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REVIEW ARTICLE

Prospects for gene therapy of haemophilia

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Summary. That gene therapy offers the promise of a cure for haemophilia was apparent more than a decade ago. After years of failure, substantial progress in the efficiency of gene transfer technology has recently resulted in impressive success in animal models with haemophilia. However, fears of the risks intrinsic to such therapy have been raised by the fate of two children cured of immune deficiency by gene transfer who have, however, subsequently developed leukaemia as a result of insertional mutagenesis. The purpose of this review is to outline the current status of gene therapy in light of recent successes and tragedies and to consider the prospects for curing haemophilia in the short-to-medium term.

Keywords: gene therapy, haemophilia, insertional mutagenesis

Introduction

Through the introduction of a functional gene into a target cell, gene therapy aims to restore, modify or enhance cellular functions. The haemophilias were recognized early as ideal candidates for gene therapy because their clinical manifestations are attributable to the lack of a single protein that circulates in minute amounts in the plasma. Additionally, years of clinical experience indicates that an increase of 1–2% in circulating levels of the deficient clotting factor can significantly modify the bleeding diathesis, therefore, the therapeutic goal for gene therapy is modest. Although gene therapy was originally conceptualized as a treatment for inherited genetic disorders, of the 918 registered clinical trials involving over 3000 patients, two-thirds (Journal of Gene Medicine Clinical Trial Database: http://www.wiley.co.uk/genetherapy/clinical) have focused on acquired disorders including cancer, vascular disease, degenerative neurological conditions and infectious diseases such as HIV/AIDS. However, very few studies have progressed beyond the phase I stage of assessing safety despite exciting preclinical data, primarily due to the lack of vehicles (or vectors) capable of efficiently 'introducing' the therapeutic gene into the target cells of humans. Expectations were further dampened, in 1999, when a patient with ornithine transcarbamylase deficiency (OTC) died following gene transfer with a vector based on adenovirus [1]. The inquisition that followed culminated in more stringent oversight of the design of gene therapy studies together with more robust means of reporting toxicity.

The turn of this century saw the first success of gene therapy in which retrovirus-based treatment of infants with life-threatening X-linked severe combined immune deficiency (X-SCID) resulted in impressive immune reconstitution [2,3]. These children who did not have suitable bone marrow donors were able to assume normal lives instead of near total isolation because of the risks of life-threatening infections. Just when hopes were being raised for a variety of catastrophic disorders, two of the SCID patients treated with retroviral vectors developed leukaemia, 3 years after gene transfer. This was shown to be due to insertional mutagenesis [2,3], which from being a theoretical risk, has now become a reality. Consequently, further research is now being undertaken to design safer vectors, to understand the critical interactions of

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gene transfer on host genes, and to define toxicity. Also needed is a careful risk-benefit assessment of gene therapy in the context of haemophilia where there are other well-established and successful therapeutic options.

An outline of vectors used for haemophilia gene therapy

The success of gene therapy depends on the development of vehicles, known as vectors, which introduce the therapeutic genes into target somatic cells, a process referred to as transduction. The target cells can either be in culture (e.g. fibroblasts), thereby allowing *ex vivo* gene transfer, or reside in organs, which requires *in vivo* delivery of vector. A number of gene transfer vehicles have been developed that can broadly be divided into two categories – non-viral and viral vectors. Non-viral vectors include naked DNA and DNA encapsulated with cationic lipids known as liposomes. These non-viral vectors offer the advantages of relative ease of production and reduced toxicity and therefore have been evaluated in a variety of preclinical models and in humans. They are, however, not very effective at delivering therapeutic genes *in vivo* and gene expression mediated by non-viral vectors is often transient.

