

REVIEW

Progress and Prospects: Gene Therapy Clinical Trials (Part 1)

Over the last two decades gene therapy has moved from preclinical to clinical studies for many diseases ranging from single gene disorders such as cystic fibrosis and Duchenne muscular dystrophy, to more complex diseases such as cancer and cardiovascular disorders. Gene therapy for severe combined immunodeficiency (SCID) is the most significant success story to date, but progress in many other areas has been significant. We asked 20 leaders in the field succinctly to summarize and comment on clinical gene therapy research in

their respective areas of expertise and these are published in two parts in the Progress and Prospect series.

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In brief

Progress

- Gene therapy clinical trials have been carried out for a large number of single gene and complex disorders over the last two decades.
- Understanding the strength and weaknesses of gene transfer vectors and the choice of the appropriate vector for the individual disease has significantly advanced.
- Gene therapy for SCID shows clear clinical benefit, and is the most significant success story to date.
- Early hype has largely been replaced by the necessary stepwise progress needed to realize clinical benefit.

Prospects

- Immune responses to both vector and transgene may have to be better understood and overcome.
- Placebo-controlled trials are crucial to document clinical benefits of gene therapy; uncontrolled trials should be interpreted cautiously.
- Replacing hype with reasonable expectation may help return gene therapy to its appropriate place in the public's thinking.

Eye diseases

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The eye has unique advantages as a target organ for gene therapy. In particular, its compartmentalized anatomy enables local vector delivery with low likelihood of systemic dissemination. Intraocular tissues comprise small stable cell populations that can be transduced efficiently and stably by small volumes of vector suspension. The eye is readily accessible for *in vivo* assessment by optical imaging and electrophysiological techniques. Significant progress has been made during the last decade in the development of ocular gene therapy in experimental and preclinical models. The

results of the first clinical trials for ocular cancer and angiogenic disease have now been reported. One trial of gene replacement therapy for inherited retinal degeneration commenced recently and further such trials are expected to begin imminently.

Retinoblastoma is the commonest primary intraocular tumour of childhood. A phase I clinical trial has been conducted to evaluate the feasibility and safety of adenovirus-mediated 'suicide' gene therapy for vitreous tumour seeds in one eye of children with bilateral retinoblastoma refractory to conventional treatment.¹ This trial evaluated the effects of repeated intravitreal injections of an adenoviral vector (group C serotype 5) expressing a herpes simplex thymidine kinase gene followed by systemic administration of ganciclovir. The vitreous tumour seeds appeared to respond to the intervention in all eight children. However, mild-to-moderate inflammation was reported and all eyes were ultimately enucleated for progression of the primary tumour, making it difficult to assess the efficacy of this intervention.

Age-related macular degeneration (AMD), which is one of the commonest causes of blindness, is typically

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characterized by pathological angiogenesis under the macula. A phase I study has been conducted to evaluate the safety and tolerability of a single intravitreal injection of an adenoviral vector expressing the anti-angiogenic cytokine pigment epithelium-derived factor (PEDF).² In this multicentre dose-escalation trial that involved 28 patients, a single intravitreal injection of adenoviral vector (E1-, partial E3-, E4-deleted) was generally well tolerated. Signs of mild-to-moderate transient intraocular inflammation were reported in 25% of patients but no dose-limiting toxicities or serious adverse events attributed to the study drug were identified. Although the study was not designed to identify a therapeutic effect, the results suggest that the intervention results in a possible dose-dependent anti-angiogenic effect. A further trial is planned to investigate the therapeutic efficacy of Ad.PEDF in patients with neovascular AMD.

