

Recent developments in gene therapy: applications for the treatment of pituitary tumours

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Pituitary tumours are normally benign, highly differentiated and slow growing neoplasms. Nevertheless, as many as half of them will show evidence of local invasion into the surrounding structures. Despite their benign growth characteristics and slow clinical progression, pituitary tumours commonly cause serious morbidity. The mass effects of large tumours, including headache and visual failure from optic chiasm compression, may cause lifelong disability. Hormone hypersecretion or deficiency causes major clinical problems that often require expensive and long-term medical therapy. Major advances have been made in the therapy of pituitary tumours over the past 20–30 years, but despite this, their treatment often remains an unsatisfactory compromise in practice. There is, therefore, a place for improvements in therapy, and to this end, gene therapy may come to hold a significant place in the future treatment of human pituitary tumours. With the development of new gene delivery vehicles, this concept can now be explored with a view to treating specific types of pituitary tumours.

Key words: viral vectors; transcriptional targeting; hormones; anterior pituitary gland; regulatable expression.

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Human gene therapy can be defined as directing the expression of genes, or DNA sequences, with the intention of correcting a disease phenotype. This new therapeutic approach came to fruition as a result of advances in molecular biology and genetics, and also because of the unravelling of the pathophysiology of a vast number of genetic and sporadic life-threatening diseases. Although the scientific concept of gene therapy is now widely accepted by the scientific and medical communities, the implementation of human clinical trials has proved to be a very challenging undertaking.

The increasing interest in gene therapy as a novel approach to treating disease has stemmed from the fact that current treatment modalities have mostly addressed the consequences of the underlying defects, or tried to relieve the symptoms, as opposed to trying to eliminate or correct the cause of the disease. Gene therapy therefore aims to bridge the gap between disease pathogenesis and effective therapy, aiming to correct the underlying causes of disease. The use of gene therapy to treat symptoms adds a further value to this novel alternative to classical small-chemicals pharmacology.

In this chapter, we will review the pathophysiology of pituitary tumours, current treatment modalities and possible gene therapy approaches aimed at treating tumours that do not respond to classical therapeutic approaches.

PITUITARY TUMOURS

Pituitary tumours are unusual neoplasms and, unlike most other intracranial tumours, are normally benign, highly differentiated and slow growing. Even so, as many as half of them will show evidence of local invasion into the surrounding structures. Their differentiated nature means that they often retain many of the characteristics of the normal cell type, including the regulatory mechanisms controlling hormone production. There is a wide spectrum of clinical presentation, depending on the size of the tumour and whether or not it produces enough mature hormone to generate a clinical syndrome.

Tiny non-functional pituitary microadenomas are commonly seen as incidental findings on high-resolution magnetic resonance imaging scans of the brain.¹ Small microadenomas secreting prolactin or adrenocorticotrophin are commonly less than 5 mm in diameter yet generate substantial morbidity through the clinical syndromes of amenorrhoea and galactorrhoea, or Cushing's disease. Larger tumours of 5–10 mm in diameter may present with a mixture of endocrine syndromes caused by relative hypopituitarism together with variable degrees of hormone excess, for example somatotroph tumours causing acromegaly. Tumours larger than 10 mm generally extend outside the pituitary fossa to become locally invasive macroadenomas that cause compression of local brain structures and bony erosion, even occasionally being reported to extend extracranially or to develop true distant metastases.^{2,3}

Despite their benign growth characteristics and slow clinical progression, pituitary tumours commonly cause serious morbidity. The mass effects of large tumours, including headache and visual failure from optic chiasm compression, may cause lifelong disability, and hormone hypersecretion or deficiency causes major clinical problems that often require expensive and long-term medical therapy. Major advances have been made in the therapy of pituitary tumours over the past 20–30 years, but despite this, their treatment often remains an unsatisfactory compromise in practice. There is, therefore, a place for improvements in therapy, and to this end, gene therapy may come to hold a significant place in the future treatment of human pituitary tumours.

CURRENT TREATMENT MODALITIES: SCOPE AND LIMITATIONS

The general aim of pituitary adenoma therapy is to reduce the tumour mass and to normalize or reduce hormone hypersecretion. Reduction of the tumour mass is often essential in order to reduce symptoms of headache arising from compression of the surrounding structures, and especially to relieve pressure on the optic nerve from suprasellar extensions. Normalization of the endocrine abnormality is recognized as being equally important for long-term health and even long-term mortality. An ideal therapy should also reduce hormone hypersecretion to normal or 'safe' levels (see, for example, discussions relating to acromegaly in Sheppard and Orme et al.^{4,5}) We will now review the current available therapies to assess to what extent they can achieve these goals, considering in turn surgery, pituitary irradiation and receptor-mediated pharmacotherapy.

Surgery

Pituitary adenectomy can achieve long-term cure by a total excision of a pituitary adenoma, leaving intact the remaining normal pituitary gland. A surgical approach may also sometimes be important in offering a tissue diagnosis in cases where this is not obvious. There have been a number of significant advances in surgical techniques in recent years that have improved the success of surgery in pituitary disease (see Chapter 10). The pituitary surgeon has the choice of transnasal or transcranial routes, and trans-sphenoidal pituitary microadenectomy is increasingly seen as a very safe procedure, with a low risk of damage to the normal pituitary and other neighbouring structures.

The success of surgery for pituitary disease can be judged both in terms of the effect on tumour volume and regrowth, and (for functioning tumours) in terms of correction of the endocrine abnormality. This latter criterion is hard to satisfy as even tiny amounts of tumour residue may continue to hypersecrete hormones, leaving a persistent endocrine syndrome despite a seemingly satisfactory removal of tumour bulk.

In a recent series of patients with growth hormone (GH) secreting tumours operated by a specialist surgeon, 14% of patients developed new pituitary dysfunction as a result of the surgery, and 7% were left with permanent diabetes insipidus.⁶ Fistula formation with CSF leakage is rare⁶ but may in some cases require further surgery; meningitis can occur but is now very unusual.

In general, therefore, surgery is a safe procedure, but many published series report a disappointingly low cure rate for the endocrine abnormality, the results being worse the larger the tumour. In addition, the endocrine criteria for cure, especially for acromegaly, have become more stringent in recent years, and this adversely affects the outcome figures.

In the case of acromegaly, adequate treatment now demands a target GH concentration of less than 5 mU/l (2.5 ng/ml), probably together with a normal insulin-like growth factor-I value, as this reverses the increased mortality from vascular disease, respiratory disease and colonic cancer.^{5,7} A number of surgical series have been reported in recent years, and it is now clear that the outcome depends greatly on the size of the tumour, also improving with increasing experience of the surgeon.⁶ Results from non-specialist pituitary surgeons are likely to be poorer than those reported in the literature, as suggested by a recent audit of results for acromegaly.⁸ In

areas where a single surgeon has taken over all the operations for pituitary disease, dramatic improvements in the therapeutic outcome have been achieved.⁹

For prolactin-secreting tumours, endocrine cure is essential to restore fertility without the need for long-term dopamine agonist treatment, and thus the endocrine criteria for successful surgical outcome are very exacting, demanding normalization of the serum prolactin level. As in the case of acromegaly, this depends on tumour size and invasiveness. For microprolactinomas, different surgical series report a long-term rate of overall endocrine 'cure' varying between 45%¹⁰ and 73%¹¹, a meta-analysis of surgical series in the USA reporting an overall cure rate of only 53%.¹² For prolactin-secreting macroadenomas, the rate of endocrine cure is much worse, being at best only 13–17%.¹³

In summary, therefore, surgery has the potential to cure and therefore remains a first choice of therapy in many circumstances, but the actual outcome is often disappointing, even in specialist series. Newer surgical techniques and the increasing specialization of surgeons are likely to improve the results seen, but many tumours will still remain virtually impossible to cure.