Viruses succeed in nature as infectious agents because they have evolved the ability to gain access to specific cells (including bacteria, plants and animals) and exploit the host's cellular machinery to facilitate their replication. Recombinant viral vectors are designed to harness the native viral infection pathway. They are, however, modified so that they lack the ability to replicate in the host cell because non-essential viral coding sequences including those required for viral replication are replaced with the gene of interest such as human factor IX (hFIX) (Fig. 1). The number of different viruses that are under development as gene-therapy vectors is steadily increasing, but can be divided into two general categories – integrating and non-integrating. At present, retroviral vectors based on oncoretro-, foamy- or lenti-virus are the only gene transfer systems that can mediate efficient integration of the transgene into recipient cells' nuclear DNA. In contrast, the genome of vectors based on herpes, adeno-associated or adenovirus vectors is maintained mainly outside the nucleus as episomes. Since episomes are lost with each cell division, expression from non-integrating vectors is often transient, especially in tissues/organs with a high cell turnover. The properties and efficacy of the commonly used vectors are considered below.

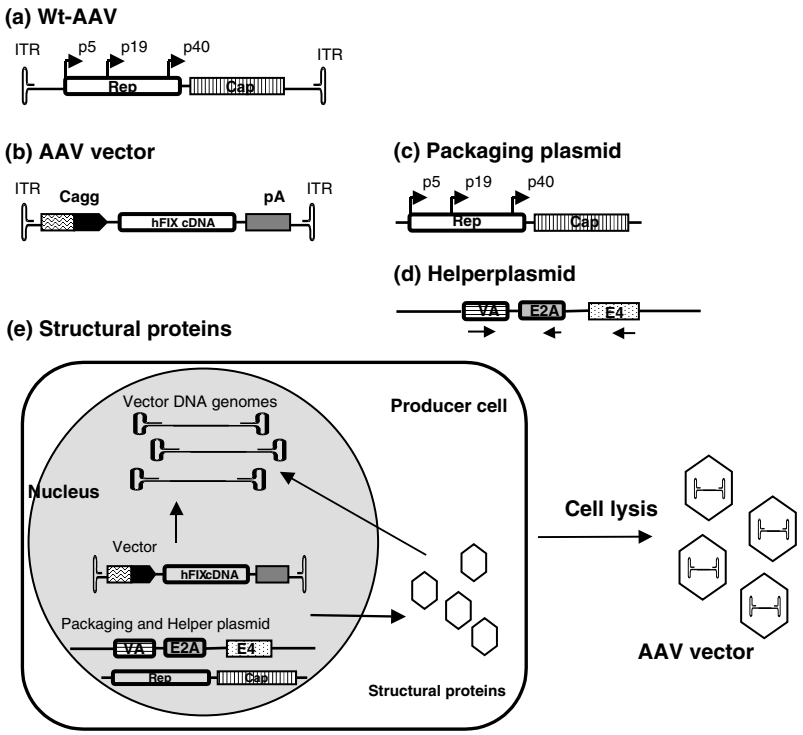

Fig. 1. Production of recombinant adeno-associated viral (AAV) vectors for treating haemophilia B: (a) The wild-type AAV (Wt-AAV) genome containing the rep and cap genes flanked by inverted terminal repeats (ITR). (b) In the AAV vector, the rep and cap genes are replaced by promoter (CAGG) and transgene (human FIX, hFIX) sequences. (c) The rep and cap genes are expressed from a separate packaging plasmid. (d) The adenoviral helper plasmid expresses helper genes E2, VA and E4, which are required for rescue and replication of the AAV vector genome. (e) Recombinant AAV is produced by simultaneous expression of the viral vector, packaging and helper constructs in 293 producer cells, which provide complimenting adenoviral E1 proteins. The vector genome is packaged into the structural capsid proteins in the nucleus and recovered from the producer cell by lysis.

