regulated expression of immunomodulatory genes.

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Two conclusions derive from the fact that gene therapy works, and what untoward effects to further our understanding of how gene therapy is pharmacology, as with all drugs, 'side effects' will occur. It thus becomes of importance to study and look for the 'side effects' of gene therapy to further our understanding of how gene therapy works, and what untoward effects to predict.

The basic science of brain-tumour gene therapy

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Gene therapy

Two conclusions derive from the fact that gene therapy is pharmacology using nucleic acids as drugs. First, a realization that even if most current implementations of gene therapy aim at the use of single therapeutic strategies, it will be more likely to succeed when several strategies are used in combination, as in the classical pharmacological treatment of disease. Further, this contradicts the approach to gene therapy as a new (and single) 'magic bullet'. Gene therapy does not provide 'magic bullets'; it provides a novel way of implementing the ideas and principles of pharmacology for the treatment of human disease because it uses nucleic acids instead of small chemical compounds. Thus, instead of administering an active compound, gene therapy administers nucleic acids encoding the active chemicals, harnessing the combinatorial power of modern molecular biology. Secondly, these premises also predict the existence of 'side effects' of gene therapy. If gene therapy is pharmacology, as with all drugs, 'side effects' will occur. It thus becomes of importance to study and look for the 'side effects' of gene therapy to further our understanding of how gene therapy works, and what untoward effects to predict.

Implementing gene therapy for the treatment of neurological disease: the rationale for gene therapy for brain tumours

Several reasons demonstrate that high-grade malignant brain gliomas are a reasonable target for gene therapy. The disease is life threatening. Patients diagnosed with glioblastoma multiforme survive between 6 and 12 months, even when
treated with aggressive combined surgery, chemotherapy and radiotherapy [1]; thus, treatment ought to be started as soon after the diagnosis is made as possible.

Concerning which area of the brain needs to be transduced in clinical trials of glioblastoma, two scenarios can be envisaged. Either tumour tissue is removed, and vectors injected into the peritumoural tissue, or tumours could be injected directly with vectors. If much of the tumour mass can be removed, the vector should be injected into the peritumoural area, where the presumed infiltrating tumour cells are located. If however, the tumour mass cannot be removed, direct injections into tumour tissue will attempt to reduce tumour mass. Direct transduction of tumour cells will be the aim of most tumour strategies; however, immune-stimulatory strategies aimed at increasing the brain levels of cytokines may not require a direct transduction of tumour cells [2]. Also, viral vectors can encode for secreted tumour-specific cytotoxins. Thus, direct transduction of tumour cells is not a necessary requirement for gene therapy.

Achieving an adequate level of transgene expression does not appear to be a limiting step for either experimental or clinical gene-therapy trials, since high levels of expression can be obtained using various available vectors and promoters. Currently, there is much interest in the transcriptional targeting and regulated expression of transgenes. This will allow the restricted expression of transgenes to either tumour cells or particular brain cells, i.e. astrocytes. Also, direct viral-vector targeting is constantly improving our capacity to selectively transduce predetermined cell types.

Lack of stable, long-term transgene expression currently limits the implementation of gene therapy for chronic neurodegenerative disorders. Whether long-term gene expression is needed for the treatment of brain tumours remains unclear. Current available gene-transfer vectors (either retroviruses or adenoviruses) mainly provide short-term gene expression. Nevertheless they have been used, following the rationale that only short-term transgene expression is needed to kill the main tumour mass. However, it is likely that longer term, stable, and inducible expression of proteins toxic for tumour cells will enhance the therapeutic prospects of brain-tumour gene therapy, and especially tumour recurrence. Adeno-associated virus (AAV) vectors and lentiviral vectors should achieve long-term stable transgene expression. They have not yet been used in brain-tumour gene-therapy applications.

Mechanisms responsible for the failure to achieve long-term expression remain unclear. This is especially since up until now both integrating and non-integrating vectors have shown short-term expression. Importantly, integration of vector genome into the target cell's genome is not a prerequisite for long-term expression. This is most clearly demonstrated by herpes simplex virus type 1 (HSV1) latency in dorsal root ganglion neurons. In these cells, the large HSV1 genome remains episomally within the nuclei of non-dividing neurons for the life of the host. Under various conditions, the virus reactivates, causing renewed disease. Latent genomes, however, cannot be eliminated from the dorsal root ganglion neurons.