Pituitary irradiation

External beam irradiation of the pituitary is effective in reducing tumour growth in the long term, often being used as an adjunct to surgery when this has failed to achieve an adequate cure. Large series have been published concerning the efficacy and safety of external pituitary irradiation, and it is a widely accepted treatment for residual tumour (reviewed by Plowman¹⁴). However, although radiotherapy clearly reduces the risk of tumour progression, and appears to cause very little associated tissue damage, it does damage normal pituitary tissue and frequently results in progressive hypopituitarism¹⁵, which is irreversible and requires lifelong multiple-hormone replacement therapy. Thus, patients require regular screening for progressive hypopituitarism and eventually need substitution therapy with corticosteroids, GH, thyroxine and sex steroids (or gonadotrophins to achieve fertility). Radiotherapy is thus rarely used as a sole treatment for pituitary adenomas, but it can be an important adjunctive therapy. The impact of stereotactic radiosurgery (the 'gamma knife') remains to be seen (see Chapter 9), but it is probable that the long-term endocrine consequences will be similar.

Receptor-mediated pharmacotherapy

Pharmacological therapy based on the endocrine manipulation of prolactin or GH secretion has brought about a revolution in our expectations of treatment for pituitary disease. In many cases, drug treatment alone can prove adequate without recourse to any other therapy. Key medical therapies include dopamine agonists for hyperprolactinaemia, and somatostatin analogues and GH antagonists for acromegaly. The current state of these therapies will only be briefly considered here as they are covered in detail elsewhere in this book.

The astonishing success of dopamine agonists in both reducing serum prolactin level and causing the shrinkage of prolactinomas has allowed drugs such as bromocriptine, cabergoline and quinagolide to be used as the sole therapy for many patients with hyperprolactinaemia.¹³ Despite their success in 85–90% of patients, however, they have a high rate of side-effects, notably nausea and vomiting, postural hypotension and dizziness, headache and constipation. Depressive reactions may also be seen in some patients. At least 20% of patients experience significant nausea while taking bromocriptine, and even the less nauseating drug cabergoline had to be stopped in 3% of

patients in a double-blind trial.¹⁶ Thus, these drugs, although remarkably effective, commonly need to be taken for very many years, are often disliked by patients and sometimes cannot be used at all because of their side-effects.

Somatostatin analogues are discussed in Chapter 5. They generally do not cause a clinically useful shrinkage of somatotroph tumours, but they frequently reduce the GH level, if not to normal, then to at least a level that appears to be 'safe' in terms of normalizing long-term mortality. Their clinical use has been much eased by the introduction of depot preparations of octreotide and lanreotide that can be given at 2–4-week intervals, but there is still a requirement for uncomfortable repeated injections over long periods of time, and treatment can involve great expense over many years.

GH antagonists, reviewed in Chapter 6, are currently undergoing clinical trials and may be an important advance, although they are unlikely to affect the size or growth of the underlying pituitary tumour. So far, they appear to be well tolerated, and information on side-effects is likely to emerge from current trials. For small tumours, both somatostatin analogues and GH antagonists may find a place as a sole therapy for acromegaly, even if the size of the pituitary tumour frequently necessitates surgical treatment.

In summary, therefore, pituitary tumour therapy, despite important advances, is often unsatisfactory at present. Surgery is commonly inadequate to achieve endocrine cure, medical therapy is associated with significant long-term side-effects and expense, and irradiation causes hypopituitarism, necessitating lifelong replacement therapy. It is thus timely to consider whether recent advances in pituitary cell and molecular biology may be used to design new therapies, with the aim of the selective ablation of tumour cells without damaging the normal pituitary gland.

With a substantial background of knowledge concerning the regulation of pituitary hormone gene expression, there is a strong case for now applying this information to developing new tools for therapy. The tight transcriptional regulation of pituitary hormone genes could in principle be exploited to direct the expression of a desired transgene to specific cell types only within the mixed cell population found in the intact pituitary gland. With the development of new gene delivery vehicles, this concept can now be explored with a view to treating specific types of pituitary tumour.

GENE DELIVERY VEHICLES

The gene delivery systems available for gene transfer and therapy include non-viral vectors, viral vectors and ex vivo engineered cells. The most commonly used non-viral delivery systems available for gene transfer in vivo include the direct injection of naked DNA, lipofection and the gene gun. The major drawback of these methods is their low transduction efficiency (reviewed by Felgner¹⁷).

Viral vectors have high delivery efficiency and have been successfully used to deliver genes directly into the pituitary gland both in vitro^{18–23} and in vivo.²⁴ We will not describe in depth the characteristics of the main viral vectors systems available for gene therapy since they have been the subject of extensive recent reviews.^{20,25–27} The main characteristics of the most commonly used viral vectors are summarized in Table I. The most important features that must be taken into consideration before deciding which is the optimum viral delivery system to use include infectivity, cytotoxicity and length of transgene expression. The most common viral vectors in current use for gene therapy applications include replication incompetent adenovirus

Table I. Comparison between gene transfer vehicles: gene therapy applications.

Vectors	Adenovirus	'Gutless' adenovirus	HSV-1/r	HSV-1/a	Adeno-associated virus	Retrovirus (murine and human derived)	Vaccinia virus	Microinjection	Transfection
Size (kb)	36	36	152	10.30	4.68	3.5-9.2	186	Unlimited	Unlimited
Cloning capacity (kb)	7.5	~30	30	10.30	2-4.5	~8	30	Unlimited	Unlimited
In vivo?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
In vitro?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Very long-term gene expression	Yes in CNS	Yes	Yes	Yes	Yes	Yes	No	?	Yes
Gene therapy	No in periphery	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Vaccination	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No
Vector titres (pfu/ml)	10 ¹²	10 ¹⁰	10 ⁸	10 ⁸	10 ⁹	10 ⁷	10 ⁶ -10 ⁸	-	-

Reviewed by Stone et al (1999).²⁷ HSV-1/r = herpes simplex type 1-recombinant vector; HSV-1/a = herpes simplex type 1-amplicon; pfu/ml = plaque forming units per ml; 'gutless' = helper-dependent adenovirus vector; CNS = central nervous system.

(RAAd), replication-deficient herpes simplex type 1 virus (HSV-1), adeno-associated virus (AAV), retrovirus vectors derived from Moloney murine leukaemia virus (MMLV), lentivirus and gutless adenovirus.