Non-viral vectors

The use of physical or chemical methods to introduce DNA into target cells has attracted considerable attention as DNA complexes can be assembled relatively cheaply in cell-free systems from well-defined components that are potentially less immunogenic than viral vectors. Persistent expression of hFIX following liver-targeted delivery of plasmid encoding the hFIX cDNA (Table 1) either directly or in the context of 'Sleeping Beauty transposase' [4–7] has been observed in murine models. However, these approaches are dependent on hydrodynamic force for efficient gene transfer, which causes significant tissue damage, and morbidity that can hopefully be overcome with advances in technology.

A phase I trial evaluating gene transfer by electroporation of a B-domain-deleted factor VIII gene (FVIIIΔB) to skin-derived autologous fibroblasts of patients with severe haemophilia A (HA) has recently been concluded (Table 1) [8,9]. This was a dose-escalation study in which hFVIII expressing clonal cells were implanted laparoscopically into the omentum of 12 subjects. The procedures were well-tolerated without any serious side-effects. A transient rise in FVIII activity (range: 0.5–4% of normal) was detectable in some of the patients that lasted for a period of a year. Extension of this study with higher cell doses and refinement of implantation techniques is planned although the reasons for abrogation of transgene expression have not been fully established.

Retroviral vectors

Moloney murine leukaemia virus (MoMLV)-based vectors were amongst the first viral vectors to be evaluated in clinical gene therapy trials as they offer the potential for long-term gene expression by virtue of their ability to integrate stably into the host genome. However, efficient transduction of the liver with these vectors requires a partial hepatectomy to induce cell proliferation as oncoretroviral vectors cannot efficiently transduce quiescent tissues (Table 2) [10]. Other investigators have taken advantage of the active proliferative state of the liver in newborn animals to establish long-term physiological expression of hFIX in haemophilic animals after systemic administration of high titres of oncoretroviral vectors [11].

The first gene therapy trial for haemophilia, performed in 1991 in China, involved transduction of *ex vivo* expanded autologous skin fibroblasts using an oncoretroviral vector encoding the hFIX gene. Implantation of $0.4–3.0 \times 10^8$ transduced cells over 3–5 months resulted in a twofold increase in hFIX activity that persisted for over a year before

Table 1. Haemophilia gene therapy with non-viral vectors.

Packaging capacity	Ease of production	Integration into host genome	Duration of expression	Transduction of postmitotic cells	Pre-existing host immunity	Safety concerns	Germ-line transmission
Properties							
Unlimited	+++	Rarely	Usually transient	++	None	–	–

Ex vivo/in vivo	Route of vector delivery	Transgene	Promoter	Species	Expression (% of normal)	Duration	References
Summary of preclinical studies							
In vivo	IP	FIX	HCR/α1AT	Mouse	40	>18 months	[5]
In vivo	IP	FIX	HCR/α1AT	Mouse	253	>15 weeks	[6]
In vivo	IP	hFVIIIΔB	HCR/α1AT	Mouse	750	<4 weeks	[7]
In vivo	IP (transposon)	FIX	EF1α	Mouse	3	>5 months	[4]

Sponsor	Transgene	Promoter	Inclusion criteria	Method of vector delivery	Safety	Peak transgene expression	Current status
Summary of phase I–II studies							
Transkaryotic Therapies, Cambridge, MA	hFVIIIΔB	Fibronectin	Adults with severe HA	Electroporation of autologous fibroblast *ex vivo* followed by implantation into omentum	No significant side-effects	Transient increase in FVIII activity to a maximum of 4%	Closed

FVIIIΔB: B-domain-deleted factor VIII gene.

Table 2. Haemophilia gene therapy with retroviral vectors.