Inherited retinal degenerations resulting from single gene defects affect approximately 1 in 3000 of the population and have no treatment option currently available. The potential of gene therapy for these conditions has benefited from significant progress in the mapping and cloning of retinal disease genes, of which more than 120 have been identified to date. Proof-of-principle for gene therapy of inherited retinal disease has been demonstrated in broad range of experimental models. Proposals for clinical trials of gene therapy have focused on inherited retinal degenerations that cause severe visual disability, yet offer a realistic chance of success. Early-onset severe retinal degeneration (Leber's congenital amaurosis) is related in approximately 10% of cases to defects in the gene encoding RPE65, a protein critical for normal retinal cycling of vitamin A. The demonstration of long-term functional improvement following gene replacement of RPE65 in a preclinical model has supported proposals for clinical trials of rAAV-mediated gene therapy in human subjects.³⁻⁵ A phase I/II, dose-escalation clinical trial of AAV2/2 for severe early-onset retinal degeneration due to mutations in RPE65 was started in the United Kingdom in 2007. Proposals for other studies have received approval in the United States. These first clinical trials will have a strong emphasis on evaluating safety and toxicity but will also begin to address the potential for benefit in terms of visual function. Younger subjects are considered more likely to benefit since they have less advanced retinal degeneration and better preservation of photoreceptor cells. Differences in study protocol between these trials in terms of vector titre, promoter sequences and inclusion criteria, are expected to yield complementary data that will help inform optimal vector design and define a window of opportunity for the timing of intervention.

Cystic fibrosis

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Cystic fibrosis (CF) is a single gene disorder with insufficient treatment options and non-invasive access to the target organ, the lung. Thus, it is not surprising that from early on, this disease has been at the forefront of gene therapy research. Since cloning of the CF gene

(Cystic Fibrosis Transmembrane Conductance Regulator) in 1989, 25 phase I/II clinical trials, involving approximately 400 CF patients, have been carried out using a variety of viral and nonviral gene transfer agents (GTAs).⁶ Most early trials focused on the nasal epithelium as a surrogate for the lung, to allow for easy access and sampling and, importantly, to ensure safety. Once an acceptable safety profile had been established GTAs were administered directly into the lung. Early trials focused on adenoviral vectors until it became apparent that, although efficient in mouse models, transfection efficiency into human airway epithelial cells, the target cells for CF gene therapy, was low, due to the absence of the adenovirus receptor at the apical membrane of these cells. In parallel, it emerged that first-generation adenoviral vectors could not be repeatedly administered,⁷ likely to be a global problem for viral vectors, but crucial for the treatment of a life-long chronic disease such as CF.

Adenoviruses were superseded by first-generation adeno-associated (AAV) vectors. Targeted Genetics Inc. led the development of AAV2 vectors for CF gene therapy for many years.⁸ However, most recent phase I/II trials were unsuccessful and the CF programme at Targeted Genetics was discontinued in 2005. Nine clinical trials have evaluated nonviral gene transfer agents. In general, the majority of these studies have shown proof-of-principle for correction of approximately 25% of the molecular defect towards non-CF values. In contrast to viral GTAs, nonviral formulations are more likely to be repeatedly administrable and proof-of-principle for this has been demonstrated in man.⁹ Importantly, both groups (The UK CF Gene Therapy Consortium and Copernicus Inc.) currently preparing for clinical studies to be carried out over the next 3 years are focusing on nonviral GTAs.

In general, industrial and academic interest in CF gene therapy has reduced over the past decade, likely because the development of CF gene therapy has turned out to be more difficult, and slower, than originally anticipated. It is important to note that most CF gene therapy research is currently funded by private charities such as the CF Trust in the United Kingdom and the CF Foundation in the United States.

In summary, successful gene transfer into airway epithelial cells, and partial correction of CFTR-mediated chloride transport has been demonstrated in many clinical studies using viral and nonviral GTAs. The key question now is whether this level of efficiency is sufficient to improve clinical parameters. To address this important question, clinical trials will need to be carried out for long enough to have a realistic chance of altering the underlying pathophysiology (thus requiring repeated administration), coupled with the development of better clinically relevant end points. These trials will be expensive, time-consuming and will require fairly large patient numbers.

α -1-Antitrypsin deficiency

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α -1 antitrypsin (AAT) has been seen as a suitable target for clinical gene therapy, since the gene product

functions as a circulating plasma protein and a clear end point for therapeutic effect (approximately 570 µg/ml of AAT) has been established. AAT is a 52 kDa glycoprotein secreted from hepatocytes, which has multiple functions as an antiprotease, anti-inflammatory and antiapoptotic agent. The lung disease which affects 90% of AAT-deficient individuals is due to the lack of normal AAT function, and it is this disease that is the primary target of gene augmentation therapy. Since therapy for the lung disease is dependent upon replacement of plasma levels, gene augmentation could target the wild-type allele to any number of tissues capable of secreting AAT. Preclinical studies with various viral and nonviral vectors have demonstrated the feasibility of expression from liver, muscle, lung, pleura and other sites. Of these, only muscle and nasal administration have been attempted in humans to date.

