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## Progress in gene therapy for neurological disorders

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### Author contributions

All authors researched the data for the article, and provided substantial contributions to discussions of the content and writing of the article. M. Simonato and G. C. Glorioso reviewed and edited the manuscript before submission.

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## Abstract

Diseases of the nervous system have devastating effects and are widely distributed among the population, being especially prevalent in the elderly. These diseases are often caused by inherited genetic mutations that result in abnormal nervous system development, neurodegeneration, or impaired neuronal function. Other causes of neurological diseases include genetic and epigenetic changes induced by environmental insults, injury, disease-related events or inflammatory processes. Standard medical and surgical practice has not proved effective in curing or treating these diseases, and appropriate pharmaceuticals do not exist or are insufficient to slow disease progression. Gene therapy is emerging as a powerful approach with potential to treat and even cure some of the most common diseases of the nervous system. Gene therapy for neurological diseases has been made possible through progress in understanding the underlying disease mechanisms, particularly those involving sensory neurons, and also by improvement of gene vector design, therapeutic gene selection, and methods of delivery. Progress in the field has renewed our optimism for gene therapy as a treatment modality that can be used by neurologists, ophthalmologists and neurosurgeons. In this Review, we describe the promising gene therapy strategies that have the potential to treat patients with neurological diseases and discuss prospects for future development of gene therapy.

## Introduction

The nervous system is a complex and difficult organ system to study, and the brain is an organ where many of the most pervasive disease processes arise, for which the cause remains elusive. These diseases encompass a broad spectrum of pathological states and can have global or local effects on metabolism, and neural development and function. Drugs and neurosurgical procedures have generally not proven effective in the treatment of these disorders owing to the complexity and limited understanding of the pathophysiology involved. In addition, the blood–brain barrier (BBB) limits the use of systemic treatments as it impedes widespread delivery of therapeutic agents to the CNS.

Genetic interventions to supply gene products that permanently restore function and even induce replacement of lost cells could represent an alternative to standard pharmacological approaches. Such approaches, in which DNA or RNA is used as the pharmacological agent, are defined as gene therapy. Despite its tortuous development, the field of gene therapy has matured, emerging as a legitimate and promising choice for the treatment of many nervous system disorders. Improvements in gene transfer methods can largely be attributed to the development of sophisticated delivery vehicles that have been evaluated in animal models of human disease. On the basis of numerous preclinical studies, early clinical trials have been carried out to test the safety and, in some cases, efficacy of gene therapy. Some results have been encouraging, suggesting that this approach will soon be translated to the clinic.

In this Review, we describe some of the most promising emerging gene therapy approaches for the treatment of various nervous system disorders. We begin by describing the most common gene delivery systems and how vector design and biology fits their application. We discuss progress in the treatment of retinal degeneration, neuropathic pain and polyneuropathy in the PNS, and in the CNS we focus on lysosomal storage diseases, Parkinson disease (PD), epilepsy and glioblastoma. Progress has been made in the field of gene therapy to treat other disorders, but advances for the diseases described above are representative of the development of nervous system gene therapy. Our opinion is that gene therapy has the potential to prevent the onset or slow progression of neurological diseases and possibly to restore normal function. Our hope is that in the future, some of these gene therapy approaches will become available for patients.

## Gene therapy vectors

The success of gene therapy depends on effective gene delivery. Over the past two decades, vectors to deliver the therapeutic gene have undergone remarkable changes in design to meet the complex demands of transgene delivery to the host. Great effort has gone into the creation of nonviral gene delivery vehicles, including naked DNA or RNA, liposomes, and nanoparticles, owing to their low cost and ability to deliver a large cargo. Therapeutic gene expression with such vectors, however, is typically low and of limited duration.<sup>1</sup> None of these nonviral vectors contain the highly evolved mechanisms that wild-type viruses use to insert their genetic material into host cells and to alter cell functions. The use of gene vectors to treat patients with nervous system disorders has a complex history that in part mirrors the history of vector development. The most common CNS gene therapy vector is an adeno-associated virus (AAV), but lentiviral vectors have an increasing role in CNS gene therapy, and have the advantage of a larger transgene capacity. Herpes simplex virus (HSV) and adenoviral vectors have also been used to treat CNS disorders, especially tumours.

### Viral vectors

The key to development of an effective viral vector is to harness the virus biology for transgene expression rather than viral replication after host transduction. Achievement of this goal has not been easy and, indeed, suitable vectors are still in development and vary with respect to level and duration of transgene expression, cellular specificity and safety issues (Table 1).

Current design strategies for the most common viral vectors make use of the unique technical advantages of each vector (Figure 1). For example, vectors constructed from an AAV are safe, nonpathogenic and afford long-term gene expression. However, such vectors have limited transgene capacity, can be difficult to target to the appropriate location, require a high dose for effective gene expression, and are readily eliminated by humoral immune responses in patients previously exposed to the virus.<sup>2</sup> Other vectors, such as lentiviruses and retroviruses, can insert novel genetic material into the host cell chromosome, which is essential to avoid therapeutic gene loss in dividing cells. However, oncogenesis resulting from chromosomal insertion of the vector DNA poses a potential problem with the use of these viral vectors.<sup>3</sup>

Vectors with a large transgene capacity include those constructed from adenoviruses and HSV, which have the potential for effective gene targeting and sustained transgene expression. These vectors can, however, cause toxicity and inflammation stemming from 'leaky' expression of viral genes and reaction to the vector coat. Avoidance of these adverse effects requires complete vector genome silencing, which can affect transgene expression. These vectors can all be targeted by innate immune responses that, together with humoral immune responses, might trigger immune-mediated inflammatory processes that limit vector delivery, gene expression and the potential for redosing.<sup>4</sup> Despite these limitations, many of these vectors have proven to be highly effective gene delivery tools if used in a careful manner that takes advantage of their natural biology and strengths.

### Vector targeting through capsid modifications

Considerable advances in vector targeting have been made in terms of the overall efficiency of transduction, delivery to a wide target area, and in some cases delivery to a specific tissue or cell type. Improvements to AAV vectors over the past decade serve as a good example of how targeting can be improved. Alternative serotype capsids (the protein shell of a virus), rational mutagenesis of the capsid, insertion of targeting peptides into the capsid, and derivation of novel capsids by directed evolution have all been used to improve targeting of

AAV vectors. Over 100 AAV capsid variants have been identified, each with a potentially different cell tropism that provides a broad toolkit of vectors for optimized transgene delivery. For example, AAV9 could be used for CNS applications owing to its ability to cross the BBB after intravenous injection.<sup>5–7</sup> Enthusiasm for the use of AAV9 as a vector might be tempered by its high liver tropism (relative to its CNS tropism), but this tendency can be reduced by introduction of point mutations into the capsid,<sup>8</sup> or via the introduction of micro-RNA target sequences that respond to microRNAs highly expressed in the liver, but not in the CNS, into viral genes or virally delivered transgenes to limit their expression in the liver, thereby reducing toxicity to nontarget areas.<sup>9</sup>

Peptide insertions can confer novel features to AAV capsids and, by using a phage display library to generate novel peptides, modified AAV2 capsids were developed that specifically targeted the cerebral vasculature after intravenous injection.<sup>10</sup> To generate entirely new AAV capsids, DNA shuffling of capsid genes can be combined with directed evolution to select for novel traits.<sup>11</sup> In one study, a novel AAV capsid was generated that, after intravenous injection, showed increased expression at sites of epilepsy damage, with almost no expression in liver, heart and muscle; that is, a favourable safety and bio-distribution profile.<sup>12</sup> Similar to drug optimization, virus capsid engineering can increase vector potency and cell specificity and reduce the potential for adverse effects.

### Transgene expression control

The level of transgene expression and cell-specific expression can be directed by *cis*-acting elements contained within the vector genome or by the innate tropism of the virus itself. For example, the choice of 5' untranslated region (UTR), 3' UTR, enhancer, promoter and polyadenylation signal can affect cell specificity and level of transgene expression.<sup>13–20</sup> Through changes to the vector genome design, transgene expression can be modulated across at least a 1000-fold range and be restricted to specific cell populations.

### Gene therapy strategies

A good example of progress toward gene therapy strategies is the work done in retinal diseases. At least six strategies for transgene delivery and expression have been explored, extending the use of gene therapy from autosomal recessive sensorineural diseases (Leber congenital amaurosis) to complex inherited and acquired diseases, such as age-related macular degeneration.

Gene augmentation and/or gene knockdown is aimed at correcting gene expression in the context of a loss-of-function mutation by introducing the wild-type cDNA,<sup>21</sup> or at reducing expression of or eliminating a toxic gain-of-function gene product.<sup>22–24</sup> Gene augmentation is often limited by a narrow therapeutic window owing to the progressive nature of the disease, whereby the therapeutic target cell often degenerates and dies.

