003) inflammation and adaptive immune responses to adenoviral vectors injected into the brain: peculiarities, mechanisms, and consequences. Gene Ther 10(11): 946–954
85. Lowenstein PR (2004) Immunological needles in the gene therapy haystack: applying a genetic paradigm to gene therapy. Gene Ther 11(1): 1–3
86. Lüders JC *et al* (2000) Adenoviral gene transfer of nitric oxide synthase increases cerebral blood flow in rats. Neurosurgery 47(5): 1206–1217
87. Lundstrom K *et al* (2001) Semliki Forest virus vectors: efficient vehicles for in vitro and in vivo gene delivery. FEBS Lett 504(3): 99–103
88. Luo J *et al* (2002) Subthalamic GAD gene therapy in a Parkinson's disease rat model. Science 298: 425–429
89. MacMillan JC *et al* (1994) Clinical considerations in gene therapy of Huntington's disease. Gene Ther 1[Suppl] 1: 88
90. McCown TJ (2004) The clinical potential of antiepileptic gene therapy. Expert Opin Biol Ther 4(11): 1771–1776
91. McPhee SW *et al* (2005) Effects of AAV-2-mediated aspartoacylase gene transfer in the tremor rat model of Canavan disease. Brain Res Mol Brain Res 135(1–2): 112–121
92. Manome Y *et al* (1996) Gene therapy for malignant gliomas using replication incompetent retroviral and adenoviral vectors encoding the cytochrome P450 2B1 gene together with cyclophosphamide. Gene Ther 3(6): 513–520
93. Markert JM *et al* (2000) Conditionally replicating herpes simplex virus mutant, GS07 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 7(10): 867–874
94. Marr RA *et al* (2003) Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. J Neurosci 23(6): 1992–1996
95. Marr RA *et al* (2004) Neprilysin regulates amyloid Beta peptide levels. J Mol Neurosci 22(1–2): 5–11
96. Mattson MP (2004) Pathways towards and away from Alzheimer's disease. Nature 430: 631–639
97. McBride JL *et al* (2003) Structural and functional neuroprotection in a rat model of Huntington's disease by viral gene transfer of GDNF. Exp Neurol 181(2): 213–223

98. Miller CR *et al* (1998) Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fibre receptor-independent gene transfer. Cancer Res 58(24): 5738–5748
99. Miller CR *et al* (2002) Intratumoral 5-fluorouracil produced by cytosine deaminase/5-fluorocytosin gene therapy is effective for experimental human glioblastoma. Cancer Res 62(3): 773–780
100. Miller DG *et al* (1990) Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 10: 4239–4242
101. Mohanam S *et al* (2002) Modulation of invasive properties of human glioblastoma cells stably expressing amino-terminal fragment of urokinase-type plasminogen activator. Oncogene 21(51): 7824–7830
102. Monahan PE, White GC 2nd (2002) Hemophilia gene therapy: update. Curr Opin Hematol 9(5): 430–436
103. Moolten FL (1994) Drug sensitivity (''suicide'') genes for selective cancer chemotherapy. Cancer Gene Ther 1: 279–287
104. Morishita R *et al* (2004) Safety Evaluation of Clinical Gene Therapy Using Hepatocyte Growth Factor to Treat Peripheral Arterial Disease. Hypertension Jul 6 [Epub ahead of print]
105. Morishita R *et al* (2004) Therapeutic angiogenesis using hepatocyte growth factor (HGF). Curr Gene Ther 4(2): 199–206
106. Moriuchi S *et al* (1998) Enhanced tumor cell killing in the presence of ganciclovir by herpes simplex virus type 1 vector-directed coexpression of human tumor necrosis factor-alpha and herpes simplex virus thymidine kinase. Cancer Res 58(24): 5731–5737
107. Mountain A (2000) Gene therapy: the first decade. TIBTECH 18: 119–128
108. Naldini L *et al* (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272: 263–267
109. Neuwelt EA *et al* (1995) Gene replacement therapy in the central nervous system – viral vector-mediated therapy of global neurodegenerative disease. Behav Brain Sci 18(1): 1–9
110. Neuwelt G *et al* (1980) Reversible osmotic blood-brain barrier disruption in humans: implications for the chemotherapy of malignant brain tumors. Neurosurgery 7(1): 44–52