There is a theoretical advantage to expression of AAT from the respiratory tract, if higher local AAT levels were achieved. On the basis of this rationale a study was performed by Brigham *et al.*¹⁰ in which a plasmid-cationic liposome complex was used to deliver AAT to the nasal epithelium in five AAT-deficient individuals. This study showed AAT mRNA and protein expression at 5 days after administration, along with a decrease in the pro-inflammatory cytokine, interleukin-8.

Recent studies have attempted to achieve prolonged AAT expression by use of recombinant adeno-associated virus (rAAV) vector delivery to muscle.¹¹ The original preclinical and clinical studies were performed with the rAAV serotype 2 vector. A phase I clinical trial of rAAV2–AAT intramuscular injection showed a favourable safety profile with only humoral immune responses to AAV2 capsid protein, and transient AAT expression in one patient at levels more than 100-fold below therapeutic.¹² Preclinical studies have indicated a substantial efficacy advantage of rAAV serotype 1 vectors, and a phase I trial of rAAV1–AAT has now been initiated. Once again, there is careful focus on the potential that immune responses to AAV capsid proteins could limit transgene expression.¹³

Future directions for clinical AAT gene therapy are likely to rest with either a greater distribution of vector to a larger muscle mass through limb perfusion technology¹⁴ or with some form of immune modulation, should immune responses prove to be a limiting factor. There is also considerable promise from a large number of new AAV serotypes that might be used to transduce either muscle or a range of other tissues with higher efficiency.

Duchenne and other muscle wasting diseases

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The muscular dystrophies (MDs) are a heterogeneous group of inherited disorders characterized by progressive muscle weakness and degeneration, with a broad phenotypic spectrum, mostly severe and often fatal. The first gene transfer trial for a MD occurred 15 years after

cloning of the dystrophin gene responsible for Duchenne muscular dystrophy (the most prevalent inherited MD). Three Duchenne and six Becker patients, aged above 15 years, were injected in a small area of the radialis muscle, with low doses of a naked plasmid carrying the large, full-length, 79 exon dystrophin cDNA. Weak and patchy dystrophin expression was found in biopsies taken 3 weeks later in six out of the nine patients. Long-term expression is expected with naked DNA and repeated administrations can be carried out if necessary, although it remains to be seen if immune responses to the transgene become a problem with larger doses. Regional hydrodynamic delivery of the plasmid preparation leads to widespread transfection, and is expected to be evaluated in patients in the coming years.¹⁵

Adeno-associated viruses (AAVs) have a much larger muscle transduction efficiency, but lower cloning capacity than plasmids. A miniversion of the dystrophin gene, thought to retain part of the function of the protein was inserted, and this AAV1-microdystrophin vector is currently being evaluated in an intramuscular clinical trial for safety in six Duchenne patients between 5 and 15 years of age. If immune rejection of the vector can be overcome, either regional or systemic delivery will be assessed using different pseudotyped AAV vectors. Concomitantly, a similar open-label, dose-escalation phase I trial has been launched for the evaluation of the safety of local intramuscular injection of AAV1-γ-sarcoglycan in three cohorts of three patients with limb girdle muscular dystrophy 2C (LGMD 2C), as attempted in a previous trial.¹⁶

In addition to gene replacement, exon skipping is gaining attention for the restoration of the reading frame, which in Duchenne patients may allow expression of a shorter but in-frame dystrophin transcript and a potentially functional Becker-like, immune-tolerant dystrophin protein. Seventy per cent of Duchenne patients and other MDs may ultimately benefit from this strategy. A 10-year-old Duchenne patient defective in exon 20 of the dystrophin gene received a 4-week course of 0.5 mg/kg i.v. infusion of an antisense phosphorothioate oligonucleotide against the splicing enhancer sequence of exon 19. Low amounts of dystrophin were seen in peripheral lymphocytes and in muscle biopsies.¹⁷ Four Dutch patients (aged 10–13 years) with deletions in the exons 48–52 region have been recently injected intramuscularly with tailored antisense phosphorothioate oligonucleotides.¹⁸ Intravenous administration of larger doses is expected by early 2008. A similar trial using more stable morpholino oligonucleotides is under preparation in the United Kingdom. Interestingly, these trials are considered as gene therapy by UK regulatory bodies, but as conventional pharmacology in the Netherlands. An alternative approach, based on AAVU7-driven long-term *in situ* expression of oligonucleotides, which offers the potential also to target the heart, is expected to reach clinical trials in the near future.