This raises the question of whether other large DNA viruses will also be able to remain episomally in the nuclei of target cells. This has not yet been described, and thus remains to be explored. Adenoviruses, for example, are not known to establish latency in their human hosts. However, adenovirus-derived vectors need not behave as wild-type adenovirus. Adenoviral vectors are able to deliver their genome to the nuclei of infected cells. While a wild-type genome will initiate replication which leads to the death of the infected cells, a viral vector can only express those transgenes containing appropriate promoter sequences. Thus, in the absence of replication-induced death of host cells, the hypothesis has to be considered that genomes of viral vectors could remain episomally within nuclei of infected cells. Whether they continue to express transgenes will then depend on the characteristics of their genomes, and transcriptional control regions contained within the viral vectors' genome.

**Which brain tumours could be treated by gene therapy?**

Most, but not all, brain tumours carry a dire prognosis (see Table 1). The main brain-tumour classification is based on tumour histology. Brain tumours are further classified into malignant or benign forms, according to the differentiation of constituent cell types [3-6]. However, even histologically benign tumours can be inaccessible to resective surgery, because of their intracranial location close to essential brain centres or large blood vessels. Thus, although benign according to histology, they can be highly malignant due to anatomical location.
Table 1

Classification of brain tumours

<table>
<thead>
<tr>
<th>Classification</th>
<th>Peak age incidence</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Astrocytic tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 Pilocytic astrocytoma (grade I)</td>
<td>0–20</td>
<td>Good</td>
</tr>
<tr>
<td>1.2 Low-grade diffuse astrocytoma (grade II)</td>
<td>20–50</td>
<td>Poor</td>
</tr>
<tr>
<td>1.3 Anaplastic astrocytoma (grade III)</td>
<td>20–70</td>
<td>Poor</td>
</tr>
<tr>
<td>1.4 Glioblastoma multiforme (grade IV)</td>
<td>30–70</td>
<td>Very poor</td>
</tr>
<tr>
<td>1.5 Giant cell glioblastoma</td>
<td>30–80</td>
<td>Poor</td>
</tr>
<tr>
<td>1.6 Gliosarcoma</td>
<td>40–70</td>
<td>Poor</td>
</tr>
<tr>
<td>1.7 Pleomorphic xanthoastrocytoma</td>
<td>0–18</td>
<td>Poor</td>
</tr>
<tr>
<td>2. Oligodendroglial tumours and mixed gliomas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 Oligodendroglioma</td>
<td>20–70</td>
<td>Ip deletion, good; otherwise, poor</td>
</tr>
<tr>
<td>2.2 Anaplastic oligodendroglioma</td>
<td>20–80</td>
<td>Very poor</td>
</tr>
<tr>
<td>2.3 Oligoastrocytoma</td>
<td>20–50</td>
<td>Very poor</td>
</tr>
<tr>
<td>2.4 Anaplastic oligoastrocytoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Ependymal tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 Ependymoma and variants</td>
<td>0–60</td>
<td>Very poor</td>
</tr>
<tr>
<td>3.2 Anaplastic ependymoma</td>
<td>0–60</td>
<td>Very poor</td>
</tr>
<tr>
<td>3.3 Myxopapillary ependymoma</td>
<td>0–60</td>
<td>Good</td>
</tr>
<tr>
<td>3.4 Subependymoma</td>
<td>0–60</td>
<td>Good</td>
</tr>
<tr>
<td>4. Choroid plexus tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.1 Choroid plexus papilloma</td>
<td>0–20</td>
<td>Very good</td>
</tr>
<tr>
<td>4.2 Choroid plexus carcinoma</td>
<td></td>
<td>Poor</td>
</tr>
<tr>
<td>5. Neuroepithelial tumours of uncertain origin</td>
<td>Young adults</td>
<td></td>
</tr>
<tr>
<td>5.1 Astroblastoma</td>
<td>Children</td>
<td>Very poor</td>
</tr>
<tr>
<td>5.2 Polar spongioblastoma</td>
<td>0–80</td>
<td>Very poor</td>
</tr>
<tr>
<td>5.3 Gliomatosis cerebri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Neuronal and mixed neuronal-glial tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1 Gangliocytoma and glioglioma</td>
<td>0–40</td>
<td>Good</td>
</tr>
<tr>
<td>6.2 Desmoplastic infantile astrocytoma and ganglioglioma</td>
<td>3–10</td>
<td>Good</td>
</tr>
<tr>
<td>6.3 Dyssembryoplastic neuroepithelial tumours</td>
<td>0–30</td>
<td>Benign</td>
</tr>
<tr>
<td>6.4 Central neurocytoma</td>
<td>10–40</td>
<td>Benign</td>
</tr>
<tr>
<td>6.5 Paraganglioma</td>
<td>20–70</td>
<td>Good</td>
</tr>
<tr>
<td>7. Pineal tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.1 Pineoblastoma</td>
<td>0–40</td>
<td>Poor</td>
</tr>
<tr>
<td>7.2 Pineocytoma</td>
<td>10–70</td>
<td>Good</td>
</tr>
<tr>
<td>8. Embryonal tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.1 Medulloepithelioma</td>
<td>0–5</td>
<td>Very poor</td>
</tr>
<tr>
<td>8.2 Central neuroblastoma and ganglioneuroblastoma</td>
<td>0–5</td>
<td>Very poor</td>
</tr>
<tr>
<td>8.3 Ependymoblastoma</td>
<td>0–5</td>
<td>Very poor</td>
</tr>
<tr>
<td>8.4 Medulloblastoma</td>
<td>0–40</td>
<td>Poor</td>
</tr>
<tr>
<td>8.5 Medulomyoblastoma</td>
<td>0–10</td>
<td>Very poor</td>
</tr>
<tr>
<td>8.6 Melanotic medulloblastoma</td>
<td>0–10</td>
<td>Very poor</td>
</tr>
<tr>
<td>8.7 Lipomatous medulloblastoma</td>
<td>40–60</td>
<td>Good</td>
</tr>
<tr>
<td>8.8 Supratentorial PNET</td>
<td>0–10</td>
<td>Poor or very poor</td>
</tr>
<tr>
<td>8.9 Atypical teratoid/rhabdoid tumours</td>
<td>0–18</td>
<td>Very poor</td>
</tr>
<tr>
<td>9. Meningeal tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.1 Meningiomas</td>
<td>30–80</td>
<td>Good</td>
</tr>
</tbody>
</table>