Vectors based on RAAd, HSV-1, AAV and lentivirus have wide tropism and can also infect slowly proliferating and post-mitotic cells, i.e. endocrine pituitary cells.^{18,19,21} Retrovirus vectors based on MMLV can only enter the target cell nucleus and integrate into chromosomal DNA during cell division, so they are not useful for gene transfer into endocrine cells, although they could be capable of transducing pituitary tumour cells. Lentivirus vectors, on the other hand, can mediate stable gene transfer in post-mitotic cells, and this makes them an attractive delivery system for gene transfer into endocrine pituitary cells.²⁷

Adenoviral vectors are very efficient for gene delivery: they can be grown to high titres – approximately 10^{12} – of infectious units per ml (IU/ml)²⁸ (reviewed by Stone et al.²⁷). They are less toxic than HSV-1 vectors both *in vitro* and *in vivo*, and can infect dividing and quiescent or post-mitotic cells.²⁹ RAAd vectors used in gene therapy applications are mostly derived from Ad serotypes 2 and 5. They are also safe in humans; i.e. they have been used as vaccines without major adverse side-effects. The recently developed gutless adenovirus vectors have a larger cloning capacity, up to about 32 kbp, and since they are devoid of all virus open reading frames, they elicit a much lower immune response, which should allow readministration of the therapeutic agent.^{30–33}

The AAV vectors are defective parvoviruses derived from the human AAV serotype 2, which is not associated with disease (reviewed in Stone et al.²⁷). AAVs have been used to transfer genes into slowly proliferating and post-mitotic cells, and they can also integrate into the host's genome. They could thus be potential vehicles for gene transfer into endocrine anterior pituitary cells. A major limitation of this vector is that it has a small cloning capacity – approximately 4.0 kbp of foreign DNA (see Table 1 above) (reviewed by Stone et al.²⁷).

HSV-1 vectors also have characteristics that make them attractive vehicles for gene transfer into the anterior pituitary gland. HSV-1 enters its target cells by fusion to the plasma membrane. The viral capsid is then transported to the nucleus, the viral genome being released into the nucleus, where it can be maintained in an episomal state. HSV-1 has a large double-stranded DNA genome (approximately 150 kbp in length), and this allows the creation of several types of replication defective vector as well as the incorporation of large fragments of foreign DNA.^{34,35} HSV-1 vectors have been successfully used to express transgenes within anterior pituitary cells grown in primary culture.¹⁹

In summary, it is now possible to engineer viruses to disable them and make them replication defective and non-pathogenic, at the same time endowing them with desired characteristics so that they can be manufactured at reproducibly high titres and used as efficient and safe gene delivery vehicles for both *in vitro* and *in vivo* applications.

GENE THERAPY APPROACHES FOR THE TREATMENT OF PITUITARY TUMOURS

One of the most promising gene therapy approaches for the treatment of pituitary tumours is the use of genes that activate prodrugs (Table 2). The most common of these genes is the thymidine kinase (TK) gene from HSV-1.^{36,37} HSV1-TK converts

Table 2. Gene therapy strategies for the treatment of pituitary tumours.**Cyto-reductive approaches***Conditional cytotoxicity*

Herpes simplex type I-thymidine kinase (HSV1-TK)/ganciclovir
Escherichia coli guanine phosphoribosyl transferase (gpt)
Escherichia coli cytosine deaminase (cd)
 Rat cytochrome P450 2B1
Escherichia coli nitroreductase
 Bacterial carboxypeptidase G2

Direct cytotoxicity

Diphtheria toxin A
 Pseudomonas exotoxin A
 Tetanus toxin
 Fas ligand
 Caspases

Anti-angiogenic strategies

Angiostatin, endostatin
 Antisense VEGF; dominant negative VEGF receptors
 Antisense basic FGF
 Antisense EGF; dominant negative EGF receptors

Oncolytic viruses

HSV1 1716
 HSV1 G207
 Onyx-15 adenovirus

VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor;
 EGF = epidermal growth factor.

nucleoside analogues, i.e. acyclovir or ganciclovir, into their monophosphate metabolites, which are further phosphorylated by mammalian kinases to their triphosphate metabolites, these then acting as competitive inhibitors of endogenous nucleotides for incorporation into replicating DNA, and therefore causing cell death of the proliferating cells. The cytotoxicity of this system is enhanced by the 'bystander effect', which occurs via the transfer of ganaciclovir (GCV) metabolites via gap junctions. In vivo, this effect can be further enhanced by the presentation of tumour antigens to professional antigen-presenting cells at the tumour site as a result of tumour cell killing. This approach has been successfully used for the gene therapy treatment of experimental pituitary induced lactotroph hyperplasia in rats^{22,24} and a subcutaneous transplantable prolactinoma in nude mice.³⁸

Other prodrug-activating enzymes include²⁵:

1. the rat cytochrome P450 2B1 gene, which activates CB 1954;
2. the *Escherichia coli* guanine phosphoribosyl transferase gene, which activates 6-thioxanthine and 6-thioguanine;
3. the mammalian deoxycytidine kinase gene, which activates cytosin arabinoside;
4. the *E. coli* cytosine deaminase gene, which activates 5-fluorocytosine;
5. the bacterial carboxypeptidase G2 gene, which activates DNA alkylating agents;
6. nitroreductase, which metabolizes the prodrug 5-(aziridin-1-yl)-2,4-dinitrobenzamide.

These prodrug-activating approaches could be used on their own or in combination.^{39,40} It has recently been reported that conditional cytotoxicity in combination with radio- and/or chemotherapy could have synergistic anti-tumoural effects. Current

tumour chemotherapy also uses combinations of different chemotherapeutic agents with differing mechanisms of action to increase treatment efficiency. Equally, the successful treatment of AIDS infection requires the combined use of various anti-viral agents. Thus, the combination of gene therapy approaches with more classical surgical, chemo- and radiotherapy is very likely to lead to enhanced therapeutic results, which could also be applicable for pituitary tumours.^{41,42}

The process of new blood vessel formation in growing pituitary tumours also offers a potential target for gene therapy modalities (see [Table 2](#) above). It has been demonstrated experimentally that it is possible to use gene transfer methods *in vivo* to disrupt the angiogenic process in tumours. The main targets would be vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1 or flt-1, and VEGFR-2 or flk-1), basic fibroblast growth factor and its receptor, epidermal growth factor and its receptor, and also transforming growth factor (TGF- β and TGF- α). Both antisense and dominant negative variants could be used as gene therapy approaches to target angiogenesis.^{43,44} A selective toxicity could be achieved using this approach if the molecular targets were located preferentially within the tumour blood vessels; blocking these pathways should mainly affect proliferating cells in need of continuous blood flow for oxygen and nutrients.

The recent discovery of angiostatin⁴⁵ and endostatin⁴⁶ has provided anti-angiogenic therapy that could not only regress large tumours *in vivo*, but also maintain them quiescent at microscopic size for as long as the therapy was present.⁴⁷ These anti-angiogenic inhibitors proved not to be toxic, nor did they induce drug resistance. It is conceivable that anti-angiogenic therapy for cancer will require the long-term administration of inhibitors at frequent intervals. Gene therapy could provide a means by which endostatin and/or angiostatin could be delivered systematically or locally, providing a constant supply of angiogenesis inhibitors. Recently, Pawliuk et al⁴⁸ have used stably transduced bone marrow cells with retroviral vectors expressing angiostatin and endostatin, which were grafted into the bone marrow to assess whether the bone marrow could become a source of circulating angiogenic inhibitors. One could also envisage the direct gene transfer of these inhibitors into solid tumours to inhibit their growth. Only migrating vascular endothelial cells in the tumour bed should be inhibited: the remaining endothelium in the body, which is quiescent, should not be affected. This therapeutic approach will therefore pose a low risk to normal tissue.