With the remarkable progress in complementary areas, gene therapy of MDs has suddenly become a rapidly moving field. Considering the complexity of the MDs and the need to overcome hurdles such as long-lasting body-wide therapy and immune issues, combined approaches (gene replacement/repair and strategies to increase muscle mass) will probably be necessary.

Lysosomal storage disorders

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The lysosomal storage disorders (LSDs) are a group of inherited metabolic diseases that result from a loss or deficiency of one or several lysosomal enzymes. This deficit leads to the intralysosomal accumulation of non-metabolized macromolecules and subsequent cellular and organ dysfunction, including the brain. LSDs are attractive candidates for gene therapy as the production of modest amounts of the affected enzymes into the systemic circulation, by virtue of their ability to confer metabolic cooperativity, is expected to provide clinical benefit. These observations encouraged one of the earliest applications of this emerging technology to treat non-neuropathic Gaucher disease,¹⁹ and subsequently, mucopolysaccharidosis type I and II diseases.

All of these early investigational protocols involved transplanting *ex vivo* retrovirus-modified autologous CD34⁺ cells into non-conditioned subjects. Although there was evidence of transduction and expression of the lysosomal hydrolases, and of engraftment of the transplanted cells, the levels of enzymes observed in the clinical studies were low. Recent and ongoing research activities in gene therapies have been directed at improving vector designs to increase transduction efficiencies and at incorporating myeloreductive conditioning regimens to facilitate greater engraftment of transplanted cells.^{20,21} The possible use of partial- or reduced-conditioning regimens *in lieu* of full myeloablation is an important consideration, particularly for treating LSDs for which there is the option of clinically approved enzyme or small molecule drug therapies. Nevertheless, there is continued interest in evaluating this *ex vivo* gene therapeutic strategy, as illustrated by the recent submission of a clinical protocol to the Recombinant DNA Advisory Committee for treating mucopolysaccharidosis type VII disease.²⁰ This proposal seeks to use lentiviral-transduced autologous hematopoietic progenitor cells in combination with a myeloreductive-conditioning regimen. In addition to *ex vivo* approaches, there is also significant interest in the potential use of recombinant AAV vectors for *in vivo* gene therapy of the visceral pathologies of several LSDs.²² A multitude of successful preclinical studies in animal models of LSDs have been performed and await translation to clinical trials.

Another application of recombinant AAV vectors has been directed at addressing the CNS manifestations frequently associated with LSD. The purported ability of certain AAV serotypes to transduce post-mitotic neurons efficiently has encouraged an evaluation of direct administration of the vector into the brain to prevent or delay disease progression. Supported by safety and efficacy studies in rodents and non-human primates, a clinical study of AAV2-mediated gene transfer of tripeptidyl peptidase I to 10 children with late infantile neuronal ceroid lipofuscinosis has been initiated.²³ Two groups, one with the severe form and another with a moderate form of the disease will be studied. To effect widespread biodistribution of the therapeutic, multiple (via six burr holes) intracranial injections of the vector into the brain parenchyma will be performed. Alternative routes of CNS delivery under active preclinical

investigation include intrathecal and intracerebroventricular injections of a variety of AAV and lentiviral vectors.

It is evident that there are reasonable scientific and medical rationales for developing gene therapy for LSDs as indicated by the measured progression of the various therapeutic strategies to the clinic.