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Most cell types in the brain can originate brain tumours. The most common and aggressive ones are those derived from the brain astroglial cells, the gliomas [7]. Age is usually a good prognostic factor in gliomas (younger patients survive longer), although neuronal and neuroepithelial tumours of young children do carry a very poor prognosis. Even so, median survival in high-grade glioblastoma is 6–12 months post-diagnosis. Thus, there is no doubt that new treatments are needed for these aggressive, or inoperable, brain tumours.

Gene therapy offers the possibility of developing completely novel approaches to therapy [8]. Thus, a whole new variety of ways of disposing of tumour cells have been developed by this highly active, newly emerged field, which is now being adapted to the treatment of brain tumours. Indeed, the first randomized, double-blind, clinically controlled trial of gene therapy versus conventional therapy has been recently performed for the treatment of glioblastoma. This multicentre trial has been implemented by Novartis; although it is widely known that this trial has now been stopped it is not known when results will be made available to the scientific public.

Even in cases of major resection, tumours recur within 3–6 months. 80% of recurrences lie within the margins of the resection. This is mostly taken as evidence for the existence of locally infiltrating tumour cells located beyond the surgical margins. However, extension of glioblastoma resection is not associated with extended survival. Heroic major hemispherectomies in the past have not improved survival. Alternatively, glioblastoma multiforme is a diffuse disease of brain astrocytes. Thus, removing the main mass only allows the disease to appear at a novel site. The existence of diffuse gliomatosis could be used to support this argument, which has not yet been explored thoroughly.