Another important therapeutic target for tumour gene therapy comprises the genes involved in the apoptotic pathway, which can be used in addition to direct cytotoxins to achieve cell death (see [Table 2](#)) (reviewed by Watson and Lowenstein⁴⁹). Examples of targeting pro-apoptotic targets include the use of the potent pro-apoptotic molecule Fas-L (CD95L). Fas-L is a 40 kDa type II transmembrane protein, a member of the tumour necrosis factor (TNF) family of cytokines. When it binds to its receptor CD95 (Fas, APO-1), a glycosylated 45 kDa transmembrane protein that belongs to the TNF receptor family, it induces very quick apoptosis.⁵⁰ This system is involved in the deletion of activated T-lymphocytes, in terminating immune responses and in mediating T-lymphocyte-induced cytotoxicity.⁵⁰⁻⁵² Under physiological conditions, the expression of CD95 and CD95L is very tightly regulated to prevent the undesired induction of apoptosis.

The mechanism by which the CD95/CD95L system induces apoptosis has recently been elucidated and has suggested some potential therapeutic targets. The binding of CD95L to CD95 induces the formation of a 'death-inducing signalling complex' (DISC) containing the proteins FADD or RIP and RAIDD, leading to the recruitment and activation of caspase 8 or caspase 2 depending on the components of the DISC.^{53,54} The

activation of either caspase ultimately leads to apoptotic cell death. Upregulation of the CD95/CD95L system results in cell suicide by the cross-linking of these molecules on the same cell, or fratricide by cross-linking between neighbouring cells.^{55,56}

Transcriptionally targeted adenoviruses expressing Fas-L have been recently constructed^{57,58} and used as a gene therapy approach for the treatment of an intracranial glioblastoma tumour model in rats.⁵⁹ Thus, gene therapy strategies may be developed that directly act on the CD95/CD95L signal transduction pathway. Such an approach could also be developed for devising gene therapy strategies for the treatment of pituitary tumours.

Apoptosis is also regulated by enzymes that cleave interleukin-1 β (ICE), known as ICE proteases or caspases (reviewed by Watson and Lowenstein⁴⁹). The expression of active caspases is capable of inducing apoptosis. In addition, the activation of the TNF receptor, TNFR2, leads to the activation of caspases and apoptotic cell death. These molecules could also constitute attractive targets for pro-apoptotic gene therapy for the ablation of pituitary tumours.

Direct cytotoxins such as pseudomonal exotoxin A or diphtheria toxin, although potentially useful, have to be used very cautiously because of the potential of damage to neighbouring non-tumour cells, which could have dramatic physiological implications (Table 2).⁶⁰ A possible strategy to improve the safety of this approach would be to express the toxic gene products under the control of cell-type (i.e. tumour cell) specific promoters, as inducible promoter cassettes or engineered to be synthesized as fusion proteins to secreted antibody regions so that the toxic moiety is directed towards the secretory pathway of infected cells. This latter approach yields a secreted toxic fusion protein that binds to specific receptors or antigens and is internalized into neighbouring tumour cells, causing their death by, for example, inhibiting protein synthesis.^{61,62}

Tumour gene therapy has been successful in experimental animals but less so in human clinical trials. Thus, much developmental work in gene therapy has been aimed at making viral vectors tissue specific. Such transcriptional targeting can now be achieved in several different forms. Virion targeting has also been successful in restricting viral vector entry into specified predetermined cells. For cancer therapy, however, tumour killing needs to be very efficient, and while virion and transcriptional targeting makes vectors more specific, there is a price to pay in terms of a reduction in tumour cytotoxicity.

Replication-competent viruses have been developed, several of which are now undergoing clinical trials. The replication of viral vectors is restricted to tumour cells, through the use of viral mutants that can replicate only in dividing cells. Strategies used to achieve this are reflected by the adenovirus Onyx-15, which only replicates in cells lacking functional p53 protein, and the HSV-1 vector 1716, and G207, which can only replicate in tumoural glioma cells but not in normal post-mitotic neurones.⁴² Further developments include the expression of proteins essential for viral replication under the control of tumour cell type specific or inducible transcriptional control elements. In this way, viruses can only replicate in tumour cells. Again, this approach would be amenable for pituitary tumour gene therapy.

CELL-TYPE-SPECIFIC AND REGULATED TRANSGENE EXPRESSION IN THE PITUITARY GLAND

For safe and efficient gene-based therapy, it is important to achieve an adequate level of expression of the therapeutic gene as well as to restrict its expression to the desired

cell types. The adverse side-effects of inappropriate gene expression in normal tissue surrounding the tumour mass of, for example, a cytotoxic gene product could be fatal. Such non-specific toxicity would depend on the location of the tumour, the nature of the transduced normal tissue and the toxicity of the therapeutic gene used.

A number of groups are actively trying to address the above issues by improving the design of the gene delivery vectors and gene therapy protocols. One approach used is to identify and develop gene-regulatory elements that would provide a cell-type-specific and simultaneous regulatable expression of a therapeutic transgene.^{20,63} Some examples of the use of tumour cell-type-specific promoters to restrict expression to tumour cells are the melanocyte-specific tyrosinase promoter to target melanoma cells, the c-erb-B₂ promoter to target breast cancer cells, the alpha-fetoprotein promoter to target hepatocellular carcinoma, the colon embryonic antigen promoter to target colon carcinoma, the prostate-specific antigen to target prostate cancer and the glial fibrillary acid protein promoter to restrict expression to glioma cells (reviewed in Reeves⁶⁴).

For targeting specific cell populations within the anterior pituitary gland, that give rise to pituitary tumours, one could use the promoter elements that drive hormone gene expression. Examples of such an approach used within recombinant adenovirus vectors are the GH and α -glycoprotein promoters, which drive transgene expression in somatotrophs and gonadotrophs/tyrotrophs respectively³⁸, as well as the prolactin promoter^{20,23}, which drives transgene expression in lactotrophs and a subpopulation of GH-producing anterior pituitary cells.

In addition, complex gene-regulatory systems have recently been developed and inserted into viral delivery vectors that allow not only cell-type-specific transgene expression, but also a tight regulation of gene expression levels. Such regulation of transgene expression can be controlled upon the administration of a small effector molecule such as the antibiotic drug tetracycline. The tetracycline-controlled transactivator responsive promoter (Tet system) has been adapted for use in mammalian cells by constructing a promoter containing elements of the prokaryotic tetracycline-sensitive operon and a strong transcriptional activation domain from the HSV transactivator VP16.⁶⁵

The initial Tet system is contained in two plasmids, one containing the response element and transgenes of interest, the other containing the regulatory components (Figure 1). The response unit is composed of the *Escherichia coli*-derived tetracycline-resistance operon regulatory elements (tet O) embedded within a minimal cytomegalovirus (minCMV) promoter. The regulatory component encodes the transactivator hybrid protein (tTA or rtTA), consisting of the tetracycline repressor (tet R) fused to the HSV-1 transactivator VP16 (Figure 1). The expression of a gene inserted downstream of the tet O/minCMV promoter depends on tTA or rtTA, which binds to the tet O sequences through the tet R domain and recruits strong cellular transcription factors through the transcriptional activator domain of VP16. Gene expression in the tTA system is inhibited by the addition of tetracycline, which, by binding to the transactivator protein, causes its dissociation from the tet O/minCMV complex.