Packaging capacity	Ease of production	Integration into host genome	Duration of expression	Transduction of postmitotic cells	Pre-existing host immunity	Safety concerns	Germ-line transmission
Properties							
Approximately 8.0 kb	No reliable producer cell lines for lentiviral vectors	Yes	Long-term	Lentiviral vectors ++ Oncoretroviral vectors −	None	Insertional mutagenesis	−/+

Vector	Route of delivery	Transgene	Promoter	Species	Expression (% of normal)	Duration	Reference
Summary of preclinical studies							
MoMLV	IP	FIX	LTR	HB dogs	0.2	9 months	[10]
MoMLV	IP	FIX	hAAT	HB dogs	36	>14 months	[11]
FIV	IV	hFVIIIΔB	RSV	FVIII KO mice	74	>22 weeks	[15]
HIV	IP	FIX	PGK	Mouse	7	>4 months	[14]
HIV	Bone marrow	hFVIIIΔB	CAGG	FVIII KO mice	5	3 months	[16]

Sponsor	Transgene	Vector	Inclusion criteria	Method of vector delivery	Safety	Peak transgene expression	Current status
Summary of phase I–II studies							
Chiron Corp., Emeryville, CA, USA	hFVIIIΔB	Oncoretroviral	Adults with severe HA, negative for HCV	IV	Erroneous detection of vector genome in semen	Isolated increase in FVIII activity at low levels	Trial terminated. No future plans
Fudan University, China	hFIX	Oncoretroviral	Adults with moderate HB (baseline FIX of 2%)	3 monthly injections of *ex vivo* modified autologous fibroblasts	No significant side-effects	Transient (<16 months) increase in FIX activity to 4%	Closed

MoMLV, Moloney murine leukaemia virus; FIV, feline immunodeficiency; FVIIIΔB: B-domain-deleted factor VIII gene; HCV, hepatitis C virus; HB, haemophilia B.

declining to baseline levels [12]. Chiron Corporation (Emeryville, CA, USA) conducted a phase I dose-escalation study involving systemic administration of oncoretroviral vectors encoding FVIIIΔB gene in 13 subjects with haemophilia A [13]. Doses as high as 8.8×10^8 transduction units kg^{-1} were well-tolerated, with gene transfer confirmed by the presence of vector sequences in peripheral blood mononuclear cells for as long as 1 year post-treatment. Modest increase in hFVIII activity (around 1% of normal) was transiently detected in six of 13 subjects, however, clinical response did not correlate with vector dose. There are currently no plans to extend this study.

Recently attention has turned to lentiviral vectors because of their ability to transfer complex genomes into quiescent cells without rearrangement (Table 2) [14,15]. Theoretical safety concerns including the mobilization of vector genome by wild-type HIV, a common comorbid condition in adults with haemophilia, have lead to the development of self-inactivating (SIN) vectors and vectors based on non-primate lentiviruses, which cannot replicate in human cells. An additional safety concern relating to all retroviral vectors is that of insertional oncogenesis which is discussed further in the section on safety. Target selection will be critical as demonstrated by a recent study in which neutralizing antibodies to FVIII were generated in mice after transplantation with bone marrow transduced with lentiviral vector encoding the hFVIII gene [16].

Adenoviral vectors

Adenoviral vectors are highly efficient at transducing postmitotic tissues, such as liver, leading to therapeutic and supraphysiological factor levels after a single administration of vector particles (Table 3) [17–21] Early generation adenoviral vectors contained a large proportion of wild-type viral genes. Their expression resulted in a robust inflammatory response, toxicity, and short-term transgene expression. Although local adenovirus-mediated inflammatory response can be an asset in the treatment of

Table 3. Haemophilia gene therapy with adenoviral vectors.

Packaging capacity	Ease of production	Integration into chost genome	Duration of expression	Transduction of postmitotic cells	Pre-existing host immunity	Safety concerns	Germ-line transmission
Properties							
Approximately 30.0 kb	+/−	No	Transient	Very efficient	+++	Inflammatory response	−