111. Nguyen TT *et al* (2003) Convective distribution of macromolecules in the primate brain demonstrated using computerized tomography and magnetic resonance imaging. J Neurosurg 98(3): 584–590
112. Ogris M, Wagner E (2002) Targeting tumors with non-viral gene delivery systems. Drug Discov Today 7(8): 479–485
113. Onoue H *et al* (1998) Expression and function of recombinant endothelial nitric oxide synthase gene in canine basilar artery after experimental subarachnoid hemorrhage. Stroke 29: 1959–1966
114. Palmer AM *et al* (2002) Pharmacotherapy for Alzheimer's disease: progress and prospects. Trends Pharmacol Sci 23: 426–433

115. Palmer DH *et al* (2004) Virus-directed enzyme prodrug therapy: intratumoral administration of a replication-deficient adenovirus encoding nitroreductase to patients with resectable liver cancer. J Clin Oncol 22(9): 1546–1552
116. Pardridge WM (2002) Drug and gene delivery to the brain: the vascular route. Neuron 36: 555–558
117. Pardridge WM (2002a) drug and gene delivery to the brain: the vascular route. Neuron 36(4): 555–558
118. Pardridge WM (2002b) Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 1(2): 131–139
119. Pompl PN *et al* (2003) A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. FASEB 17(6): 725–727
120. Prados MD *et al* (2003) Treatment of progressive or recurrent glioblastoma multiforme in adults with herpes simplex virus thymidine kinase gene vector-producer cells followed by intravenous ganciclovir administration: a phase I/II multi-institutional trial. J Neurooncol 65(3): 269–278
121. Puduvalli VK (2004) Inhibition of angiogenesis as a therapeutic strategy against brain tumors. Cancer Treat Res 117: 307–336
122. Puumalainen AM *et al* (1998b) Beta-galactosidase gene transfer to human malignant glioma in vivo using replication-deficient retroviruses and adenoviruses. Hum Gene Ther 9(12): 1769–1774
123. Puumalainen AM, Vapalahti M, Ylä-Herttuala S (1998a) Gene therapy for malignant glioma patients. Adv Exp Med Biol 451: 505–509
124. Qureshi NH *et al* (2000) Multicolumn infusion of gene therapy cells into human brain tumors: technical report. Neurosurgery 46(3): 663–668; discussion 668–669
125. Rabino I (2003) Gene therapy: ethical issues. Theor Med 24: 31–58
126. Rainov NG (2000a) A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 11(17): 2389–2401
127. Rainov NG *et al* (2000b) Immune response induced by retrovirus-mediated HSV-tk/GCV pharmacogene therapy in patient with glioblastoma multiforme. Gene Ther 7(21): 1853–1858
128. Rainov NG, Kramm CM (2001) Vector delivery methods and targeting strategies for gene therapy of brain tumors. Curr Gene Ther 1: 367–383
129. Rainov NG, Ren H (2003) Oncolytic viruses for treatment of malignant brain tumours. Acta Neurochir [Suppl] 88: 113–123
130. Rainov NG *et al* (1999) Intraarterial delivery of adenovirus vectors and liposome-DNA complexes to experimental brain neoplasms. Hum Gene Ther 10(2): 311–318
131. Regulier E *et al* (2002) Dose-dependent neuroprotective effect of ciliary neurotrophic factor delivered via tetracycline-regulated lentiviral vectors in the quinolinic acid rat model of Huntington's disease. Hum Gene Ther 13(16): 1981–1990

132. Richichi C *et al* (2004) Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. J Neurosci 24(12): 3051–3059
133. Saito R *et al* (2003) Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. Cancer Res 64: 2572–2579
134. Samii A, Nutt JG, Ransom BR (2004) Parkinson's disease. Lancet 363: 1783–93
135. Sanchez-Pernaut R *et al* (2001) Functional effect of adeno-associated virus mediated gene transfer of aromatic L-amino acid decarboxylase into the striatum of 6-OHDA-lesioned rats. Mol Ther 4(4): 324–330
136. Sandmair A-M *et al* (2000a) Thymidine kinase gene therapy for human malignant glioma using replication-deficient retroviruses or adenoviruses. Human Gene Ther 11(16): 2197–2205
137. Sandmair A-M (2000b) Gene therapy for malignant glioma. Thesis work. University of Kuopio
138. Schlachetzki F *et al* (2004) Gene therapy of the brain. Neurology 62: 1275–1281