The system has recently been modified so that transcription can be induced in the presence of tetracycline or its analogues, for example doxycycline. A mutant transactivator protein (rtTA) was selected that must bind to tetracycline before it can bind to tet O/minCMV. Our laboratory and others have developed such a regulatable system within a dual adenovirus system^{63,66,67} and have determined that the dose of tetracycline/doxycycline needed to induce gene expression is non-toxic both in vitro

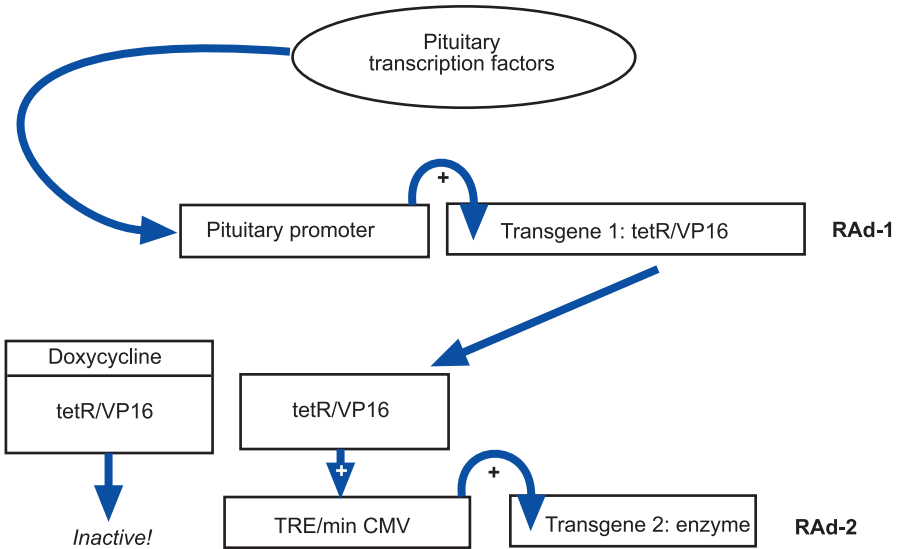


Figure 1. Combined pituitary cell-type-specific and inducible transgene expression using recombinant adenovirus vectors (RAAd). The system is based on two recombinant adenovirus vectors that have to be used in combination. RAAd-1 encodes the first transgene, i.e. tetR/VP16, driven by a pituitary-specific promoter. RAAd-2 encodes the second transgene, i.e. HSV1-TK or another cytotoxic enzyme or therapeutic transgene whose expression is driven by the tetracycline-resistance operon regulatory elements embedded within a minimal CMV promoter (TRE/min CMV).

and *in vivo*. For gene therapy applications, it would be ideal to develop these regulatable expression systems within a single delivery vector.⁶⁸

It is also possible to regulate transgene expression using radiation-responsive gene-regulatory elements in combination with the effects of ionizing radiation. The radiation-inducible promoter region of the early growth response-1 (Egr-1) gene has been used to regulate gene expression in response to ionizing radiation.⁶⁹ An adenovirus vector encoding for TNF α under the control of the Egr-1 promoter was constructed and injected into a human epithelial tumour xenograft. Tumour volume decreased after the administration of ionizing radiation, with a 10-fold increased expression of TNF α . The possibility of coupling the effects of ionizing radiation with the expression of cytotoxic gene products is a very attractive prospect for cancer gene therapy applications.

An alternative approach to regulating therapeutic transgene expression would be to use a regulatory element controlled by a physiological parameter common to a broad range of tumours. Tissue hypoxia is a characteristic of several diseases, including solid tumours.⁷⁰ Tissue and cellular responses to hypoxia are mediated through changes in the expression of a number of genes, including tyrosine hydroxylase, erythropoietin, VEGF and the glycolytic enzyme phosphor-glycerate kinase.⁷¹ These genes contain one or more copies of a sequence known as the hypoxia response element (HRE), 5'-TACGTGC-3'. These sequences act as binding sites for a subfamily of the helix-loop-helix family of transcription factors. The best characterized of these proteins are hypoxia-inducible factor-1 α (HIF-1 α) and EPAS1 (HIF-1 α -like factor).⁷² Based on these observations, synthetic hypoxia-responsive promoters that use multimeric HREs in combination with heterologous promoters have been developed.^{73,74} Both *in vitro* and

in vivo data suggest that therapeutic gene expression under hypoxia-regulated control could be developed into a useful gene therapy strategy for solid tumours.^{73,75}

GENE THERAPY: ETHICAL IMPLICATIONS

As previously stated, gene therapy is the use of nucleic acids as drugs. Thus, as for any drug, its uses will be mainly, but not solely, restricted to therapeutic applications. Four different actual or potential types of uses of the novel gene therapy technology can be defined⁷⁶:

1. somatic gene therapy;
2. somatic genetic enhancement;
3. germline gene therapy;
4. germline genetic enhancement.

Gene therapy refers to the use of the gene transfer technology to treat human diseases. Genetic enhancement encompasses all the non-therapeutic uses of gene transfer technology to improve on non-disease-related characters (for example, muscular strength in an otherwise normal person). Somatic gene therapy refers to the gene therapy engineering of any cells, tissue or organ, excluding reproductive (germ) cells, with therapeutic intent. An ethical line has been drawn beyond somatic gene therapy, stating that somatic gene therapy is permissible, while any uses of gene therapy for enhancement purposes, as well as germline engineering, should for the time being remain out of bounds. Thus, these non-therapeutic uses of gene therapy are considered 'unethical' at the present time.

The ethical discussion has informed the legal bodies, which have thus ruled that the purposeful modification of germline cells is specifically forbidden by law in the UK and in many other countries. Even though this ethical and legal debate has been described as defining clear-cut boundaries, the dividing line between somatic gene therapy and enhancement is not very clear, for example when considering certain human attributes such as height. Furthermore, animal experiments on the development of the necessary technology to achieve germline modifications through gene therapy continue to be undertaken.⁷⁷ Mainly because of imposed technical limitations, current clinical gene therapy trials all involve somatic gene therapy applications. However, technological as well as ethical issues related to fetal in utero gene therapy, and germline gene therapy, continue to be actively discussed.

In spite of much discussion on gene therapy, several misconceptions still remain, even in academic circles, regarding what gene therapy really is and the nature of clinical trials. The first misconception is that gene therapy is something completely new. The prospects of clinical gene therapy have been discussed for at least the past 30 years, if not more. Recently, however, Friedmann²⁶ convincingly argued that gene therapy is now past the 'conceptual phase' and has entered the 'implementation phase'. Prior to this turning point, the conceptual basis of gene therapy was still being hotly debated (for example, 'Will it ever work?'). Now, with the rapid growth in the number of gene therapy clinical trials, dedicated professional gene therapy societies, gene therapy journals and gene therapy biotechnology companies, the implementation phase ('How do we get it to work clinically?') is firmly in place.