Vector	Route of vector delivery	Transgene	Promoter	Species	Peak expression (% of normal)	Duration	References
Summary of preclinical studies							
ΔE1ΔE3	IV	hFVIIIΔB	Albumin	HA dogs	800	1–2 weeks	[18]
Gutless	IV	hFVIIIΔB	Albumin	FVIII KO mice	150	>9 months	[20]
Gutless	IV	hFVIIIΔB	ApoE/ApoCII	HA dogs	3.5	2–3 weeks	[21]
ΔE1	IV	hFIX	CMV	Rhesus	>80	22 days	[17]
Gutless	IV	hFIX	HCR/α1AT	FIX KO mice	820	>2 months	[19]
Gutless	IV	cFVIII	HNF-FVIII	HA dogs	190	Approximately 6 months	[47]
Adeno-spliceosome	IV	mFVIII	CMV	FVIII KO mice	100	>2 months	[25]

Sponsor	Transgene	Vector	Inclusion criteria	Method of vector delivery	Safety	Peak transgene expression	Current status
Summary of phase I–II studies							
GenStar Therapeutics, Alameda, CA, USA	Full-length FVIII	Gutless adenoviral vector containing the albumin promoter	Adults with severe HA with low titres of antiadenovirus antibodies	Peripheral venous infusion	Elevation of liver enzymes observed in the first patient	Transient increase in FVIII activity to 3%	Closed

hFVIIIΔB: human B-domain-deleted factor VIII gene; HCV, hepatitis C virus; HA, haemophilia A.

malignant disorders, systemic administration of adenoviral vectors is associated with hepatic and pulmonary inflammation that proved fatal in a patient with OTC deficiency [1]. A new generation of adenoviral vectors which lack wild-type genes ('gutless' adenoviral vectors) appear to be less immunogenic and are able to mediate sustained transgene expression in a variety of tissues *in vivo* [21,22]. However, evaluation of these vectors in a phase I/II study involving patients with severe HA has been hampered by the occurrence of transient transaminitis and natural immunity to the adenoviral capsid proteins [23]. Strategies to transiently block the immune response to capsid proteins as a means to enhance safety and efficacy of adenoviral vectors is currently being evaluated [21,24].

Recently adenoviral vectors have been used in an innovative approach to gene therapy of HA, which involves correction of the genetic mutation at the transcript level using the spliceosome-mediated mRNA repair technology [25]. Results are highly encouraging and this approach offers an added advantage in that repair of FVIII will only occur in tissues expressing the endogenous transcript thus reducing the risk of ectopic expression. This technology, however, needs to be formally evaluated in larger animal models before transition to the clinic.

Adeno-associated viral vectors

Currently, recombinant adeno-associated viral vectors (rAAV) are the most promising vectors for gene therapy of haemophilia B. They have the best safety profile among gene transfer vectors of viral origin, as wild-type AAV has never been associated with human disease. Safety is further enhanced by the dependence of AAV on co-infection with helper virus (usually adeno- or herpes-virus) for productive infection. Additionally recombinant vectors are entirely devoid of wild-type viral genes thus reducing the potential for invoking cell-mediated immune response to foreign viral proteins. Most importantly, these vectors can direct long-term transgene expression from a variety of postmitotic tissues including the liver and muscle (Table 4). Interesting stable expression is mediated predominantly by episomally retained rAAV transgenes through mechanisms that are not clearly understood [26]. Two clinical gene therapy trials for haemophilia B have been performed with rAAV vectors based on serotype 2 [27,28]. The first study was a dose-escalation phase I/II study entailing single i.m. injections of AAV-2 at a dose ranging from 2 to 20×10^{11} vg.kg^{-1}. Vector administration was not associated with serious adverse events [27].

Table 4. Haemophilia gene therapy with AAV vectors.