Severe combined immunodeficiency

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Severe combined immunodeficiencies (SCID) are a heterogeneous group of inherited disorders characterized by a profound reduction or absence of T lymphocyte function. Other lineages, including B lymphocytes and natural killer cells, may also be affected depending on the molecular defect. Mismatched allogeneic haematopoietic stem cell transplantation (HSCT) is associated with significant morbidity and mortality. HSC gene therapy is an attractive option as corrected cells may have a significant growth and survival advantage. The most common form of SCID is an X-linked form (SCID-X1) that accounts for 40–50% of all cases. SCID-X1 is caused by defects in the common cytokine receptor γ chain (γ c), which was originally identified as a component of the high-affinity interleukin-2 receptor (IL-2RG) but is now known to be an essential component of the IL-4, -7, -9, -15 and -21 cytokine receptor complexes.

Two studies in a total of 20 infants with classical presentation have demonstrated that gene therapy can be highly effective.^{24,25} Both utilized an MFG-based gammaretroviral vector encoding a *IL2RG* cDNA (regulated by intact Moloney murine leukaemia virus long terminal repeat (LTR) sequences), to transduce autologous CD34⁺ cells *ex vivo* which were re-infused into the patients in the absence of pre-conditioning. With some variation, which may relate to the age of the patients treated, dosage of transduced cells, and clinical status, the number and distribution of these T cells increased rapidly (often more rapidly than observed following haploidentical transplantation), usually achieving normal numbers compared to age-matched control values. In both studies, evidence exists for long-term engraftment of transduced HSCs at low levels (estimated at 0.1–2% based on marking in myeloid lineages), which may have significant implications for maintenance of thymopoiesis long term and for persisting B cell functionality.

In one study, four patients developed T-cell acute lymphoblastic leukaemia-like complications, primarily as a result of insertional mutagenesis. Remarkably, LTR enhancer-mediated activation of the same protooncogene *LMO-2* occurred in at least two of these patients.²⁶ Other contributing factors have not been clearly defined and although non-physiological expression of the transgene has been proposed, this remains controversial.²⁷ Two older patients have been treated using the same protocols but without effect, suggesting that there are age-related restrictions to the efficacy of HSC or gene therapy that may reflect inability to retrieve thymic function. Recent results from a US study in older patients would appear to support this contention.

Adenosine deaminase deficiency (ADA-SCID) accounts for approximately 15% of SCID and has been the subject of a number of clinical trials of T cell and HSC gene therapy using gammaretroviral vectors. Two recent studies in a total of 13 patients to date have also reported remarkable efficacy, which in part has been due to the absence or withdrawal of concomitant enzyme replacement therapy (PEG-ADA), and the administration of low-intensity bone marrow conditioning to facilitate HSC and multilineage engraftment.^{28,29} Other similar trials are ongoing. Interestingly, the kinetics of immunological reconstitution have been considerably slower compared to that seen in SCID-X1 patients, and adverse events due to insertional mutagenesis have not been reported. Newer studies are currently in preparation for SCID-X1 and other molecular forms of SCID using less mutagenic vector configurations, but the proof-of-principle for effective gene therapy of these diseases is clearly established.

Chronic granulomatous disease

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Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency characterized by recurrent, often life threatening bacterial and fungal infections due to a functional defect in the microbial-killing activity of phagocytic neutrophils.³⁰ It occurs as a result of mutations in genes encoding a multicomponent enzyme complex, the NADPH oxidase, that catalyses the respiratory burst. The majority of patients have an X-linked form of the disease and mutations in a membrane-bound component gp91phox. HLA-matched allogeneic haematopoietic stem cell (HSC) transplantation can be curative, but for patients without donors, genetic modification of autologous hematopoietic stem cells is an attractive alternative. The first phase I/II gene therapy trials for CGD were conducted in the United States almost 11 years ago.^{31–33} Although the level of correction achieved was low (0.004–0.6% functionally corrected cells), they provided clear evidence that a gene therapy approach for CGD was feasible and paved the way for further clinical trials. One common feature of these initial trials was the lack of bone marrow conditioning to enhance engraftment of transduced HSCs. As gene corrected myeloid cells are not predicted to have a proliferative advantage in this condition, it is now acknowledged that engraftment of sufficient numbers of HSC to provide long-term correction will depend on bone marrow conditioning or on the coexpression of a gene for *in vivo* selection.