A second misconception is that therapeutic applications of gene therapy will be restricted to the treatment of inherited disease. In reality, the great majority of current clinical protocols are for the treatment of non-inherited conditions such as

cancer.⁷⁸ Historically, gene therapy arose as a possible novel treatment for hitherto incurable inherited diseases⁷⁹, but nowadays most gene therapy is intended for the treatment of cancer, AIDS and other life-threatening non-inherited pathologies. This will change as new advances in human molecular genetics continue to identify genetic predispositions to cancer or diabetes. In the future, it can safely be predicted that gene therapy will attempt to eradicate such genetic predispositions, avoiding the recourse to so far heroic measures such as preventative mastectomies in women with a high risk of developing breast cancer. Essentially, for the gene therapy of cancer:

1. There is a larger availability of gene transfer vectors.
2. It is easier to define a therapeutically useful starting point for the therapy.
3. The organ(s) to be targeted are easily identified as those bearing the tumour.
4. The cells to be targeted are initially (although not exclusively) the tumour cells.
5. The level of transgene expression needed is related to the amount needed to kill tumour cells.
6. The transgene expression needs to remain active only until the tumour cells are killed, i.e. a relatively short time.
7. The clearly defined therapeutic objective is to kill the tumour cells, and this can be monitored clinically.

Thus, it is more straightforward, using currently available technology, to devise a successful scientifically sound strategy for the treatment of cancer than to do the same for an inherited disorder.⁷⁹

The third misconception is that gene therapy is 'different' from other current therapeutic approaches, i.e. that it will provide the elusive magic bullets to cure serious diseases. Gene therapy, however, is not 'different' from other kinds of novel medical treatments: it is merely a new branch of pharmacology. Where classical pharmacology has been (and will always remain) limited to modifying existing cellular functions, gene therapy can ask the cells to perform new functions. Gene therapy techniques, but not classical pharmacology, allow the clinician to engineer and effectively reprogramme the function of adult somatic cells in individual patients, even if the relevant function is not normally performed by the target cells being modified. As with current pharmacology, gene therapy is being applied to treat a wide variety of both inherited and sporadic diseases, even in the absence of a detailed understanding of underlying disease pathophysiology.

However, the regularly asked question 'How long will it take to work?' still remains. Many phase I clinical trials are currently in progress, and as these move into phase II–IV clinical trials and randomized controlled trials, we will have more accurate information on the strengths and weaknesses of individual gene therapy trials. As in pharmacology, it will be impossible to predict which gene therapy strategy will succeed and which will fail, in the same way as it is impossible to predict which drug will still be used in 100 years and which will be superseded in the next 12 months. As no-one would ever suggest that pharmacology itself has failed if a single drug fails, the same is true of gene therapy. We should remember that while for childhood acute leukaemias, 5-year survival is currently achieved in over 80% of cases, only as recently as 1948 the corresponding figure was zero. It took classical cancer chemotherapy almost 50 years to arrive at such a remarkable achievement. Although we expect and hope that gene therapy will succeed more quickly than this, the list of outstanding cancers incurable in spite of all the pharmacological developments of this century should provide us with some humbling realizations as we become aware of the fact that we are dealing with extremely serious and aggressive diseases.

How does the ethical discussion apply to the development of gene therapy for pituitary diseases? Even if many pituitary tumours can be treated by currently available surgery, radiotherapy and pharmacology, the long-term outcome is in some cases still problematic. Gene therapy will provide novel, flexible ways of engineering not only tumour cells to eliminate their growth, but also the possibility of modifying normal surrounding cells to inhibit tumour regrowth. Furthermore, gene therapy techniques could also be used to replace hormone secretion that might have been lost during tumour treatment. In summary, gene therapy will offer new approaches to treatment, management and ongoing hormonal control in patients whose pituitary function has been seriously compromised.^{81,83}

CONCLUSION

Gene delivery methods that allow the expression of both marker and/or therapeutic genes within somatic cells are currently available and are being constantly improved. Their potential uses for gene therapy applications to treat both hereditary and sporadic disorders are enormous. Thus, some untreatable cases of pituitary tumour that do not respond to the currently available treatment strategies constitute a plausible target for gene therapy approaches. The major limitations that we face in terms of making gene therapy a clinically viable strategy are the relative inefficiency of the gene-delivery vehicles, both viral and non-viral, the lack of a vector allowing a targeted expression following the systemic delivery of the therapeutic entity⁸⁴, the immune response of the host to the delivery vehicles and therapeutic transgenes, the limited duration of transgene expression, the putative cytotoxic side-effects of the therapy, the production of delivery vehicles in sufficient quantities and of the required quality for clinical use, and finally the cost implications of the therapy (Table 3). In each of these areas, there is a need for a greater understanding of the basic limiting mechanisms involved in order that they can be manipulated to allow the development and implementation of effective gene therapy protocols to treat human disease.⁸⁵

Table 3. Factors affecting the implementation of clinical gene therapy protocols.

Effectiveness	The efficient delivery and expression of therapeutic transgenes leading to the regression of the disease phenotype. The adequate duration of transgene expression
Safety	A lack of unwarranted vector spread <i>in vivo</i> . The elimination of adverse immune responses and other cytotoxic reactions, both local and/or systemic, within the host
Implementation	Large-scale production and quality control of delivery vehicles (viral/non-viral). Compliance with local gene therapy advisory committee regulations. Need to take into account the cost of the therapy versus the population to be treated. Benefits to health-care provision

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REFERENCES

1. Hall WA, Luciano MG, Doppman JL et al. Pituitary magnetic resonance imaging in normal volunteers: occult adenomas in the general population. *Annals of Internal Medicine* 1994; **120**: 817–820.
2. Davis JRE, Sheppard MC & Heath DA. Giant invasive prolactinoma: a case report and review of nine further cases. *Quarterly Journal of Medicine* 1990; **74**: 227–238.
3. Pernicone PJ, Scheithauer BW, Sebo TJ et al. Pituitary carcinoma: a clinicopathological study of 15 cases. *Cancer* 1997; **79**: 804–812.
4. Wass JAH (ed.) *Aims of Treatment and Definition of Cure: Acromegaly – 100 Years On*, pp 17–31. Bristol: Society for Endocrinology, 1994.
5. Orme SM, McNally RJQ, Cartwright RA & Belchetz PE. Mortality and cancer incidence in acromegaly: a retrospective cohort study. *Journal of Clinical Endocrinology and Metabolism* 1998; **83**: 2730–2734.
6. Ahmed S, Elsheikh M, Stratton IM et al. Outcome of transphenoidal surgery for acromegaly and its relationship to surgical experience. *Clinical Endocrinology* 1999; **50**: 561–567.
7. Bates AS, van't Hoff W, Jones JM & Clayton RN. An audit of outcome in treatment of acromegaly. *Quarterly Journal of Medicine* 1993; **86**: 293–299.
8. Lissett CA, Peacey SR, Laing I et al. The outcome of surgery for acromegaly: the need for a specialist surgeon for all types of growth hormone secreting adenomas. *Clinical Endocrinology* 1998; **49**: 653–657.
9. Gittoes NJL, Johnson AP, Sheppard MC & Stewart PM. Outcome of surgery for acromegaly: the experience of a single surgeon. *Quarterly Journal of Medicine* 1999 (in press).
10. Soule SG, Conway GS & Jacobs HS. The outcome of hypophysectomy in the era of dopamine agonist therapy. *Clinical Endocrinology* 1996; **44**: 711–716.
11. Thomson JA, Davies DL, McLaren EH & Teasdale GM. 10-year follow-up of prolactinoma treated by transphenoidal surgery. *British Medical Journal* 1994; **309**: 1409–1410.
12. Molitch ME, Thorner MO & Wilson C. Management of prolactinomas. *Journal of Clinical Endocrinology* 1997; **82**: 996–997.
13. Bevan JS, Webster J, Burke CW & Scanlon MF. Dopamine agonists and pituitary tumour shrinkage. *Endocrine Reviews* 1992; **13**: 220–240.
14. Plowman PN. Pituitary adenoma radiotherapy – when, who and how? *Clinical Endocrinology* 1999; **51**: 265–271.
15. Little MD, Shalet SM, Beardwell CG et al. Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Quarterly Journal of Medicine* 1991; **70**: 145–160.
16. Webster J, Piscitelli G, Polli A et al. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhoea. *New England Journal of Medicine* 1994; **331**: 904–909.
17. Felgner PL. Nonviral strategies for gene therapy. *Scientific American* 1997; **276**: 102–106.
- *18. Castro MG, Goya RG, Sosa YE et al. Expression of transgenes in normal and neoplastic anterior pituitary cells using recombinant adenoviruses: long term expression, cell cycle dependency, and effects on hormone secretion. *Endocrinology* 1997; **138**: 2184–2194.
19. Goya R, Rowe J, Sosa YE et al. Use of recombinant herpes simplex virus type 1 vectors for gene transfer into tumour and normal anterior pituitary cells. *Molecular and Cellular Endocrinology* 1998; **138**: 199–207.
20. Castro MG, Windeatt S, Smith-Arica J & Lowenstein PR. Cell-type specific expression in the pituitary: physiology and gene therapy. *Biochemical Society Transactions* 1999; **27**: 858–863.
21. Neill JD, Musgrove LC, Duck LW & Sellers JC. High efficiency method for gene transfer in normal pituitary gonadotropes: adenoviral-mediated expression of G protein-coupled receptor kinase 2 suppresses luteinising hormone secretion. *Endocrinology* 1999; **140**: 2562–2569.
22. Windeatt S, Perone M, Dewey R et al. Gene therapy using herpes simplex type 1 thymidine kinase (HSV1-TK) for the treatment of prolactin-secreting adenomas. *Journal of Endocrinology* 1999; **160S**: P152.
23. Windeatt S, Perone M, Southgate T et al. Development of a cell-type specific recombinant adenovirus for transgene expression in pituitary lactotrophic cells. *Journal of Endocrinology* 1999; **160S**: P156.
- *24. Windeatt S, Southgate TD, Dewey F et al. Adenovirus-mediated HSV-1 thymidine kinase gene therapy suppressed oestrogen induced pituitary prolactinomas. *Journal of Clinical Endocrinology and Metabolism* 2000 (in press).
25. Chiocca EA & Breakefield XO (eds) *Gene Therapy for Neurological Disorders and Brain Tumours*, p 458. Totawa, NJ: Humana Press, 1998.