Packaging capacity	Ease of production	Integration into host genome	Duration of expression	Transduction of postmitotic cells	Pre-existing host immunity	Safety concerns	Germ-line transmission
Properties							
4.6 kb	Cumbersome	Rarely	Long-term in postmitotic cells	Efficient	++	–	+/–

Vector	Route of vector delivery	Transgene	Promoter	Species	Expression (% of normal)	Duration	References
Summary of preclinical studies							
2 (Split)	IP	hFVIIIΔB	CAGG	No SCID mice	4	>4 months	[35]
2 (two vectors)	IP	hFVIIIΔB	EF1α	Mice	200	>9 months	[36]
2	IP	cFVIIIΔB	TTR	HA dogs	4	>14 months	[33]
8	IP	hFVIIIΔB	TGB	FVIII KO mice	100	>12 months	[34]
2	IM	cFIX	CMV	HB dogs	3	>8 months	[48]
2	IP	FIX	CAGG	Mice	12	>7 months	[29]
2	IP	cFIX	ApoE/α1AT	HB dogs	12	>17 months	[49]
2	IP	hFIX	CAGG	Rhesus macaques	10	>24 months	[41]
5	IP	hFIX	EF1α	Mice	20	>28 weeks	[50]
8	IP	cFIX	TBG	Mice	340	>2 weeks	[31]

Sponsor	Transgene	Vector	Inclusion ccriteria	Method of vector delivery	Safety	Expression (% of normal)	Current status
Summary of phase I–II studies							
Avigen, Alameda, CA, USA	hFIX	AAV-2	Adults with severe HB	IM	No significant side-effects	Transient <1.6%	Study closed
Avigen, Alameda, CA, USA	hFIX	AAV-2	Adults with severe HB	Bolus infusion into hepatic artery	Vector in semen of patients	Transient hFIX at 2–3% in two patients given 5×10^{12} vg.kg^{-1}	Open

hFVIIIΔB: human B-domain-deleted factor VIII gene; AAV, adeno-associated viral vectors; HB, haemophilia B; SCID, severe combined immune deficiency.

Human FIX was detected immunohistochemically at the site of injection in all patients although systemic levels were below the therapeutic range. There are no plans to extend this study because it appears that muscle targeted delivery of rAAV is more likely to evoke an immunological response to hFIX [29,30]. A second clinical trial with AAV vectors has focused on liver directed delivery of rAAV-2 at a dose ranging from 2 to 50×10^{11} vg.kg^{-1}. In all patients vector genomes were transiently detected in the semen although there is no evidence of germ line transmission. Human FIX at therapeutic levels has thus far only been detected transiently (up to 6 weeks after gene transfer) in two patients who received the highest dose (50×10^{11} vg.kg^{-1}). One of these patients had an episode of reversible subclinical transaminitis of unknown aetiology at 4 weeks after gene transfer [28]. These results indicate that rAAV-mediated gene transfer requires further optimization before its potential is fully realized. In this respect, we and others have observed substantially greater transduction of the liver with AAV serotype 8 than observed with AAV-2 [31,32]. Another potential advantage of using this serotype is the low prevalence of pre-existing neutralizing antibodies to AAV-8 in humans.

The limited packaging capacity of AAV vectors (4680 kb) has hindered the use of these vectors for HA gene therapy although encouraging *in vivo* success has been achieved with FVIIIΔB cDNA using small promoters [33] and AAV-8 [34]. In addition, co-administration of two AAV vectors separately encoding the FVIII heavy- and light-chains whose intracellular association *in vivo* leads to the formation of a functional molecule shows promise [35]. Finally, the tendency of AAV vectors to form head-to-tail concantamers has been exploited in strategies that rely on dimerization of two AAV transgenes encoding different portions of the FVIII expression cassette to mediate FVIII expression at therapeutic levels [36] (Table 4).