Correction of the primary genetic lesion at the chromosomal level is another approach to gene therapy, but is challenging owing to limited efficiency of current gene-editing technologies. Delivery of a vector carrying a transgene that encodes a decoy protein to the target organ is a technique that has been used to treat pathological ocular neovascularization. This approach involved expression of the *sFlt1* transgene, which encodes a tyrosine kinase that binds vascular endothelial growth factor—a key driver of pathology in ocular neovascularization (Supplementary Table 1 online).<sup>25</sup>

Delivery of vectors to express genes that encode proteins with antineovascular or antiapoptotic function is also possible. For example, delivery of the pigment epithelium-derived factor-encoding gene, which has antiangiogenic properties that are not fully

characterized is in development for treatment of choroidal neovascularization (Supplementary Table 1 online). Increased expression of genes encoding growth factors has been used to enhance nerve regeneration. Induction of a photoreceptor phenotype through expression of the CRX transcription factor has been shown in retinal stem cells.<sup>26</sup> Such an approach has also been used to produce functional auditory hair cells in the cochlea in animal models.<sup>27,28</sup> Finally, molecular prosthetics is an option to restore visual function by introducing light-sensitive ion channel proteins or ion pump proteins derived from bacteria and algae, such as the channel rhodopsin or halorhodopsin subfamilies, into the retina.<sup>29</sup> This approach is unique in that it can theoretically restore some function by rendering remaining cells in the retina light-sensitive and harnessing the function of remaining circuitry in the retina or optic nerve long after the primary disease-causing cells have died. Ultimately, this kind of gene therapy might be useful for all in herited diseases or environmentally induced degenerative processes that affect retinal pigment epithelium and photoreceptors, and also for blindness due to untreated retinal detachment.

The approaches described above all require some intact CNS function or sufficient plasticity to incorporate the neural signal from the treated peripheral organs. Sufficient plasticity exists in at least some retinal degenerative diseases, as demonstrated in a functional MRI study in recipients of retinal pigment epithelium-specific 65 kDa protein (*RPE65*) retinal gene augmentation therapy, in whom light-induced cortical responses were present even after long-term (>3.5 decades) visual deprivation.<sup>30</sup>

## Gene therapy targets

### Sensory organs and the PNS

Considerable progress has been made in developing gene therapy for sensorineural disorders, in particular blinding retinal degenerative diseases, for which no treatments are available. The mammalian eye has been the target organ in a number of therapeutic trials of gene therapy, because of its accessibility, its benign immunological response to gene transfer, and the availability of noninvasive functional and structural analyses. Many of these studies have focused on rare diseases, such as retinitis pigmentosa, Leber congenital amaurosis and choroideraemia, which could provide stepping stones to treat more-prevalent blinding conditions that have limited treatment options such as age-related macular degeneration, glaucoma and diabetic retinopathy.

Gene therapy to treat retinal blindness has progressed furthest of all the therapeutic strategies discussed above, with several completed or ongoing clinical trials (Supplementary Table 1 online). Three independent clinical trials have demonstrated safety and efficacy of *RPE65* gene augmentation in patients with Leber congenital amaurosis.<sup>31–33</sup> This strategy was aided by progress in identification and cloning of the disease-associated genes, and has provided momentum for several studies of gene therapy for other inherited forms of blindness (Supplementary Table 1 online). More than 25 genes associated with blindness have been recognized after identification of the first two— the choroideraemia and rhodopsin genes—in 1990.<sup>34–36</sup> A number of the retinal disease-associated mutant genes can also cause hearing and/or vestibular disorders (Usher syndrome). Progress in the field of genetics has led to the development of animal models of blindness and an improved understanding of disease pathogenesis. Studies in these models have been used for proof of concept in gene therapy and have led to clinical trials.

Progress has been made in gene therapy for other, extraocular sensory deficits, albeit at a slower rate than for retinal applications. The clinical need here is large: hearing loss and deafness due to presbycusis is a growing problem owing to the ageing of populations. Although surgical access is more of a challenge for cochlear than for retinal disorders, proof

of concept for gene therapy in several inherited conditions leading to deafness has been demonstrated.<sup>37,28</sup> In one of these studies, cochlear hair cells were regenerated after noise-induced degeneration, by delivering a transcription factor known to be important in the development of stereocilia.<sup>28</sup> In another study, a gene encoding the missing vesicular glutamate transporter-3 was delivered to the cochlea in mice lacking this enzyme; gene augmentation therapy at least partially restored hearing in these mice.<sup>37</sup> A third pivotal proof-of-concept study showed correction of a splicing defect in one form of Usher syndrome through administration of antisense oligonucleotides to a mouse model of the disease.<sup>38</sup> Finally, although target organs such as the nose and tongue are more accessible than the retina and cochlea, very few studies have addressed disorders of smell and taste,<sup>39</sup> probably owing to the complex aetiologies of these disorders and risk–benefit ratios for these indications.

### Pain and sensory neuropathy

Nonreplicating HSV vectors are promising vehicles for delivery of therapeutic transgenes to the PNS.<sup>40</sup> Enthusiasm for the use of this vector has been prompted by the high rate of infectivity of the virus in dorsal root and trigeminal ganglia, as well as its life-long persistence in sensory neurons in a nonintegrated state that is thought to mimic viral latency. HSV vector delivery to the PNS is achieved by simple inoculation of the skin. The vector enters nerve terminals and undergoes retrograde axonal transport to the nucleus, where the therapeutic gene is expressed in the absence of viral lytic functions (Figure 2). Potential applications for HSV vectors include the treatment of peripheral pain disorders (inflammatory or neuropathic)<sup>41,42</sup> and nerve degeneration (sensory polyneuropathy).<sup>43</sup>

In preclinical studies involving rodent models of pain, subcutaneous inoculation with a nonreplicating HSV vector expressing the opioid peptide enkephalin substantially reduced pain-related behaviour caused by inflammation, nerve damage or cancer.<sup>44,45</sup> A similar vector expressing the glutamic acid decarboxylase (*GAD*) transgene to cause release of the inhibitory neuro-transmitter  $\gamma$ -aminobutyric acid (GABA) prevented neuropathic pain caused by spinal nerve trauma or diabetes.<sup>46,47</sup> In addition, HSV vectors that expressed anti-inflammatory peptides, including IL-4 and IL-10, reduced pain in models of CNS and PNS neuropathic pain.<sup>48–50</sup>

Pain control has been achieved by using an HSV vector to express the glycine receptor—a ligand-gated Cl<sup>-</sup> channel that inhibits neurotransmission—in sensory neurons, combined with application of glycine to activate nerve silencing and provide a molecular switch for pain control.<sup>42</sup> Similarly, subcutaneous inoculation of rodent models with nonreplicating HSV vectors that express conventional or atypical neurotrophic factors, including nerve growth factor, neurotrophin-3 and erythropoietin, has been shown to prevent progression of neuropathy caused by an overdose of pyridoxine, treatment with chemotherapeutic drugs, or diabetes.<sup>51–56</sup>

An HSV vector construct to express the prepro-enkephalin gene was the first to advance to clinical trials.<sup>57</sup> In phase I–II of the trial, patients with intractable pain from cancer did not experience adverse events related to vector inoculation. Studies to treat painful diabetic neuropathy using a viral vector to express a *GAD* transgene are planned.<sup>58</sup>

### Genetic diseases

A large number of human genetic diseases affect the CNS; these conditions are frequently characterized by neurodegeneration and typically have pathology that is widely distributed in the brain. Transfer of the normal gene into diseased cells can correct the biochemical defect. Other gene transfer strategies besides direct gene replacement may be needed

depending on the underlying nature of the disease; for example, neurotrophic factors may rescue diseased cells even when the gene defect is not known. Alternatively, small interfering RNA approaches may be used to suppress dominant-negative genes (for example, in Huntington disease).<sup>59</sup> However, to deliver the therapeutic gene to the entire brain is a major challenge, especially in humans, whose brains are 2,000–3,000 times larger than a mouse brain.<sup>60</sup> Potential routes of vector delivery for widespread distribution of the transgene, which have shown promise in animal models, include injections of the vector along multiple injection tracks;<sup>61</sup> transport of the vector to brain regions distal to the injection site via neural pathways;<sup>62–64</sup> intravenous injection so that the vector crosses the BBB;<sup>5,6</sup> and injection of the vector into the cerebrospinal fluid spaces for distribution within the brain via the circulation (Figure 3).<sup>65,66</sup>

The properties of some proteins, such as lysosomal enzymes, can be harnessed to achieve wider distribution of the expressed protein and, therefore, provide a broader therapeutic effect. More than 50 human lysosomal storage diseases (LSDs) exist, most of which are characterized by accumulation of storage material in somatic and nervous system cells, leading to progressive degeneration of the CNS, usually beginning in early childhood. In the normal brain, lysosomal enzymes are released from the cell and taken up by neighbouring cells. Gene therapy for LSDs makes use of this mechanism to transfer lysosomal enzymes released from a set of genetically corrected cells and taken up into mutant cells, in a process known as cross-correction.<sup>67</sup> Studies in animal models using transplantation of cells corrected for the genetic defect or direct viral vector gene transfer have shown that the levels of functional enzyme delivered are sufficient to arrest or even reverse pathology.<sup>68</sup> A major barrier to treating the CNS has been delivery of the normal protein to a sufficiently large area of the brain to have a therapeutic effect. Positive results have been shown in many experimental models of LSDs, providing a foundation for clinical trials (Supplementary Table 2 online). In naturally occurring large-animal models of LSDs, direct delivery of the transgene into the CNS after symptoms have developed has shown substantial reversal of established lesions,<sup>61,69</sup> which raises hope that treatment of even advanced disease in patients with an LSD may provide some clinical benefit.