Thus, it is currently accepted that infiltration into surrounding normal brain is the main challenge in developing a cure for gliomas. While the main tumour can be resected, infiltrating cells cannot be easily visualized, even at the histological...
level. Furthermore, infiltrating cells are thought to exit the cell cycle during migration. In theory at least, and possibly in clinical practice, this renders them resistant to all kinds of therapies which selectively target dividing cells, i.e. radiotherapy, chemotherapy and conditional cytotoxicity using retroviral vectors.

Other aspects of glioblastoma, such as the high degree of tumour vascularization, relatively slow growth and lack of distant metastasis, can be used as potential novel targets for tumour treatment. Although tumours are highly vascularized, glioblastomas also display large areas of tumour necrosis. Such areas are an index of high malignancy, and thus are an indicator of poor prognosis. The existence of necrosis suggests the presence of areas of hypoxia within the tumours. On the one hand, this will determine tumour radioresistance; on the other, this could be used to drive the expression of cytotoxic transgenes under the control of hypoxia-sensitive transcriptional elements within restricted areas of glioblastomas.

Further, patients suffering from glioblastoma are immune-suppressed. Several parameters of immune function, such as delayed-type hypersensitivity responses, are all impaired. The causes of the profound immune-suppression have not been completely elucidated. It is likely, however, that the tumours themselves secrete factors which inhibit immune responses. What is not understood is how tumour-released factors can achieve such a powerful and generalized immune-suppression. Importantly, it has been shown that glioblastomas secrete large amounts of the powerful immune-suppressant transforming growth factor-β, which can act on various inflammatory and specific immune cells to down-regulate their activity.

Many gliomas also produce Fas-ligand, which induces apoptosis of activated cytotoxic T-lymphocytes that express high levels of the apoptosis-inducing receptor, Apo-1. Further, patients suffering from brain tumours receive high doses of the powerful anti-inflammatory and anti-immune steroid dexamethasone to inhibit brain oedema, which is also caused by the tumours themselves. Dexamethasone has many effects on the tumour and peritumoural tissue. It is administered for its powerful anti-oedema actions, but it also has direct anti-tumoural effects, while it also inhibits the efficiency of chemotherapeutic agents used clinically.

Immune-stimulation has also been used clinically in patients with glioblastoma. Direct administration of interleukin-2, or lymphokine-activated killer (LAK) cells, or in vitro-modified tumour-infiltrating lymphocytes (TIL) has been attempted. So far, little clinical benefit has been detected. Nevertheless, recent strategies demonstrating that patients can be immunized to recognize specific tumour antigens raises hopes that future immune-stimulatory strategies may still work. The risk of initiating an auto-immune disease needs to be weighed against the otherwise dire prognosis of these patients.

Much work has been done on the molecular genetics of brain tumours. Although several molecular genetic lesions have been found, and correlate with progressive changes in malignity so far the clinical implications of these data remain unclear. Such lesions involve cyclin-dependent kinase inhibitor 2A (CDKN2A) deletions, mutated in advanced cancers 1 (MMAC1) alterations, epidermal growth factor receptor (EGFR) amplifications, or mutations in the tumour-suppressor gene p53. However, a recent retrospective study of oligodendroglioma has demonstrated an improved response to treatment and thus prolonged survival in patients with a deletion in the chromosome 1p. Thus, it is expected that molecular genetic characterization of tumours will eventually provide more accurate predictors for the response of individual tumours to treatment.

**Gene-therapy strategies for the treatment of brain tumours**

Essentially three different strategies have been developed as part of the gene-therapy programme for brain tumours: tumour cytotoxicity, anti-tumour immune-stimulation and inhibition of angiogenesis (Table 2) [9–13]. Cytotoxic approaches attempt to kill tumour cells directly through the expression of cellular toxins, or indirectly through the expression of conditionally cytotoxic prodrug-converting enzymes administered in combination with prodrugs. Alternatively, other strategies have proposed the correction of mutated tumour-suppressor function in gliomas.