139. Scollay R (2001) Gene therapy: a brief overview of the past, present and future. Ann NY Acad Sci 953: 26–30
140. Seki T *et al* (2004) Adenoviral gene transfer of aspartoacylase ameliorates tonic convulsions of spontaneously epileptic rats. Neurochem Int 45(1): 171–178
141. Shand N *et al* (1999) A phase 1–2 clinical trial of gene therapy for recurrent glioblastoma multiforme by tumor transduction with the herpes simplex thymidine kinase gene followed by ganciclovir. GLI328 European-Canadian Study Group. Hum Gene Therapy 10(14): 2325–2335
142. Shen Y *et al* (2000) Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. Hum Gene Ther 11(11): 1509–1519
143. Shigeru T *et al* (2004) Infiltrating macrophages as in vivo targets for intravenous gene delivery in cerebral infarction. Stroke Published online before print
144. Shimamura M *et al* (2003) HJV-envelope vector for gene transfer into central nervous system. Biochem Biophys Res Commun 300(2): 464–471
145. Shimamura M *et al* (2004) Novel therapeutic strategy to treat brain ischemia: overexpression of hepatocyte growth factor gene reduced ischemic injury without cerebral edema in rat model. Circulation 109(3): 424–431
146. Shimoura N, Hamada H (2003) Gene therapy using an adenovirus vector for apoptosis-related genes is a highly effective therapeutic modality for killing glioma cells. Curr Gene Ther 3(2): 147–153
147. Shirakura M *et al* (2004) Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils. Gene Ther 11(9): 784–790
148. Simonato M *et al* (2000) Gene transfer into neurons for the molecular anal-

ysis of behaviour: focus on herpes simplex vectors. Trends Neurosci 23(5): 183–190
149. Smitt PS *et al* (2003) Treatment of relapsed malignant glioma with an adenoviral vector containing the herpes simplex thymidine kinase gene followed by ganciclovir. Mol Ther 7(6): 851–858
150. Snyder RO, Flotte TR (2002) Production of clinical grade recombinant adeno-associated virus vectors. Curr Opin Biotechnol 13(5): 418–423
151. Steinbach JP and Weller M (2002) Mechanisms of apoptosis in central nervous system tumor: application to theory. Curr Neurol Neurosci Rep 2(3): 246–253
152. Steinwaerder DS *et al* (2001) Tumor-specific gene expression in hepatic metastases by a replication-activated adenovirus vector. Nat Med 7(2): 240–243
153. Stoodley M *et al* (2000) Effect of adenovirus-mediated nitric oxide synthase gene transfer on vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 46(5): 1193–1205
154. Sun JY *et al* (2003) Immune responses to adeno-associated virus and its recombinant vectors. Gene Ther 10(11): 964–976
155. Sun M *et al* (2003) Correction of a rat model of Parkinson's disease by co-expression of tyrosine hydroxylase and aromatic amino acid decarboxylase from a helper virus-free herpes simplex virus type 1 vector. Hum Gene Ther 14(5): 415–424
156. Tanaka T *et al* (1998) Viral vector-targeted antiangiogenic gene therapy utilizing an agiostatin complementary DNA. Cancer Res 58(15): 3362–3369
157. Tani H *et al* (2003) In vitro and in vivo gene delivery by recombinant Baculoviruses. J Virol 77(18): 9799–9808
158. Temin HM (1990) Safety considerations in somatic gene therapy of human disease with retroviral vectors. Human Gene Ther 1: 111–123
159. Tenenbaum L *et al* (2004) Recombinant AAV-mediated gene delivery to the central nervous system. J Gene Med 6: 212–222
160. Thomas CE *et al* (2003) Progress and problems with the use of viral vectors for gene therapy. Nature 4: 346–358
161. Todo T *et al* (2000) Evaluation of ganciclovir-mediated enhancement of the antitumoral effect in oncolytic, multimediated herpes simplex virus type 1 (G207) therapy of brain tumors. Cancer Gene Ther 7(6): 939–946
162. Toyoda K *et al* (2003) Gene therapy for cerebral vascular disease: update 2003. Br J Pharmacol 139: 139–147
163. Trask TW *et al* (2000) Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. Mol Ther 1(2): 195–203