Three phase I clinical trials of gene therapy for genetic diseases of the CNS have been completed.<sup>70–72</sup> The most promising results were in patients with X-linked adrenoleukodystrophy—a severe demyelinating disease caused by deficiency of the *ABCD1* gene. In this trial, a lentivirus vector was used to transfect haematopoietic stem cells *ex vivo* with the *ABCD1* gene.<sup>72</sup> These cells were subsequently infused into the patient, and the effects of the treatment were thought to be mediated by corrected monocytes migrating into the CNS. The other trials involved direct injection of AAV2 vector into the brains of patients with Canavan disease<sup>70</sup> or a form of Batten disease.<sup>71</sup> Both were phase I safety trials of AAV2 that involved too few injection sites to be therapeutic. New trials are under way to test AAV vector serotypes that may mediate increased spread of the transgene once delivered to the target area. However, true clinical improvement will probably require substantial increases in the amount of gene vector delivered, as well as significantly greater dispersion within the brain.

### Parkinson disease

The most common neurodegenerative diseases, PD and Alzheimer disease (AD), affect the ageing population and are, therefore, an expanding demographic.<sup>73,74</sup> Some neurodegenerative diseases, such as Huntington disease (HD), are entirely genetic, whereas others, including PD, AD and amyotrophic lateral sclerosis (ALS), occur more commonly in idiopathic than in familial forms.<sup>75–77</sup> Consequently, gene therapy strategies for HD have concentrated on correcting the underlying gene defect and the resultant neurodegeneration,

whereas in PD, AD and ALS, the focus has been on neuroprotection and repair. Here, we limit the discussion of gene therapy to PD, although substantial progress has been made in preclinical studies towards gene therapy for HD and ALS, and a trial for gene therapy in AD is ongoing (Table 2).

PD is characterized by neurodegeneration of dopaminergic neurons in the substantia nigra that provide input to the basal ganglia. Three gene therapy strategies have evolved in the treatment of PD: induction of dopamine production, protection of substantia nigra neurons, and inhibition of the subthalamic nucleus through enhanced GABA signalling (Figure 4). The mainstay of therapy for patients with PD is pharmacological dopamine replacement. In patients with this condition, however, dopamine production in the nigrostriatal pathway is disproportionately reduced compared with that in the mesolimbic pathway. Pharmacological replacement of dopamine in the nigrostriatal pathway causes increased mesolimbic dopamine levels, leading to adverse effects such as poor impulse control.<sup>78</sup>

**AADC, tyrosine hydroxylase and GCH1**—Replacement of dopamine in an anatomically specific fashion, targeting nigrostriatal rather than mesolimbic pathways, could be a strategy to avoid the adverse effects of standard dopamine replacement strategies. Research efforts have been directed at targeted replacement of the enzymes and cofactors required to produce dopamine from tyrosine: aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase and GTP cyclohydrolase. An equine infectious anaemia virus (EIAV) vector was created to deliver the genes encoding these proteins to rodent and primate models of PD. EIAV has a large transgene capacity, thereby enabling the vector to contain all three genes. This vector was shown to reverse functional deficits in pharmacological animal models of PD with substantia nigra lesions,<sup>79,80</sup> and was taken forward into phase I–II trials of an escalating dose of putaminal injections in patients with PD. The results of this trial have not yet been published.

Two research groups have attempted to deliver the *AADC* gene in isolation to the putamen using an AAV vector. Because AADC uses levodopa as its substrate, dopamine production can be controlled by oral levodopa treatment in patients. In dopamine-depleted rodents and primates, AAV-mediated delivery of *AADC* resulted in long-term dopamine replacement and functional recovery when paired with oral levodopatherapy.<sup>81,82</sup> A phase I trial of *AADC* delivery to the putamen using an AAV2 vector showed a 31% increase in putaminal dopamine as measured by PET scanning. Three patients were able to reduce their medication intake, but two experienced worsening of dyskinesia.<sup>83</sup> A second study showed a 56% increase in dopamine activity above baseline on PET, and a 46% improvement in scores of motor function from baseline.<sup>84</sup>

**Glutamate decarboxylase**—Injection of GABA receptor agonists into the subthalamic nucleus has been shown to reduce the symptoms of PD by suppressing neuronal activity in this region.<sup>85</sup> Prompted by this finding, researchers developed an AAV2 vector to express the *GAD* gene, which encodes glutamate decarboxylase, the enzyme that converts glutamate to GABA. Delivery of this vector to the subthalamic nucleus in rodents and primates reduced excitatory output from the subthalamic nucleus, and improved tremor or dyskinesia.<sup>86,87</sup> The improvement in motor functions might be the result of a change in the excitatory glutamatergic output from the subthalamic nucleus to inhibitory GABAergic output, increased GABA production in the subthalamic nucleus that reduced excitatory activity in the subthalamic nucleus, or increased GABAergic input to the subthalamic nucleus owing to retrograde transport of the *GAD*-containing vector from the subthalamic nucleus injection site to input nuclei that would inhibit activity in the subthalamic nucleus.<sup>86,87</sup>



A phase II trial of an AAV2 vector containing *GAD*, injected bilaterally into the subthalamic nuclei, improved Unified Parkinson Disease Rating Scale (UPDRS) scores by 36% from baseline at 6 months.<sup>88</sup> Notably, however, 27% of patients in the treatment group were not included in the analysis. Nonetheless, this study is the first to demonstrate the efficacy of gene therapy for a neurodegenerative disease in a randomized, placebo-controlled trial. Pharmacological dopamine replacement strategies and *GAD* delivery to the subthalamic nucleus provide symptomatic relief, but do not slow or prevent dopaminergic neuron loss. Such surgical strategies should, therefore, only be considered for patients with late-stage refractory PD, given the lack of alternative therapeutic options in these circumstances. Dopamine replacement can also reduce dyskinesia resulting from the shift in dopamine production that occurs in the later stages of PD.

**Neurturin**—Neurturin (NRTN) is a neurotrophic factor that protects dopaminergic neurons from degeneration. AAV2 vector-mediated expression of *NRTN* to protect the nigrostriatal dopamine system from degeneration was demonstrated in rodents and primates.<sup>89,90</sup> Neuroprotection by NRTN expression is expected to prevent neuronal degeneration, and the first clinical trial was designed to detect improvements in motor function over a 1-year period. The anticipated improvement in motor function depends on an increase in dopamine production from the substantia nigra after putaminal injection with a vector containing the *NRTN* gene. In a phase I trial, bilateral putaminal injection of the AAV2–*NRTN* vector increased UPDRS motor scores by 36% at 1 year compared with baseline,<sup>91</sup> but no significant effect was detected at 1 year in a phase II trial.<sup>92</sup> A second phase I–II trial, implementing transgene delivery to both the putamen and substantia nigra, is under way to examine higher doses of vector than in the first trial and with longer follow-up. Histochemical analysis of postmortem tissue from the first phase II trial suggested poor retrograde delivery of AAV2–*NRTN* to the substantia nigra from the putamen, which prompted direct injection of AAV2–*NRTN* into the substantia nigra in the second phase II trial. Given that *NRTN* expression protects substantia nigra dopaminergic neurons against degeneration and promotes axonal regeneration, therapeutic *NRTN* delivery should probably be used early in the course of PD to maximize preservation of the substantia nigra.

## Epilepsy

The term epilepsy encompasses disorders characterized by a persistent increase in neuronal excitability that is occasionally and unpredictably expressed as seizures.<sup>93</sup> Seizures can be generalized, when the electrical activity occurs in bilaterally distributed networks, or focal, when activity is limited to one brain hemisphere.<sup>94</sup> Epilepsies associated with generalized seizures are often caused by a genetic defect, whereas epilepsies with focal seizures generally result from a lesion in a specific brain region.