A major problem in both strategies is how to target relatively large, and infiltrating, tumours. Replication-competent viruses, which selectively replicate within tumour cells, are already being used in clinical trials. The rationale is that these viruses will replicate through tumour cells, but spare the non-tumoural cells. Tumour cells are killed because the virus replicates in them.
Gene-therapy strategies for the treatment of brain tumours

GM-CSF, granulocyte macrophage colony-stimulating factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

I. Tumour cell killing

I.1 Conditional cytotoxicity

HSV1-TK/ganciclovir
Escherichia coli cytosine deaminase and 5-fluorocytosine
E. coli nitroreductase and CB1954
Cytochrome P450 and cyclophosphamide
Carboxypeptidase G2 and 5-{[(2-chloroethyl)-5-fluorocytosine}(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid (CMDA)

I.2 Direct cytotoxicity

Tetanus toxin
Pseudomonas exotoxin A
Diphtheria toxin A
Fas-ligand

I.3 Correction of tumour-suppressor function

p53; p16; p21; retinoblastoma

I.4. Replication-competent viruses

HSV1 I17; HSV1 G207: adenovirus-derived Onyx-15

2. Immunomodulation

2.1 Cytokines

Interleukins 2 and 4; GM-CSF; interferon γ

2.2 Inhibition of TGFβ

Antisense TGFβ; TGFβ-soluble receptors; decorin

2.3. Dendritic cell priming

In vitro priming; DNA vaccines; viral transduction with tumour antigens

3. Anti-angiogenesis

Endostatin; angiotatin; antisense VEGF; antisense bFGF

Immune-stimulation has been attempted before in gliomas, but no clear responses have yet been observed. Furthermore, the potential breaking of tolerance against brain-specific antigens and the development of auto-immune responses as a consequence constitute a real risk. Nevertheless, activated lymphocytes would be powerful cells to identify small islands of infiltrating tumour cells amid normal brain cells. The recent demonstration that breaking of tolerance for normal melanocyte antigens results in clinical benefit in melanoma patients makes this a realistic prospect. Moreover, it has been argued that an auto-immune brain disease may be a small price to pay for eradicating glioblastoma multiforme.

Brain tumours are highly vascularized, and thus, the blood vessels have been identified as a further target. Both drugs and gene-therapy approaches aiming to inhibit blood-vessel growth have been proposed and are under clinical investigation. Recent progress in our discovery of anti-angiogenic peptides clearly opens up a promising field for gene-therapy approaches.

First generation gene-therapy clinical trials

The failure of brain tumours to respond to combined aggressive treatments encourages the use of novel therapies, such as gene therapy. What gene therapy can provide are alternative ways of either killing the tumour cells directly, inhibiting tumour blood vessel growth, sensitizing cells to conventional radio- and chemotherapy, or stimulating the immune response to brain tumours. In addition, selective replication of viruses within tumours could provide tumour elimination by direct tumour cell killing during viral replication. We will now briefly provide an overview of current clinical gene-therapy approaches to brain-tumour therapy.

The main strategies used clinically so far have been: (i) to inject defective viral vectors into the margins of the resected tumour (i.e. retroviral and adenoviral vectors); (ii) to inject defective viral vectors directly into inoperable tumours (i.e. retroviral and adenoviral vectors); and (iii) to inject tumour-restricted replication-competent viral vectors directly into the tumours [i.e. adenoviruses (Onyx-15) and HSV1 vectors (i.e. HSV1 1716; G207)]. Anti-angiogenic, directly cytotoxic and immune-stimulatory strategies are currently in development. Importantly, our laboratory is currently testing the effectiveness of various gene-therapy strategies for the killing of primary cell lines of human glioblastoma in vitro [14]. We expect these experiments to assess the sensitivity of particular human glioma cells to various gene-therapy strategies.

Retroviral-mediated brain-tumour gene therapy

Retroviruses predominate as the vector system of choice for the majority of human gene-therapy clinical trials, including those to treat the primary brain tumour. Retroviruses are RNA viruses which have the ability to stably integrate their genome into the host genome of many cell types.
virus have been heavily exploited. They also have viruses such as the Moloney murine leukaemia virus been heavily exploited. They also have an inability to integrate their DNA into non-dividing cells [16], but for the treatment of cancer this can be advantageous, as the growing tumour is selectively targeted. New research is focusing on the development of lentivirus vectors based on HIV [17].