26. Friedmann T. The origins, evolution and directions of human gene therapy. In Friedmann T (ed.) *The Development of Human Gene Therapy*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1996.
27. Stone D, Bolognani F, David A et al. Viral vectors for gene delivery/therapy in the endocrine system. *Journal of Endocrinology* 2000; **164**: 103–118.
28. Lowenstein PR, Shering AF, Bain D et al. The use of adenovirus vectors to transfer genes to identified target brain cells *in vitro*. In Lowenstein PR & Enquist LW (eds) *Protocols for Gene Transfer in Neuroscience: Towards Gene Therapy of Neurological Disorders*, pp 93–114. Chichester: John Wiley & Sons, 1996.
29. Shering AF, Bain D, Stewart K et al. Cell-type specific expression in brain cell cultures from a short human cytomegalovirus major immediate early promoter depends on whether it is inserted into herpesvirus or adenovirus vectors. *Journal of General Virology* 1997; **78**: 445–459.
- *30. Cartmell T, Southgate T, Poole S et al. IL-1 mediates a rapid inflammatory response following adenoviral vector delivery into the brain. *Journal of Neuroscience* 1999; **4**: 1517–1523.
31. Kochanek S. High-capacity adenoviral vectors for gene transfer and somatic gene therapy. *Human Gene Therapy* 1999; **10**: 2451–2459.
32. Thomas CE, Schiedner G, Kochanek S et al. Adenovirus-mediated gene transfer to the brain: a comparison between gutless and first-generation viral vectors. *Abstracts of the Society for Neuroscience Part 1*, 1177, Abstract No. 476.1, 1999.
33. Thomas CE, Schiedner G, Kochanek S et al. Adenovirus-mediated gene transfer to the brain: 'gutless' vectors versus first generation vectors. *British Neuroscience Association* 1999; **15**: 57.
34. Wilkinson GWG, Darley RL & Lowenstein PR. Viral vectors for gene therapy. In Latchman DS (ed.) *From Genetics to Gene Therapy*, pp 161–192. Oxford: BIOS Scientific Publishers, 1994.
35. Tomasec P, Bain D, Castro MG et al. Herpes simplex virus 1 temperature-sensitive mutant tsK vector for neuronal gene transfer. In Lowenstein PR & Enquist LW (eds) *Gene Transfer into Postmitotic Neurons: Towards Gene Therapy of Human Neurological Disorders*, pp 169–186. Chichester: John Wiley & Sons, 1996.
- *36. Dewey RA, Morrissey G, Cowsill C et al. Chronic brain inflammation and persistent HSV1-TK expression in survivors of syngeneic glioma treated by adenovirus-mediated gene therapy: implications for clinical trials. *Nature Medicine* 1999; **5**: 1256–1263.
37. Cowsill C, Southgate TD, Morrissey G et al. Central nervous system toxicity of two adenoviral vectors encoding variants of herpes simplex virus type 1 thymidine kinase: reduced cytotoxicity of a truncated HSV1-TK. *Gene Therapy* 2000 (in press).
- *38. Lee EJ, Anderson LM, Thimmapaya B & James JL. Targeted expression of toxic genes directed by pituitary hormone promoters: a potential strategy for adenovirus-mediated gene therapy of pituitary tumors. *Journal of Clinical Endocrinology* 1999; **84**: 786–794.
39. Karpati G, Li HW, AlonsoVasnegas MA et al. Combination therapy for experimental malignant glioma using adenovirus-mediated transfer of p53 and cytosine deaminase suicide gene. *Neurology* 1999; **52**: A474–A475.
40. Wang ZH, Zagzag D, Zeng BJ & Kolodny EH. *In vivo* and *in vitro* glioma cell killing induced by an adenovirus expressing both cytosine deaminase and thymidine kinase and its association with interferon- α . *Journal of Neuro pathology and Experimental Neurology* 1999; **58**: 847–858.
41. Brust D, Farnsworth J, Braddus WC & Valerie K. Radiosensitization of rat glioma with bromodeoxycytidine and an adenovirus expression herpes simplex virus thymidine kinase (HSV-TK) delivered by slow, rate-controlled positive pressure infusion. *Cancer Gene Therapy* 1998; **5**: S5054.
42. Nemunaitis J, Khuri F, Ganly I et al. Phase II trials of intratumoral ONYX-015, an E1B 55-kDa gene-deleted adenovirus, alone and in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Cancer Gene Therapy* 1998; **55**: O-78.
- *43. Milauer B, Shawyer LK, Plate KH et al. Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature* 1994; **367**: 576–578.
44. Fakhrai H, Dorigo O, Shawler DL et al. Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy. *Proceedings of the National Academy of Sciences of the USA* 1996; **93**: 2909–2914.
- *45. O'Reilly MS, Holmgren L, Shing Y et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 1994; **79**: 315–328.
46. O'Reilly MS, Boehm T, Shing Y et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997; **88**: 277–285.
47. O'Reilly MS, Holmgren L, Chen C & Folkman J. Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nature Medicine* 1996; **2**: 689–692.
48. Pawliuk R, Bachelot T & Boehm T. Bone marrow engraftment of murine hematopoietic cells transduced with retroviral vectors expressing the angiogenesis inhibitors angiostatin and endostatin. *Proceedings of the American Association of Cancer Research* 1998; **39**: 555.