Gene therapy could be an option for patients with epilepsy, most probably for epilepsies caused by a lesion rather than those caused by a genetic defect. Genetic epilepsies usually result from inheritance of multiple susceptibility genes, and the associated pathology affects large brain areas, which would require widespread transfection of the brain with multiple genes. Lesional epilepsies are more amenable to gene therapy: first, a causal event is often identified, which provides a therapeutic window for prevention of disease during the latency period before spontaneous seizures occur (antiepileptogenic effect); second, seizure-generating areas in the brain are restricted and easily identified. The unmet medical needs of patients with epilepsy include antiepileptogenic therapy (available drugs do not prevent the development of epilepsy in at-risk patients); new antiseizure therapies (available drugs fail to control seizures in one-third of patients and can have debilitating adverse effects); and disease-modifying therapies (available drugs do not prevent disease progression or the associated comorbidities).<sup>95</sup> Gene therapy could help to address these needs.

Gene therapy has been used to produce antiepileptogenic and antiseizure effects in experimental models of epilepsy (Table 3). Epileptogenesis may be alleviated by limiting the associated tissue damage.<sup>96</sup> An HSV vector containing transgenes encoding two neurotrophic factors, fibroblast growth factor 2 and brain-derived neurotrophic factor was injected into the lesion area to supplement growth factor expression in the epileptogenic region during latency, which led to attenuation of cell loss and reduction of epileptogenesis.<sup>97–99</sup>

Antiseizure effects can be obtained by targeting the threshold for neuronal excitability; that is, by increasing the strength of inhibitory signals or reducing that of excitatory signals. Gene therapy intervention leading to re arrangement of GABA or glutamate receptor composition, so as to either increase or reduce the responsiveness of the receptors, produced antiseizure effects in animal models of epilepsy.<sup>100,101</sup> These effects were, however, dependent on which cell population expressed the transgene: selective inhibition of excitatory, but not inhibitory, neurons produced antiseizure effects.<sup>91</sup> Consistent with this finding, lentivirus vector-mediated overexpression of the potassium channel Kv1.1 preferentially in excitatory neurons, which reduced neuronal excitability, suppressed seizures in a rodent model of neocortical epilepsy.<sup>102</sup>

One way to circumvent the problem of cell-population selection is to induce constitutive secretion of seizure-inhibiting factors—for example, inhibitory neuropeptides—from transduced cells. Seizure control can be achieved without targeting specific cells if the receptors for these factors are present in brain tissue affected by epileptiform activity. Promising results for gene therapy in epilepsy have been obtained by local injection of vectors containing transgenes encoding the neuropeptides galanin or neuropeptide Y (NPY). In particular, NPY-expressing AAV vectors produced robust antiseizure effects and did not have adverse effects,<sup>103–111</sup> which supports their application in the clinic.

Patients with partial epilepsies who have been selected for surgical resection of the epileptogenic area are ideal candidates for gene therapy. In such cases, brain pathology is focal, medical treatment has failed, and gene transfer of seizure-inhibitory factors (such as NPY) into the seizure-generating area might silence epileptic hyperactivity. These patients undergo implantation of depth electrodes for diagnosis before surgery, thereby obviating the need for *ad hoc* surgical intervention to inject the vector. In the event that gene therapy fails to prevent seizures, patients could undergo surgery as originally planned. Studies of gene therapy for epilepsy to date have been carried out in experimental animal models by injecting vectors directly into the epileptogenic region. However, a recombinant vector in which the capsid is a mixture of various AAV serotypes has been created that crosses only the seizure-compromised BBB,<sup>11</sup> which suggests that selective targeting of seizure-generating areas after intravenous administration of the vector may become possible.

## Brain tumours

Glioblastoma multiforme, WHO grade IV, is the brain tumour with the most aggressive disease course. Advances in surgical techniques, radiotherapy and chemotherapy have increased the median survival of patients from 6–9 months to 18–21 months.<sup>112</sup> Fatal tumours that recur are thought to originate from surviving glioma cells and/or glioma-initiating cells after therapy. Advances in viral vector development in the 1980s, coupled with the idea that vectors injected into the brain might reach tumour cells not killed by other therapies, led to the development of gene therapy approaches for brain tumours in the 1990s (Table 4).<sup>113</sup>

Initial clinical trials used a nonreplicating retrovirus vector containing an HSV thymidine kinase gene, which sensitizes transfected cells to ganciclovir treatment.<sup>114–118</sup> Researchers

aimed to transduce tumour cells with thymidine kinase, and to kill the transfected cells with a systemically administered ganciclovir prodrug toxic to tumour cells. Trials moved rapidly from early phase I to a randomized controlled phase III trial, which failed to find improvement in patient survival, possibly owing to poor intratumoural distribution of the retroviral vector and subsequent immune responses to vector-producing cells.<sup>119</sup>

Developments in the technology to produce adenoviral vectors to high titres, and data that showed extensive intratumoural vector diffusion, led to phase I–II trials of adenoviral vectors containing the thymidine kinase transgene.<sup>120–123</sup> The results of these early trials prompted a randomized control phase III trial that was completed in 2009. This trial failed to demonstrate a significant therapeutic effect.<sup>124</sup> Other gene therapy strategies have included adenovirus-mediated expression of the tumour suppressor gene *p53* and augmentation of the localized immune response through adenoviral delivery of *IFN-β*. These approaches were not developed further, however, possibly owing to limited transduction of tumour cells with *p53* or toxicity from the adenovirus-*IFN-β* construct.<sup>125,126</sup> Nevertheless, the safety of viral vectors, which has been demonstrated in clinical trials, has led to the proposed use of replication-competent oncolytic viruses to increase intratumoural vector diffusion and tumour killing. Replication-competent HSV-1, adenovirus, reovirus, measles virus, retrovirus, and Newcastle disease virus are currently being tested as vectors in early-phase clinical trials.<sup>127–130</sup>

Research continues to improve vector delivery and transgene expression, as well as vector specificity for tumour cell delivery and targeting. A promising method is the use of MRI to guide viral vector and transgene delivery, and to track vector distribution. Strategies include construction of a vector to express the ferritin reporter gene, which is detectable by MRI, and covalent binding of the viral vector to superparamagnetic iron oxide nanoparticles for detection by MRI.<sup>131,132</sup> Focused ultrasound combined with MRI to focally disrupt the BBB has been proposed to increase delivery of viral vectors to specific brain regions in a noninvasive manner via the bloodstream.<sup>133</sup> Such strategies allow controlled and focused therapeutic delivery to brain tumours.

Despite technical advances, changes to regulatory procedures, and promising results from translational studies in the past 20 years, a breakthrough in gene therapy for treatment of patients with glioblastoma multiforme is still awaited. Gene therapy strategies currently in phase I–II clinical trials include oncolytic wild-type viruses (measles virus), oncolytic viruses containing molecular therapeutics (retroviruses encoding cytosine deaminase), and adenoviral vectors that provide a combination of genes encoding cytotoxic factors and immune-stimulatory cytokines (HSV-1-thymidine kinase and Flt3L; IND14574—study NCT01811992).<sup>134–141</sup> Safety of these gene therapy strategies in early phase I–II trials provides hope for success in randomized phase III trials and improved therapeutic options for glioblastoma multiforme.

## Conclusions

The aim of this Review has been to provide an overview of promising gene therapy strategies for diseases of the nervous system. PNS diseases are the most likely to have approved treatments available within the next decade. These diseases include sensory nerve degeneration due to diabetes or chemotherapy; functional deficits of vision, hearing and smell; and chronic pain conditions. Development of gene therapy for CNS diseases is far more challenging as gene delivery trials have required surgical procedures, and the pathogenesis of many of these diseases is multifactorial and poorly understood. Moreover, CNS diseases often involve large brain regions or even the entire brain, suggesting the need for widespread gene delivery. Results in animal models, however, indicate that alternative

routes of delivery to intraparenchymal injection, combined with novel properties of vectors and proteins, might enable correction of whole-brain pathology. We anticipate that most of the scientific and technical hurdles that remain to the clinical application of gene therapy for neurological disorders will be overcome.

Other barriers to the development of gene therapy approaches include regulatory and commercial issues. It is recognized that current regulations in Europe and the USA make clinical trials of gene therapy very costly and time-consuming. In addition, we suggest that in a competitive commercial environment, the potential for disruption of existing markets by new gene therapy applications might render the biopharmaceutical industry reluctant to contribute to the development of innovative technological advances.