Clinical trials examining the efficiency of retroviral vectors encoding transgenes for the treatment of malignant gliomas have been performed. Retroviral gene therapy is being tested in conjunction with classical treatments; thus, partial anti-tumour responses, stabilization of the disease and survival extended significantly beyond 24 months would be considered as a breakthrough.

Clinical trials using suicide-gene therapy with the enzyme prodrug combination HSV1 thymidine kinase (HSV1-TK)/ganciclovir are being conducted. Direct in vivo tumour-targeting with retrovirus is limited by the inefficient gene transfer using low titres of virus. Implantation of xenogeneic murine retroviral producer cell lines has thus been attempted to improve tumour transduction. The first phase-I clinical trial used suicide-gene therapy with a single stereotactic injection of producer cells into the brain tumour followed by repeated systemic ganciclovir treatment. Partial regression was observed post treatment, which following analysis of a tumour biopsy showing only 1% transduction, was attributed to the bystander effect [18]. Phase-II trials using repeat dosing every 40 days, via a catheter into the cavity created following malignant brain-tumour resection, achieved an average survival time of 25 weeks. Magnetic resonance imaging showed evidence of a modest tumour response and transient inflammatory response [19]. The results of a large phase-II randomized trial in the U.S.A., Germany and Canada are now awaited [20]. Other phase-I/II trials using HSV1-TK retroviral vector-producing cells show the treatment to be well tolerated and associated with a significant therapeutic response with 25% of the patients surviving more than 12 months [21].

**Adenoviral-mediated brain-tumour gene therapy**

Recombinant adenoviruses (rAds) possess several characteristics which make them attractive candidate vehicles for gene therapy: rAds are easily propagated, the non-enveloped virus particles are stable and therefore readily concentrated to high titres and they are potentially safe, as wild-type adenoviruses are only mildly pathogenic in humans and are generally non-integrating. A major advantage over other viral vectors, however, is the ability of rAds to mediate efficacious gene transfer to a wide variety of cell types, including post-mitotic cells.

The majority of adenoviral vectors developed to date are derived from human serotypes 2 and 5, since the biology and genetics of these viruses have been intensively investigated. Adenovirus vectors are rendered replication defective by the deletion of the E1 region (which activates the expression of all the other viral early genes) and are propagated in the human embryonic kidney-derived 293 cell line which provides the E1A and E1B functions in trans [22]. rAds encoding a transgene of interest are generated by several different mechanisms, including homologous recombination within 293 cells between the ‘backbone’ of the viral genome and a plasmid containing the expression cassette flanked by regions of viral DNA [23].

First-generation E1/E3-deleted adenoviruses have been used to successfully deliver genes to a wide variety of tissues and organs in animal models of human disease and also in human phase-I clinical trials for cystic fibrosis [24,25] and cancer [26]. The rationale for the design of less-immunogenic, second-generation rAds has been either to down-regulate the anti-virus immune response by re-introducing viral genes with immunomodulatory functions into the vector backbone, or to reduce ‘leaky’ viral gene expression by creating further deletions/mutations within additional regions of the genome, i.e. the E2 or E4 regions.

Most recently a new generation of ‘gutless’ rAds have been developed which are deleted of all viral genes [27,28]. Such vectors have a minimum requirement for the extreme termini of the linear adenovirus genome, containing simply the inverted terminal repeats and the packaging signal and, as such they have a potential cloning capacity close to the size of the native genome (36 kb). Gutless vectors are co-propagated in 293 cells with an E1-deleted helper virus, which transcomplements for all the missing viral functions. The helper virus is normally subjected to negative selection to allow the amplification of the gutless virus and, in the most sophisticated system, the loxP-flanked packaging signal within the helper virus is removed during reiterative amplification of the gutless virus in 293 cells which stably express the loxP-specific Cre recombinase from bacterio-
phage P1. The results of initial in vivo studies have demonstrated the reduced immunogenicity and enhanced safety of gutless viruses compared with first-generation ones, leading to prolonged transgene expression. Further confirmation of these advantages may signal renewed optimism in the viability of adenoviruses as safe and effective gene-delivery vehicles.