Obstacles to gene therapy and future directions

Biosafety considerations

Wild-type oncoretroviruses are known to be leukaemogenic in mice and rhesus monkeys [37,38]. However, it was generally thought that vector insertion-induced oncogenesis would be extremely rare with replication-incompetent retroviral vectors, because they only integrate at the time of target cell transduction. This belief was supported by the fact that since 1989, no instances of insertion-induced oncogenesis have been observed in over 300 patients (many with underlying malignancy) that have been enrolled in more than 50 clinical trials involving the use of oncoretroviral vectors. The two cases of leukaemia in X-SCID children have now turned into reality what was until recently just a theoretical consideration. It is clear that integration of vector into or near the *LMO2* gene, in two otherwise unrelated cases, was an important transforming event in the setting of gene therapy for X-SCID. However, the relatively long latent period before the onset of disease suggests that *LMO2* transactivation may not by itself be sufficient to cause leukaemia consistent with the current multistep models of leukaemogenesis. It is highly likely that the T-cell proliferative advantage conferred by retroviral-mediated $^{\gamma}c$ expression, and the high doses of retrovirally transduced cells received by these two patients served as compounding factors in the pathogenesis of leukaemia.

Recent studies indicate that rAAV vectors also integrate into transcriptionally active genes of the host genome following liver-targeted delivery of vector particles in mice [39]. Unlike retroviral vectors, however, rAAV integrates into the host chromosome with a frequency estimated to be below 5% [40]. Additionally integration appears to be facilitated by existing chromosomal breaks as rAAV vectors lack integrases. The safety implications of rAAV integration are unclear at present although it is apparent that the mechanism of integration is distinct from that of retroviral vectors. Additionally, the wild-type AAV usually integrates in a site-specific manner into the AAVS1 site of chromosome 19 although random integrations have also been observed in humans and macaques. Despite the endemic nature of AAV-2 in primates, malignancies as a result of AAV-mediated insertional mutagenesis have not been reported.

There are many approaches under development for reducing the risk of viral vector-induced oncogenesis. In the new generation of retroviral vectors, safety would be enhanced by deleting the endogenous viral promoter/enhancer element (U3 region of the vector's 3′-LTR) to generate SIN vectors, in which only an internal promoter would direct transgene expression. The incorporation of insulator elements, which are DNA sequences designed to shelters genes such as β-globin from external heterologous enhancer domains, would further reduce the possibility of host gene transactivation. This approach, however, needs to be formally tested in appropriate animal models.

A simple strategy to counter gene therapy induced malignancies would be to incorporate a suicide gene, such as the herpes simplex virus-1 thymidine kinase (HSVtk) as an adjunct to the vector expression cassette. After administration, the prodrug, ganciclovir, would be phosphorylated by gene-modified cells expressing HSVtk resulting in inhibition of DNA elongation. Thus, transduced cells can be selectively eliminated in the event of inadvertent malignant transformation. However, the functional reliability, transcriptional regulation and immunogenicity of this approach remain to be established.

An ideal solution would be to develop vectors that integrate in specific and safe chromosome sites. Targeted gene insertion is achievable in certain experimental settings [4], but it remains a significant challenge to establish procedures that are sufficiently efficient and reliable for clinical application. Until targeted gene delivery becomes feasible in human cells, we will continue to depend on randomly integrating recombinant viruses to transfer therapeutic genes into target cells.

Scale-up of vector production

There are a number of scale-up considerations for the production of vectors for clinical use. Efficient packaging cell lines are not available for many vector systems that are currently being considered for clinical use such as lenti- or AAV-vectors as a result of the cellular toxicity of the viral proteins required for rescue and packing of the vector genome. Currently a transient transfection method, the using highly characterized human 293 cell line that is capable of efficient uptake of exogenous DNA is used to generate clinical grade lentiviral and AAV vector. However, this is cumbersome with inherent variability of vector yields.

Derivation of producer cells and vector particles requires dedicated personnel and infrastructure to ensure that all procedures are performed under strict Good Manufacturing Practice (GMP) environment. This has significant resource implications, which are often beyond the domain of academic establish-

ments. Biotechnology companies, whilst highly interested in gene therapy for many acquired disorder such as malignancy and autoimmune disease, are often not very enthusiastic about inherited disorders, which may be catastrophic but affect a small number of patients. The lack of core central facilities for generating clinical grade vector outside of the United States, poses a significant challenge to those in academic institutions elsewhere engaged in a gene therapy programme for rare catastrophic disorders.