Opinion regarding gene therapy has evolved from it being a highly touted gene-correction strategy that can be achieved with ease, to the belief that the risk from a replication-competent vector is too great for its application to patients who are not desperately sick, to a more sober view that gene therapy might be an effective treatment or cure for some of our most difficult-to-treat diseases. Application of gene therapy to PNS disorders is rapidly maturing, whereas application to the CNS will require breakthroughs in research on targeted gene delivery, controlled transgene expression, and methods to facilitate widespread correction of brain pathology. With continued commitment from researchers in this field, gene therapy could in future make important contributions to therapeutic options for diverse neurological diseases.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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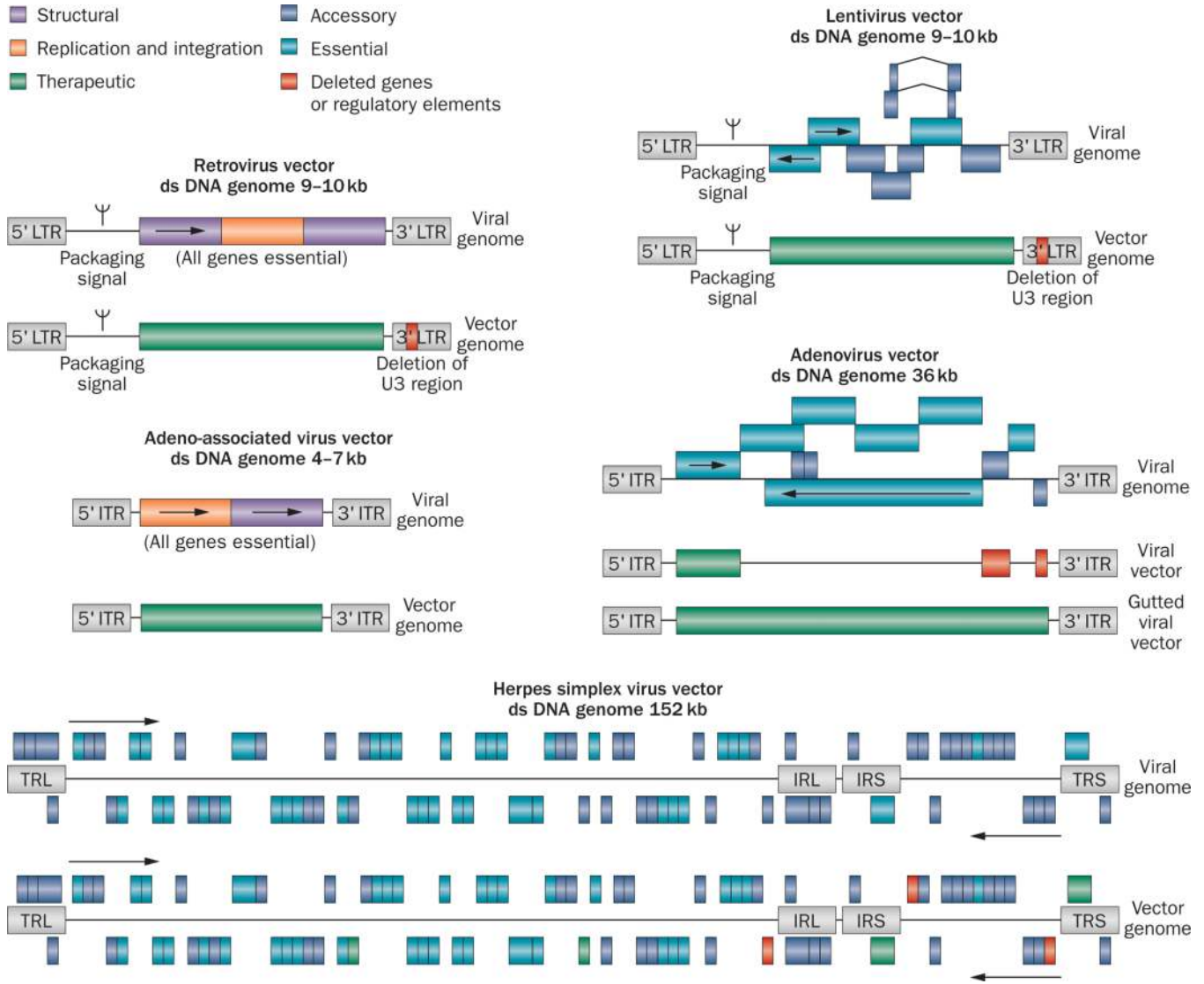
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### Key points

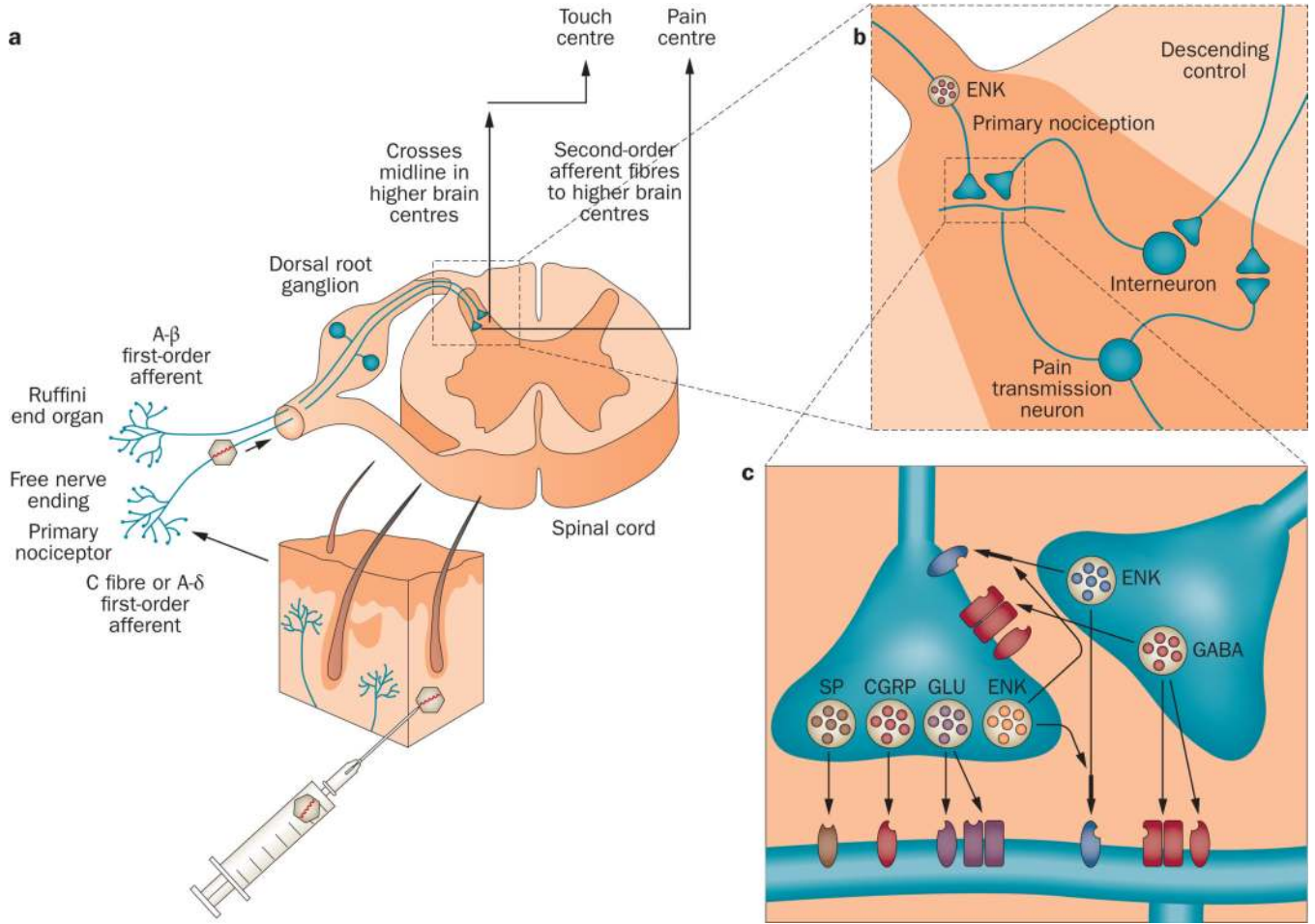
- Standard pharmacological and surgical interventions are either inadequate or unavailable for most diseases of the PNS and CNS
- Gene therapy is a viable approach to the prevention of neurological disease progression, and might offer a cure or slow down the disease process
- The efficacy of gene therapy depends on the development of gene delivery vehicles (mostly viral vectors) to target disease-modifying products to where they are needed
- Gene therapy strategies to treat some diseases that affect vision and hearing or that cause debilitating pain are at an advanced stage of development
- Gene therapy for degenerative diseases requires a more in-depth understanding of the underlying pathophysiology and, for some diseases, global brain delivery of the transgene
- With ongoing development of gene therapy applications for nervous system disease, such treatments are expected to be available to patients within 10 years

### Review criteria

Review of the literature was conducted by searching the MEDLINE database using the following terms: “gene therapy”, “genetic vectors”, “retroviridae”, “lentivirus”, “adenoviridae”, “dependovirus”, “herpesviridae”, “retina”, “cochlea”, “pain”, “brain diseases, metabolic, inborn”, “lysosomal storage diseases”, “neurodegenerative diseases”, “Parkinson disease”, “epilepsy” and “brain neoplasms”, alone and in combination. Papers were selected on the basis of title, abstract or full version (when available). The reference sections of relevant articles were checked for additional relevant articles.