In 2002, our group initiated the treatment of an X-CGD patient with gene-modified CD34+ cells using a mild immunosuppressive conditioning regimen. Similar to the previous trials, engraftment of gene-transduced cells was minimal. However, in 2004 two adult X-CGD patients were treated with a modified protocol, which included relatively low-intensity myelosuppression. In both patients, significant correction of the phagocytic defect (12 and 31%) was achieved, which contributed to

the eradication of pre-existing bacterial and fungal infections. Thus, this treatment provided a substantial therapeutic benefit to both patients early after transplantation.³⁴ Surprisingly, a gradual increase in the number of gene-corrected cells of up to 50–60% of all peripheral blood granulocytes was observed in both patients caused by retroviral-mediated insertional activation of three growth-promoting genes, namely MDS1/EVI1, PRDM16 and SETBP1.³⁴ One of the patients died 2.5 years after gene therapy from a severe sepsis. Preliminary analysis of gene-transduced cells revealed that the expression of gp91phox was almost undetectable at the time of death despite persistent high-level gene marking, suggestive of gene silencing (unpublished data). Four other patients have been treated using similar strategies, although the levels of engraftment of transduced cells have been lower.

The future of gene therapy for CGD will, therefore, depend on the development of new vectors with improved safety and efficacy profiles, and in the optimization of conditioning protocols to ensure reliable engraftment. For example, the use of self-inactivating (SIN) vectors, lacking the potent enhancer elements present within the viral LTRs, is an alternative to the conventional vectors currently in use. Moreover, transgene expression in SIN vectors is driven by an internal promoter, which allows the incorporation of tissue-specific promoters, thereby further reducing the probability of inadvertent oncogene activation in stem/progenitor cells. In conclusion, gene therapy for CGD is feasible, and may substantially contribute to improve the health conditions of the patients at least transiently.

Parkinson's disease

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Parkinson's disease (PD) is a devastating movement disorder, marked by the progressive loss of dopamine-producing cells in the substantia nigra. Traditional symptomatic therapy of PD consists of systemic administration of the precursor L-dopa to enhance dopamine levels within the brain. This treatment is efficacious in the early stages of PD but with increasing degeneration of dopamine nerve terminals and drug resistance, patients become less responsive to medication, responses become more erratic and often medication-induced adverse effects develop over time.

Several features of PD make it an attractive target for a gene therapy-based treatment. Currently, gene therapy vectors do not efficiently cross the blood-brain barrier, and even if this were more efficient, wide distribution of a transgene could have conflicting effects. Both the anatomical and neurochemical circuits within the basal ganglia have been well elucidated, allowing for focused intervention by surgical infusion of a given transgene into a specific brain region. Gene therapy has the potential to accomplish both goals.

The clinical landscape of gene therapy for PD is composed of three distinct major efforts. To date, all of these have utilized adeno-associated virus (AAV) as a vector, which was first shown to be safe and effective in the brain using an animal model of PD. The first human

trial of gene therapy for PD used AAV to transmit the gene for glutamic acid decarboxylase (GAD) into the subthalamic nucleus (STN). This strategy is based upon the most popular and successful surgical therapy for advanced human PD, which is an electrical stimulation of the STN. GAD is the rate-limiting enzyme for the synthesis of γ -aminobutyric acid (GABA), the major inhibitory transmitter in the brain. In PD, the two major inhibitory output nuclei of the basal ganglia are driven by a disinhibited and overactive STN, whose terminal projections release the excitatory neurotransmitter glutamate. AAV-GAD converts the STN from an excitatory into a predominantly inhibitory system, thereby normalizing the output of the entire basal ganglia circuit. There is also evidence that reducing STN, release of glutamate into the nigra can protect dopamine neurons from degeneration. An open label phase I clinical trial utilizing this strategy was recently completed.³⁵ Significant improvements were noted on standard clinical ratings both on and off medication, along with similar improvements in abnormal metabolism of relevant brain regions using functional imaging. A phase II study is currently being initiated.

Another approach aims to alter the natural course of disease progression by preventing degeneration of nigral neurons and promoting neuroplasticity. Here, AAV was used to transmit the gene for neurturin (NTN) to putamenal cell bodies, which are targets for dopamine neuronal projections.³⁶ NTN is a functional analog of glial-derived neurotrophic factor (GDNF), which together form a distinct TGB- β neurotrophin subfamily. A phase I clinical trial has been completed with preliminary results reported showing significant improvement in clinical ratings and this is the first approach to initiate a phase II study which is currently ongoing.