So far, only first-generation adenoviral vectors have been used for the treatment of brain tumours. Trials in the U.S.A. and Europe have utilized adenoviruses expressing the conditional cytotoxic gene TK from HSV1 [29]. Also, adenoviruses expressing the marker gene β-galactosidase have been used to demonstrate gene transfer into human glioblastoma in vivo. Trials using adenoviruses encoding HSV1-TK in conjunction with ganciclovir have been ongoing, but results of clinical trials have not yet been made available. In one recent report, it was suggested that adenoviruses appeared to be more efficient than retroviruses expressing the same HSV1-TK. Publication of these results ought to allow a thorough evaluation of the data.

Alternatively, adenovirus mutants, originally selected for unrelated scientific purposes, have now been used for tumour treatment. A particular mutant, Onyx-15, is deleted for an E1B region necessary for growth in cells containing functional p53. Thus, in the presence of functional p53, such mutants cannot replicate, while in its absence the virus is free to replicate and grow within tumour cells in vivo. Such a virus has now been used in a clinical trial for head and neck tumours, and a clinical protocol to utilize such viruses for the treatment of brain tumours has been approved in the U.S.A. recently.

**HSV1-mediated brain-tumour gene therapy**

HSVIs are large (152 kb) double-stranded DNA viruses, packaged within a protein capsid, and a lipid envelope. During its natural infectious cycle HSV1 enters into latency, normally within neurons of the dorsal root ganglia. Only a minority of transcripts are produced during latency. Although HSV1 does not integrate into the genome of host cells, it can remain indefinitely as an episome within the nuclei of infected cells. It is only due to its capacity to reactivate that its ability to establish latency was known. Infrequently, HSV1 may spread to higher regions of the central nervous system and cause encephalitis. Whether this is a new virus, or originates from virus latent in the dorsal root ganglia, is not completely understood.

Viral vectors derived from HSV1 are either replication-defective or replication-competent. Various generations of replication-defective vectors exist, based either on the deletion of individual or a series of genes, or the construction of helper-dependent ampiclon vectors. Although great efforts have been dedicated to the development of long-term expressing HSV1 vectors, based on their capacity to establish latency, the first ones to enter clinical trials have been the replication-competent viruses.

HSV1 viruses deleted in a protein called ICP34.5 are unable to replicate in non-dividing post-mitotic cells, but are able to do so in glioma cells. A mutant called 1716 is deleted for ICP34.5 and is currently being used in a clinical trial of glioblastoma in the UK. A second mutant, deleted in addition for a second gene ICP6, encoding for the major subunit of ribonucleotide reductase, virus G207, and thus more disabled, is also being tested in a clinical glioblastoma trial in the U.S.A. The rationale is that injection of these tumour-restricted replication-competent HSV1 viruses directly into the tumours will lead to tumour-cell killing and tumour-mass elimination. Although the virus may infect normal brain cells surrounding the tumour, its genetic mutations will not allow it to replicate in these cells. Clinical results from these trials are eagerly awaited.

**Future challenges for gene therapy of glioblastoma**

Glioblastoma multiforme constitutes an enormous challenge for any novel therapeutic strategy, not only gene therapy. Until now, all treatments have failed, and this apparently includes the first clinically controlled large-scale randomized gene-therapy trial of glioblastoma using retroviral-producer cells expressing HSV1-TK in combination with ganciclovir. That the first randomized clinical gene-therapy trial has been carried out on glioblastoma attests to the importance of glioblastoma as a disease that desperately needs new therapies.

Gene therapy will work, not only if it successfully eliminates tumour mass, but also if it can target those cells distant from the tumour, which infiltrate normal brain parenchyma at the time of surgical removal of the tumour. Thus, gene therapy will succeed either if the gene-therapy vectors can diffuse throughout the brain to target infiltrating cells or if it can stimulate a systemic response able to detect, identify and destroy tiny
malignant cell islands within an otherwise normal brain. Stimulation of the immune system to do so is one possibility which is receiving serious consideration and, given the successes of immune-stimulation for the treatment of melanoma, may still hold the clue to the successful eradication of an established glioblastoma.

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