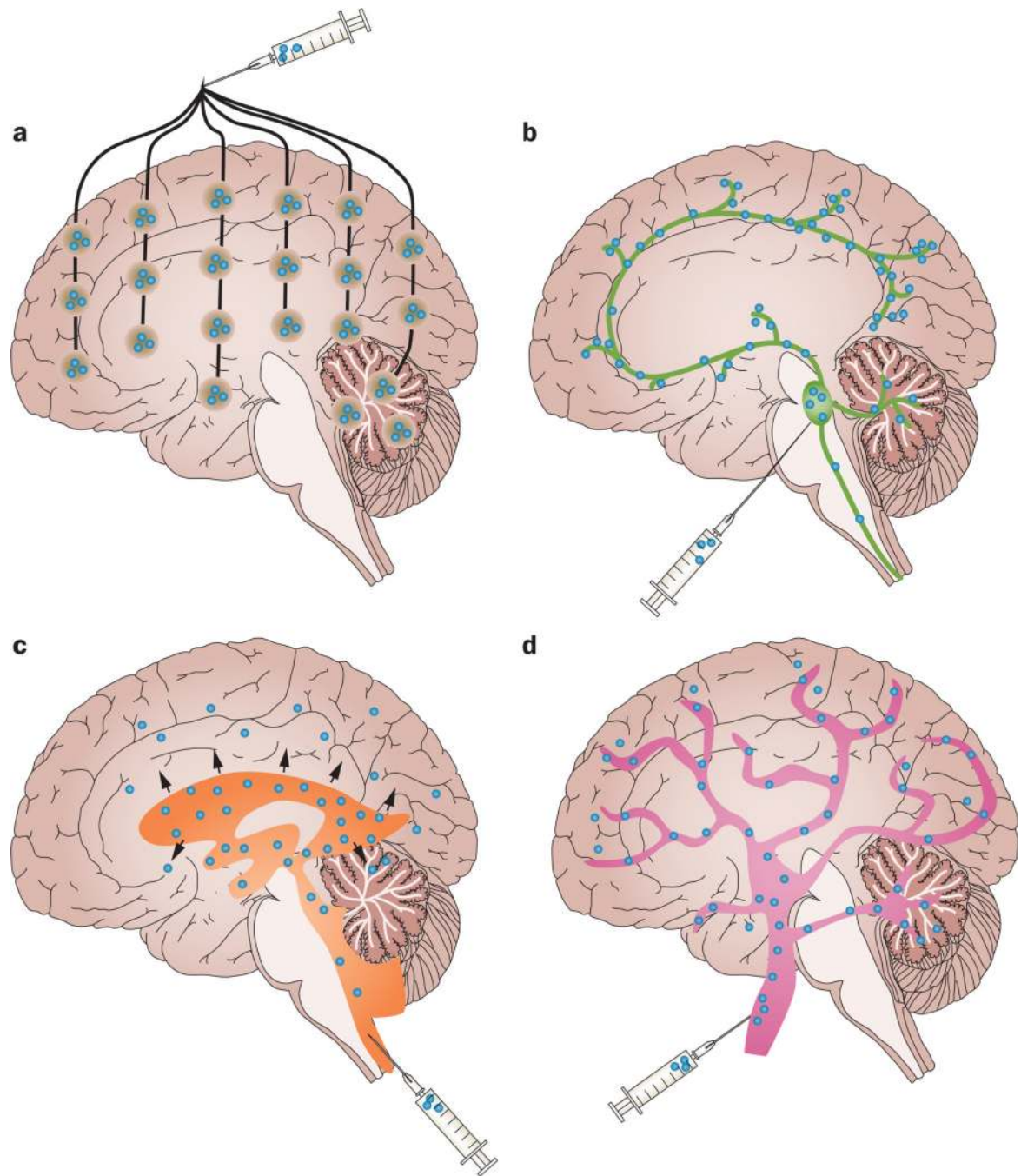


**Figure 1.** Diagrams of the genomes of various viral vectors used in gene therapy approaches. Each diagram depicts the genome of the virus along with that of the corresponding viral vector, showing viral structural genes, viral genes involved in replication, and genes essential or non-essential (accessory) for virus replication or growth. Viral genes that are transcribed in the 5' to 3' direction (rightward arrow) are depicted above the viral genome, and those transcribed in the opposite direction (leftward arrow) are depicted below the genome. Genes or regulatory elements deleted from viral vectors are shown in red and common locations for introduction of the therapeutic gene in the vector genome are depicted in green. Abbreviations: ds, double-stranded; ITR, inverted terminal repeat; IRL, inverted repeat long; IRS, inverted repeat short; LTR, long terminal repeat; TRS, terminal repeat short.



**Figure 2.** Gene therapy for pain using an HSV vector. **a,b** | Pain signalling is mediated by primary sensory afferents that connect via synapses in the spinal cord to release neurotransmitters and peptides, including glutamate, substance P and CGRP. After injection into the skin, the HSV vector is delivered to the cell bodies of primary afferents by retrograde axonal transport, enabling production and release of the transgene product (in this case ENK) from nerve terminals in the dorsal horn. **c** | ENK released from the transduced primary afferents inhibits nociceptive neurotransmission through binding to opioid receptors at presynaptic and postsynaptic sites. Abbreviations: CGRP, calcitonin gene-related peptide; ENK, enkephalin; GAD, glutamic acid decarboxylase; GLU, glutamate; HSV, herpes simplex virus; SP, substance P.