164. Tuszynski MH *et al* (1998) Targeted intraparenchymal delivery of human NGF by gene transfer to the primate forebrain for 3 months does not accelerate β-amyloid plaque deposition. Exp Neurol 154: 573–582
165. Tuszynski MH (2002) Growth-factor gene therapy for neurodegenerative disorders. Lancet Neurol 1: 51–57
166. Uchida H *et al* (2004) Adenovirus-mediated transfer of siRNA against sur-

viving induced apoptosis and attenuated tumor cell growth in vitro and in vivo. Mol Ther 10(1): 162–171
167. Van der Haute C *et al* (2003) Lentiviral vector-mediated delivery of short hairpin RNA results in persistent knockdown of gene expression in mouse brain. Hum Gene Ther 14(18): 1799–1807
168. Wakimoto H *et al* (2003) Effects of innate immunity on herpes simplex virus and its ability to kill tumor cells. Gene Ther 10(11): 983–990
169. Wang LJ *et al* (2002) Neuroprotective effects of glial cell-line derived neurotrophic factor mediated by adeno-associated virus in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 22(16): 6920–6928
170. Watanabe Y *et al* (2003) Gene transfer of extracellular superoxide dismutase reduces cerebral vasospasm after subarachnoid hemorrhage. Stroke 34(2): 434–440
171. Watson DJ, Wolfe JH (2003) Lentiviral vectors for gene transfer to the central nervous system. Applications in lysosomal storage disease animal models. Methods Mol Med 76: 383–403
172. Weedon SJ *et al* (2000) Sensitisation of human carcinoma cells to the prodrug CB1954 by adenovirus vector-mediated expression of E coli nitroreductase. Int J Cancer 86(6): 848–854
173. Weiss MD *et al* (2004) Current pharmacological management of amyotrophic [corrected] lateral sclerosis and a role rational polypharmacy. Expert Opin Pharmacother 5(4): 735–746.
174. Wickham TJ (2003) Ligand-directed targeting of genes to the site of disease. Nat Med 9(1): 135–139
175. Wildner O (1999) In situ use of suicide genes for therapy of brain tumours. Ann Med 31(6): 421–429
176. Winkler J *et al* (1998) Cholinergic strategies for Alzheimer's disease. J Mol Med 76: 555–567
177. Witham TF *et al* (2003) Expression of a soluble transforming growth factor-beta (TGFbeta) receptor reduces tumorigenicity by regulation natural killer (NK) cell activity against 9L gliosarcoma in vivo. J Neurooncol 64(1–2): 55–61
178. Wood MJ *et al* (1994) Immunological consequences of HSV-1-mediated gene transfer into the CNS. Gene Ther [Suppl] 1: 82
179. Wu K *et al* (2004) The effects of rAAV2-mediated NGF gene delivery in adult and aged rats. Mol Ther 9(2): 262–269
180. Yamanaka R *et al* (2003) Induction of antitumor immunological response by an intratumoral injection of dendritic cells pulsed with genetically engineered Semliki Forest virus to produce interleukin-18 combined with the systemic administration of interleukin-12. J Neurosurg 99(4): 746–753
181. Yang GY *et al* (1997) Overexpression of interleukin-1 receptor antagonist in the mouse brain reduces ischemic brain injury. Brain Res 751(2): 181–188
182. Yang SY *et al* (2004) Gene therapy of rat malignant gliomas using neural stem stells expressing IL-12. DNA Cell Biol 23(6): 381–389
183. Yenari MA *et al* (2003) Gene therapy and hypothermia for stroke treatment. Ann N Y Acad Sci 992: 54–68; discussion 79–81

184. Ylä-Herttuala S, Alitalo K (2003) Gene transfer as a tool to induce therapeutic vascular growth. Nat Med 9(6): 694–701
185. Ylä-Herttuala S and Martin JF (2000) Cardiovascular gene therapy. Lancet 355: 213–222
186. Yukawa H *et al* (2000) Adenoviral gene transfer of basic fibroblast growth factor promotes angiogenesis in rat brain. Gene Ther 7: 942–949
187. Zadeh G and Guha A (2003) Angiogenesis in nervous system disorders. Neurosurgery 53(6): 1362–1374
188. Zhang Y *et al* (2004) Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain tumour. Clin Cancer Res 10(11): 3667–3677
189. Zlokovic BV and Apuzzo M (1997) Cellular and molecular neurosurgery: pathways from concept to reality – part II: vector systems and delivery methodologies for gene therapy of the central nervous system. Neurosurgery 40(4): 1992–1998