49. Watson AJM & Lowenstein PR. Therapeutic manipulation of apoptosis in cancer and neurological disease. In Wilson JW, Booth C & Potten CS (eds) *Apoptosis Regulatory Genes*, pp 281–303. Boston: Kluwer Academic Publishers, 1999.
50. Nagata S. Apoptosis by death factor. *Cell* 1997; **88**: 355–365.
51. Lau HT & Stoeckert CJ. FasL: too much of a good thing? *Nature Medicine* 1997; **3**: 727–728.
52. Turvey SE, Gonzalez-Nicolini V, Kingsley CI et al. Fas ligand transfected myoblasts and islet cell transplantation. *Transplantation* 1999 (in press).
53. Medema JP, Scaffidi C, Kischkel FC et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO Journal* 1997; **16**: 2794–2804.
54. Strasser A & O'Conner L. Fas-ligand caught between Scylla and Charybdis. *Nature Medicine* 1998; **4**: 21–22.
55. Friesen C, Herr I, Krammer PH & Debatin KM. Involvement of the CD95 (Apo-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemia cells. *Nature Medicine* 1996; **2**: 574–577.
56. Muller M, Strand S, Hug H et al. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (Apo/Fas) receptor/ligand system and involves activation of wild-type p53. *Journal of Clinical Investigation* 1997; **99**: 403–413.
57. Larregina A, Morelli A, Dewey R et al. Fas L induces Fas/Apo1-mediated apoptosis in human embryonic kidney 293 cells routinely used to generate EI deleted adenoviral vectors. *Gene Therapy* 1998; **5**: 563–568.
58. Morrelli A, Larregina A, Smith J et al. Neuronal and glial cell type-specific promoters within adenovirus recombinants restrict the expression of the apoptosis-inducing molecule Fas ligand to predetermined brain cell types, and abolish peripheral liver toxicity. *Journal of General Virology* 1999; **80**: 571–583.
59. Ambar BB, Frei K, Malipiero U et al. Treatment of experimental glioma by administration of adenoviral vectors expressing Fas Ligand. *Human Gene Therapy* 1999; **10**: 1641–1648.
60. Lowenstein PR, Cowen R, Thomas C & Castro MG. The basic science of brain tumour. *Gene Therapy. Biochemical Society Transactions* 1999; **27**: 873–881.
- *61. Chen ST & Marasco WA. Novel genetic immunotoxins and intracellular antibodies for cancer-therapy. *Seminars in Oncology* 1996; **23**: 148–153.
62. Chen ST, Yang AG, Chen JD et al. Potent antitumour activity of a new class of tumour specific killer cells. *Nature* 1997; **385**: 78–80.
63. Lowenstein PR, Southgate T, Smith-Arica J & Castro MG. Gene therapy for neurological disorders: towards therapeutic intervention in the Lesch–Nyhan syndrome. In van Leuwen FH & Verhaagen J (eds) *Progress in Brain Research*, pp 481–497. Elsevier Science, 1998.
64. Reeves SA. Retrovirus vectors and regulatable promoters. In Chiocca EA & Breakfield XO (eds) *Gene Therapy for Neurological Disorders and Brain Tumours*, pp 7–38. Totowa, NJ: Humana Press, 1998.
65. Gossen M & Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences of the USA* 1992; **89**: 5547–5551.
- *66. Harding TC, Geddes BJ, Murphy D et al. Switching transgene expression in the brain using an adenoviral tetracycline-regulatable system. *Nature Biotechnology* 1998; **16**: 553–555.
67. Smith-Arica J, Morelli AE, Larregina A et al. Combining cell-type specific and inducible gene expression from first generation adenoviral vectors *in vitro*. *Cold Spring Harbor Laboratory Meeting*, 11–14 March, 1999, p 47.
68. Hu SX, Ji W, Zhou Y et al. Development of an adenoviral vector with tetracycline-regulatable human tumour necrosis factor α gene expression. *Cancer Research* 1997; **57**: 3339–3343.
- *69. Hallahan DE, Mauceri HJ, Seung LP et al. Spatial and temporal control of gene therapy using ionising radiation. *Nature Medicine* 1995; **8**: 786–791.
70. Dunn T. Oxygen and cancer. *North Carolina Medical Journal* 1997; **58**: 140–143.
71. Wenger R & Gassman M. Oxygen(es) and the hypoxia inducible factor 1. *Journal of Biological Chemistry* 1997; **378**: 609–616.
72. Crews ST. Control of cell lineage-specific development and transcription by bHLH-PAS proteins. *Genes Development* 1998; **12**: 607–620.
73. Dachs S, Patterson AV, Firth JD et al. Targeting gene expression to hypoxic tumor cells. *Nature Medicine* 1997; **3**: 515–520.
74. Prentice H, Bishopric NH, Hicks MN et al. Regulated expression of a foreign gene targeted to the ischaemic myocardium. *Cardiovascular Research* 1997; **35**: 567–574.
75. Boast K, Binley K, Iqbal S et al. Characterization of physiologically regulated vectors for the treatment of ischemic disease. *Human Gene Therapy* 1999; **10**: 2197–2208.
76. Walters LR & Palmer JG (eds) *The Ethics of Human Gene Therapy*. New York: Oxford University Press, 1997.
77. Brinster RL & Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. *Proceedings of the National Academy of Sciences of the USA* 1994; **91**: 11303–11307.

78. Roth JA & Christiano RJ. Gene therapy for cancer: what have we done and where are we going? *Journal of the National Cancer Institute* 1997; **89**: 21–39.
79. Lowenstein PR, Jaszai J & Castro MG. Gene therapy for neurological diseases: quo vadis? Achievements and expectations of the brave new technology. In Day I & Humphries S (eds) *Genetics of Common Disease: Future Therapeutic, Diagnostic, Prognostic and Screening Approaches*, pp 219–239. BIOS Scientific, 1997.
80. Anderson WF. Human gene therapy: scientific and ethical considerations. *Journal of Medicine and Philosophy* 1985; **10**: 275–291.
- *81. Castro MG. Gene therapy strategies, the treatment of pituitary tumours. *Journal of Molecular Endocrinology* 1999; **22**: 9–18.
82. Clothier C, The Department of Health. *Report on the Committee on the Ethics of Gene Therapy*, CM 1788. London: HMSO, 1993.
83. Lowenstein PR. Why are we doing so much cancer gene therapy? Disentangling the scientific basis from the origins of gene therapy. *Gene Therapy* 1997; **4**: 755–756.
84. Lowenstein PR, Wilkinson GWG, Castro MG et al. Non-neurotropic adenovirus: a vector for gene transfer to the brain and possible gene therapy of neurological disorders. In Latchman DS (ed.) *Genetic Manipulation of the Nervous Systems*, pp 11–40. London: Academic Press, 1996.
85. Wivel NA & Walters LR. Germ-line gene modification and disease prevention: some medical and ethical perspectives. *Science* 1993; **262**: 533–538.