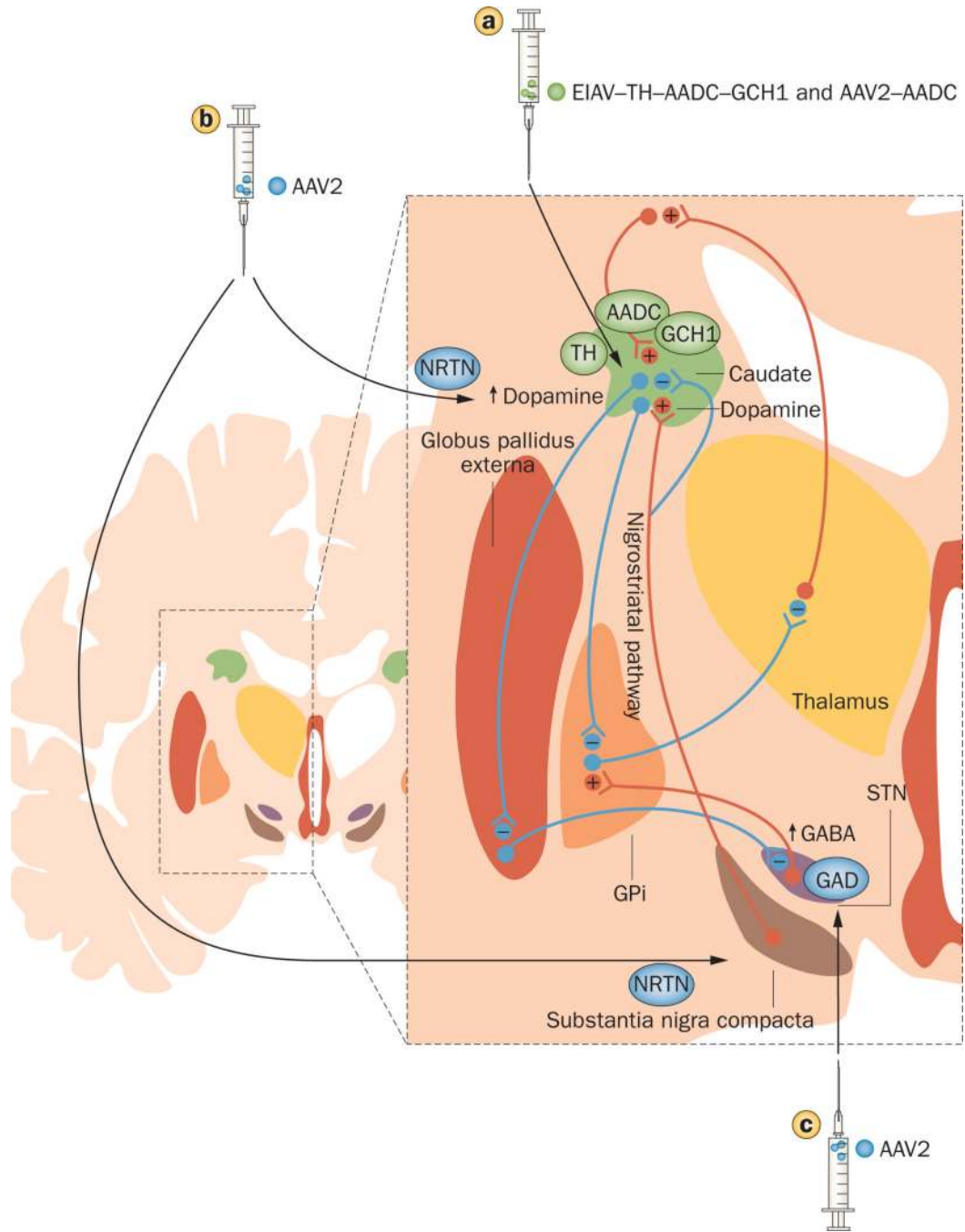




**Figure 3.**

Vector-delivery strategies for gene therapy of neurogenetic diseases. Most inherited neurological diseases have global brain pathology, which requires widespread distribution of the vector for effective treatment. Certain properties of a therapeutic gene product can enhance its therapeutic effect; for example, in diseases of lysosomal enzyme deficiency, a cell corrected by transduction with the vector can secrete the previously missing enzyme, which can then be endocytosed by neighbouring cells. Some proteins can also be transported via neural pathways within the brain, providing wide distribution. **a** | Multiple, distributed injection tracks into the brain parenchyma with multiple deposits of vector along each track.

**b** | Vector transport via axonal pathways is dependent on the specific neural system and on vector design. **c** | Injection into the cerebrospinal fluid (ventricles, cisterna magna or spinal cord) produces variable patterns and amounts of vector distribution. **d** | Vector entry into the brain via administration of herpes simplex virus to the PNS, intravenous infusion of adeno-associated virus serotypes, transplantation of lentivirus-transduced haematopoietic stem cells, or temporary osmotic opening of the blood–brain barrier.



**Figure 4.** Gene therapy targets in Parkinson disease. Excitatory connections from the cortex stimulate striatal neurons. Dopamine release regulates two populations of striatal neurons inversely: neurons that project directly to the GPi from the striatum are stimulated, and neurons that project to the GPi via the globus pallidus pars externa and STN are inhibited. Therefore, dopamine inhibits thalamic activity, which disinhibits the cortex and allows movement to occur. In PD, loss of dopaminergic neurons eliminates this cortical activation and inhibits movement. **a** | Vector injection into the caudate for the expression of dopamine producing enzymes replaces PD-related dopamine loss. **b** | Neurturin expression in the striatum and

substantia nigra might preserve dopamine neurons, and enhance their function. **c** | Delivery of *GAD* to the STN induces GABA production, changing the STN input to the GPi from excitatory to inhibitory. *GAD* expression, therefore, reverses the abnormal increase in STN activity that occurs in PD, reducing the abnormally high GPi activity that prevents movement. Abbreviations: AADC, aromatic amino acid decarboxylase; GCH1, GTP cyclohydrolase 1; GABA,  $\gamma$ -aminobutyric acid; GAD, glutamic acid decarboxylase; GPi, globus pallidus pars interna; PD, Parkinson disease; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

**Table 1**

Characteristics of viral vectors

Characteristic	Adenoviral vector	Adeno-associated viral vector		Retroviral vector		Herpes viral vector
		Murine vector	Lentiviral vector	Murine vector	Lentiviral vector	
Wild-type virus	Double-stranded linear DNA (36 kb)	Single-stranded DNA (4.7 kb)	Diploid positive strand RNA (9.2 kb)	Diploid positive strand RNA (9.2 kb)	Double-stranded linear DNA (152 kb)	
Derived from pathogenic virus	Yes	No	Yes	Yes	Yes	
Maximum size of gene insert	~7.5 kb*	~4.5 kb	~8 kb	~8 kb	~20–40 kb	
Integration into host-cell genome	No	NO <sup>‡</sup>	Yes	Yes	No	
Achievable titre	High	High	Low	Low	High	
Duration of transgene expression	Transient (days to weeks)	Long-lasting (months to years)	Long-lasting (months to years)	Long-lasting (months to years)	Transient (days to weeks) <sup>§</sup>	
Target cells	Dividing and nondividing	Dividing and nondividing	Dividing	Dividing and nondividing	Dividing and nondividing	
Safety issues	Immune and inflammatory response	Insertional mutagenesis <sup>//</sup>	Insertional mutagenesis	Insertional mutagenesis	Immune and inflammatory response	

\* 35 kb for gutless vectors.

<sup>‡</sup> Some integration at a very low frequency.

<sup>§</sup> Years in the PNS.

<sup>//</sup> Minimal compared with other vectors.

Table 2

## Gene therapy for neurodegenerative diseases

Study	Gene-delivery target	Clinical trial status	Viral vector	Therapeutic mechanism
<i>Parkinson disease</i>				
Christine <i>et al.</i> (2009) <sup>83</sup>	Stereotactic injection into putamen	Phase I	AAV2	<i>AADC</i> expression to convert levodopa to dopamine
Jarraya <i>et al.</i> (2009) <sup>80</sup> Muramatsu <i>et al.</i> (2010) <sup>75</sup>	Stereotactic injection into putamen	Phase I	Equine infectious anaemia virus	<i>AADC</i> -mediated expression of tyrosine hydroxylase and GTP cyclohydrolase 1 to stimulate autonomous dopamine production from tyrosine
Marks <i>et al.</i> (2010) <sup>92</sup>	Stereotactic injection into putamen	Phase II	AAV2	<i>NRTN</i> expression protects substantia nigra neurons, and promotes nigrostriatal regeneration and upregulation of dopamine production
Kells <i>et al.</i> (2010) <sup>142</sup>	Stereotactic injection into putamen	Phase I–II	AAV2	<i>GDNF</i> expression similar to <i>NRTN</i>
Bartus <i>et al.</i> (in press) <sup>143</sup>	Stereotactic injection into substantia nigra and putamen	Phase II	AAV2	<i>NRTN</i> expression
Lewitt <i>et al.</i> (2011) <sup>88</sup>	Stereotactic injection into subthalamic nucleus	Phase II	AAV2	Glutamic acid decarboxylase gene expression converts glutamate to $\gamma$ -aminobutyric acid, thereby increasing synaptic inhibition in the subthalamic nucleus
<i>Huntington disease</i>				
McBride <i>et al.</i> (2011) <sup>144</sup>	Stereotactic injection into striatum	Preclinical	AAV2	<i>NRTN</i> expression provides neuroprotection
Ramaswamy <i>et al.</i> (2009) <sup>145</sup>	Injection of striatum or diffuse delivery (intravenous)	Preclinical	AAV	Expression of mutant Huntingtin siRNA (allele specific)
<i>Alzheimer disease</i>				
Aravanitakis <i>et al.</i> (2007) <sup>146</sup>	Stereotactic injection into nucleus of Meynert	Phase II	AAV2	Nerve growth factor gene expression enhances cholinergic neuron protection, axonal regeneration and upregulation of acetylcholine production
Nagahara <i>et al.</i> (2009) <sup>147</sup>	Stereotactic injection into entorhinal cortex	Preclinical	AAV2	Expression of brain-derived neurotrophic factor enhances neuroprotection and axonal regeneration
<i>Amyotrophic lateral sclerosis</i>				
Suzuki <i>et al.</i> (2007) <sup>148</sup>	Injection into spinal cord	Preclinical	Lentivirus-transduced neural progenitors	<i>Ex vivo</i> gene transfer of <i>GDNF</i> to human neural progenitor cells
Franz <i>et al.</i> (2009) <sup>149</sup>	Ventral horn of spinal cord	Preclinical	AAV2	<i>IGF1</i> expression
Boulis (personal communication; trial not yet initiated)	Remote gene delivery; intravenous or intrathecal	Preclinical	AAV9	<i>IGF1</i> or <i>GDNF</i> as candidate transgenes
Kaspar <i>et al.</i> (2003) <sup>150</sup>	Remote gene delivery by retrograde axonal transport;	Preclinical	AAV2	<i>IGF1</i> or <i>GDNF</i>

Study	Gene-delivery target	Clinical trial status	Viral vector	Therapeutic mechanism
	nerve or muscle injection			Expression of mutant <i>SOD1</i> siRNA
Smith <i>et al.</i> (2006) <sup>151</sup>	Intrathecal injection	Phase I	Naked nucleic acid	Delivery of antisense oligonucleotides to target mutated <i>SOD1</i>

Abbreviations: *AADC*, aromatic amino acid decarboxylase; *AAV*, adeno-associated virus; *GDNF*, glial cell line-derived neurotrophic factor; *IGF1*, insulin-like growth factor 1; *NRTN*, neurturin; siRNA, small interfering RNA; *SOD1*, superoxide dismutase 1.