Tissue-specific vector targeting

In vivo delivery of vector often leads to wide dissemination of particles and inadvertent transduction of non-target tissues leading to side-effects such as the development of neutralizing antibodies to the transgene product [41] or severe inflammatory responses [42]. In addition, the promiscuity of vectors has raised concerns about germ-line transmission of vector genome. To realize the full potential of gene therapy it will be necessary to precisely target the vectors to diseased tissues. Restricting transgene expression to a target tissue is becoming more feasible because of a better understanding of the activity of the promoter/enhancer complex [42]. Unfortunately, the level of transgene expression mediated by some of these tissue-specific regulatory elements is often lower than that observed from constitutively active promoters. Additionally, the packaging capacity of many of the currently available vector systems restricts the size of tissue-specific regulatory element that can be included in the expression cassette. Pseudotyping of viral vectors, where a genome from one type of vector is enveloped with structural proteins from another often related virus has been widely used to retarget retroviral vectors. Similarly, the AAV-2 genome has been successfully cross-packaged into capsids of alternative serotypes. Selective modification of the head portion of the fibre protein in conventional adenoviral vectors (serotype 5) with the corresponding portion from an adenoviral 3 serotype, resulted in more efficient transduction of human stem cells [43]. The limitation of such strategies is that vector retargeting is determined by the tropism of the parental virus. Modifications of retroviral envelope protein or AAV capsids to add new or alternative binding domains have often resulted in targeted binding of the viral particle to the desired cell type but at the price of reducing vector titres. Although individual strategies to restrict vector biodistribution or transgene expression have not been fully successful, it is likely that further advances that enable a combined approach involving transductional targeting through modification of structural proteins and use of optimal promoters will reduce potential toxic effects and allow administration of lower doses of vector.

Immunological consequences of gene therapy

Many of the immunological defence systems that are used to tackle wild-type viral infections can be activated against the vectors and/or new transgene products that might be recognized as foreign. Adenovirus vectors are the most immunogenic of all the viral vector, capable of inducing both cytotoxic T-lymphocyte (CTL) as well as humoral responses. A massive systemic inflammatory response was induced by an adenovirus vector leading to the death of a patient during a 1999 trial for OTC deficiency [42]. This study clearly demonstrated that different patients have markedly different inflammatory and immune responses to the same dose of adenovirus vector. It is not yet clear how to predict which patients will mount severe inflammatory reactions. Progress has been made in reducing T-cell responses against viral gene products that are expressed by transduced cells, by engineering 'gutted' or 'helper-dependent' (HD) adenoviral vectors but these highly disabled vectors still retain the potential to induce a capsid-mediated inflammatory response [44].

Other vector systems are less inflammatory and immunogenic than adenovirus vectors. Lentivirus and AAV vectors in particular do not generate a CTL response against viral proteins, but T-cell responses can still be elicited against the expressed transgene product depending on the route of vector administration and or the ability of the vector system to transduce antigen-presenting cells such as dendritic cells [45]. Humoral immunity to the parental wild-type viruses has likely impeded success with AAV-2 based vectors, however, this obstacle may be resolved by switching capsid serotypes.

Conclusion

One of us (ET) predicted that gene therapy would become a reality for haemophilia in the first decade of the new millennium [46]. Notwithstanding the rare adverse events and disappointing expression levels associated with human trials, recent progress on gene delivery and the excellent results in haemophilic animals encourage us and others to press on with research to deliver that goal on time.

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The Avigen sponsored AAV-2 based gene therapy trial involving liver targeted delivery of vector particles has recently been closed. Transient elevation of liver enzymes were observed in another patient (patient 7) thought to be due to an immunological response to the transduced cells.

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