Table 3

## Gene therapy for epilepsy

Protein encoded by transgene	Vector	Time of treatment	Effects	References
Fibroblast growth factor 2 and brain-derived neurotrophic factor	HSV-1	Latency (4 days after epileptogenic insult [pilocarpine])	Antiepileptogenic: reduced seizure frequency and severity Disease-modifying: attenuated epileptogenesis-associated pathology; reduced cell loss, neuroinflammation and mossy fibre sprouting; increased neurogenesis	Paradiso <i>et al.</i> (2009) <sup>97</sup> Bovolenta <i>et al.</i> (2010) <sup>98</sup> Paradiso <i>et al.</i> (2011) <sup>99</sup>
$\gamma$ -aminobutyric acid receptor A subunit $\alpha 1$	AAV2	Before epileptogenic insult (pilocarpine)	Antiseizure: decreased percentage of animals with spontaneous seizures	Raol <i>et al.</i> (2006) <sup>101</sup>
<i>N</i> -methyl-D-aspartate subunit NR1*	AAV2	Before inferior colliculus stimulation	Antiseizure or proseizure, depending on promoter used and transduced cell type	Haberman <i>et al.</i> (2002) <sup>100</sup>
Galanin	AAV2	Before epileptogenic insult (kainate) Epileptic animals (fully kindled)	Antiseizure: increased seizure threshold, reduced seizure frequency and severity, reduced number of animals experiencing seizures Disease-modifying: reduced cell loss	Haberman <i>et al.</i> (2003) <sup>103</sup> Lin <i>et al.</i> (2003) <sup>104</sup> McCown <i>et al.</i> (2006) <sup>106</sup>
NPY	Chimaeric AAV1/2	Before epileptogenic insult (kainate or kindling) In chronic period (with spontaneous seizures) after epileptogenic insult (self-sustained status epilepticus)	Antiseizure: increased latency to seizure, reduced seizure frequency and duration, and slowed kindling development Disease-modifying: arrested disease progression No adverse reactions: no alterations in learning and memory, anxiety or locomotor activity	Richichi <i>et al.</i> (2004) <sup>105</sup> Foti <i>et al.</i> (2007) <sup>107</sup> Noe <i>et al.</i> (2008) <sup>108</sup> Sorensen <i>et al.</i> (2009) <sup>109</sup> Noe <i>et al.</i> (2010) <sup>110</sup>
NPY and NPY2R	Chimaeric AAV1/2	Before epileptogenic insult (kindling)	Antiseizure effects more potent than with NPY alone	Woldbye <i>et al.</i> (2010) <sup>111</sup>
Glial cell line-derived neurotrophic factor	AAV2	Before epileptogenic insult (kindling, self-sustained status epilepticus)	Antiseizure: increased seizure threshold, prevented seizure generalization, reduced seizure severity and mortality	Kanter-Schlifke <i>et al.</i> (2007) <sup>152</sup>
Adenosine kinase*	AAV8	Epileptic animals (spontaneously seizing adenosine kinase transgenic mice)	Antiseizure: reduced frequency of spontaneous seizures	Theofilas <i>et al.</i> (2011) <sup>153</sup>
ICP10PK (antiapoptotic protein)	HSV-2	Before epileptogenic insult (kainate)	Antiseizure: prevented seizures Disease-modifying: prevented neuronal loss and inflammation	Laing <i>et al.</i> (2006) <sup>154</sup>
Voltage-gated potassium channel subunit Kv1.1	Lentivirus	During or after epileptogenic insult (tetanus toxin in motor cortex)	Antiepileptogenic: prevented epileptiform events after administration during epileptogenic insult Disease-modifying: reduced frequency of epileptiform events following administration in established epilepsy	Wykes <i>et al.</i> (2012) <sup>102</sup>



\* Antisense DNA.

Abbreviations: AAV, adeno-associated virus; HSV, herpes simplex virus; NPY, neuropeptide Y; NPY2R, NPY2 receptor.

Table 4

Clinical trials of gene therapy for brain tumours

Study	Study status	Vector	Transgene	Route of administration	Efficacy
Papanastassiou <i>et al.</i> (2002) <sup>127</sup>	Phase I	HSV-1 (1716 strain)	Wild-type replication-competent virus	Intratumoural	25% of patients disease-free for 15–22 months
Chiocca <i>et al.</i> (2004) <sup>128</sup>	Phase I	Adenovirus (ONYX-015)	Wild-type replication-competent virus	Resection cavity	No significant difference in patient survival
Forsyth <i>et al.</i> (2008) <sup>129</sup>	Phase I	Reovirus	Wild-type replication-competent virus	Intratumoural	8% of patients disease-free for >6 months
Markert <i>et al.</i> (2000) <sup>134</sup>	Phase I	HSV-1 (G207 strain)	Wild-type replication-competent virus	Intratumoural	29% of patients with improved neurological status, 38% with decreased tumour volume on MRI
Rampling <i>et al.</i> (2000) <sup>135</sup>	Phase I	HSV-1 (1716 strain)	Wild-type replication-competent virus	Intratumoural	33% of patients with decreased tumour volume on MRI, 56% with stable tumour volume on MRI
Freeman <i>et al.</i> (2006) <sup>136</sup>	Phase I–II	Newcastle disease virus (HUIJ strain)	Wild-type replication-competent virus	Intravenous	7% of patients with complete response, 21% long-term survivors (range 61–66 weeks)
Ram <i>et al.</i> (1997) <sup>114</sup>	Phase I	Retrovirus	HSV-1–thymidine kinase	Intratumoural	Modest antitumour activity in 27% of patients, 7% recurrence-free for 50 months
Izquierdo <i>et al.</i> (1996) <sup>115</sup>	Phase I	Retrovirus	HSV-1–thymidine kinase	Intratumoural	No significant difference in patient survival
Palu <i>et al.</i> (1999) <sup>139</sup>	Phase I	Retrovirus	HSV-1–thymidine kinase/IL-2	Intratumoural	25% of patients showed >50% reduction in tumour volume on MRI
Chiocca <i>et al.</i> (2011) <sup>138</sup>	Phase Ib	Retrovirus	HSV-1–thymidine kinase	Resection cavity	2-year survival 33%, 3-year survival 25%
Shand <i>et al.</i> (1999) <sup>116</sup>	Phase I–II	Retrovirus	HSV-1–thymidine kinase	Resection cavity	15% of patients showed stable tumour volume by MRI at >7 months
Klatzmann <i>et al.</i> (1998) <sup>117</sup>	Phase I–II	Retrovirus	HSV-1–thymidine kinase	Resection cavity	Median survival 17.4 months, 25% of patients showed stable tumour volume on MRI at >4 months
Prados <i>et al.</i> (2003) <sup>118</sup>	Phase I–II	Retrovirus	HSV-1–thymidine kinase	Resection cavity or intraventricular	Median survival 8.4 months
Immonen <i>et al.</i> (2004) <sup>122</sup>	Phase IIIb	Retrovirus	HSV-1–thymidine kinase	Resection cavity	Median survival prolonged from 37.7 weeks to 62.4 weeks
Rainov (2000) <sup>109</sup>	Phase III	Retrovirus	HSV-1–thymidine kinase	Resection cavity	No significant difference in patient survival

Study	Study status	Vector	Transgene	Route of administration	Efficacy
Trask <i>et al</i> (2000) <sup>120</sup>	Phase I	Adenovirus	HSV-1–thymidine kinase	Intratumoural	2-year survival in 25% of patients
Germano <i>et al</i> (2003) <sup>121</sup>	Phase I	Adenovirus	HSV-1–thymidine kinase	Resection cavity	Median survival 59 weeks
Smitt <i>et al</i> (2003) <sup>155</sup>	Phase I	Adenovirus	HSV-1–thymidine kinase	Resection cavity	No objective radiological response
Chiocca <i>et al</i> (2008) <sup>125</sup>	Phase I	Adenovirus	IFN- $\beta$	Intratumoural	Median survival 17.9 weeks, median progression-free survival 9.3 weeks
Lang <i>et al</i> (2003) <sup>126</sup>	Phase I	Adenovirus	p53	Intratumoural	Median survival 10 months, 7% of patients recurrence-free at 3 years
Sandmair <i>et al</i> (2000) <sup>123</sup>	Phase IIa	Adenovirus	HSV-1–thymidine kinase	Resection cavity	Median survival 15 months versus 8.3 months in controls

Abbreviation: HSV-1, herpes simplex virus type 1.