A third effort uses adeno-associated virus (AAV) to transmit the gene for aromatic L-amino acid decarboxylase (AADC) into the striatum to increase conversion of L-dopa into active dopamine. AADC converts L-dopa into dopamine in striatal neurons as well as within residual nigral dopaminergic neurons. As the disease progresses, AADC levels decline, creating a greater demand for L-dopa. However, escalating doses of exogenous L-dopa leads to side effects due to excessive stimulation of the mesolimbic pathway, which maintains normal levels of AADC. Intra-striatal AADC gene transfer seems to enhance the sensitivity to L-dopa and widen the therapeutic window. A positive clinical response has been demonstrated in animal studies and a human phase I clinical trial is currently ongoing.³⁷ This group has also shown that rAAV can support transgene expression for more than 3.5 years in primate brain, suggesting that long-term gene transfer will be feasible in human patients.

The use of gene therapy in three phase I clinical trials, two of which have completed and are moving into phase II, clearly demonstrates that PD is leading the development of human central nervous system gene therapy. The ongoing or planned phase II studies involve blinded control patients, who undergo similar procedures, but without penetration of the brain to minimize risk, and reduce ethical concerns. These trials will be critical to confirm the safety and efficacy of any one or all of these approaches over time. The success to date in translating more than 10 years of preclinical PD gene therapy

research into numerous clinical trials should, however, provide a wealth of ongoing information to help advance the overall field of central nervous system gene therapy.

Coronary artery disease

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Coronary artery disease (CAD) is a multifactorial disorder characterized by stenosis or occlusions of large epicardial coronary arteries and their branches, leading to reduced perfusion of the downstream myocardium. Typically, CAD is managed either medically with drugs designed to reduce myocardial oxygen demand, or mechanically using either bypass surgery to literally bypass the site of stenosis or percutaneous revascularization approaches. Because a number of patients cannot be effectively managed by any of these strategies, angiogenic gene therapy has been explored as an option for these so-called 'no option/poor option' patients.

The most common genes used for these trials have been vascular endothelial growth factor (VEGF) family members VEGF-A₁₆₅, VEGF-A₁₂₁, VEGF-C, and fibroblast growth factor 4 (FGF4). Both adenoviral and plasmid-based delivery have been explored. VEGFs predominantly induce true angiogenesis, for example, growth of capillaries and venules, a process not expected to have much effect on restoration of flow caused by a proximal occlusion of a major arterial trunk. VEGF-C predominantly promotes growth of lymphatics and has relatively little effect on capillary growth. Furthermore, genes have been typically delivered by injections into ischaemic areas of the heart where expression of native VEGF is already highly increased. Thus, it is not surprising that after an initial wave of enthusiasm following reports of success in open-label trials, results of double-blind-controlled studies have been largely disappointing.^{38,39}

A series of adenoviral FGF-4 trials (AGENT 1, 2, 3 and 4) have utilized intracoronary injections of Ad-FGF4 in patients with stable CAD. FGFs possess much more of an arteriogenic activity than VEGFs and thus could be expected to be more functionally effective in stimulating arterial 'bypasses'. However, despite positive trends in two small phase I/II trials (AGENT 1 and 2), the two large double-blind placebo-controlled trials were negative. In retrospect, the result could be expected, given very poor penetration of adenoviruses across the arterial endothelial barrier.

Overall, experience with adenoviral- and plasmid-based gene therapy in CAD demonstrated this to be remarkably safe. Critical challenges remain in the areas of gene delivery, since none of the existing technologies provide for sufficiently high efficiency of delivery and tissue transduction, in identifying patients likely to respond to such interventions, and in establishing biomarkers that could specifically identify biological responsiveness as well as a functional response to therapy.^{40,41} The duration of gene expression is another very important issue, since growth and stabilization of new vasculature requires sustained presence of pro-angiogenic stimuli for an extended period of time that is well out of range of either plasmid- or adenoviral-based expression. Furthermore, it is clear that arteriogenesis, that is collateral growth and remodelling, and not

angiogenesis, is the key physiologic process that can ameliorate ischaemia in CAD population. This requires rethinking of the type of therapeutic agents used and the site of their delivery. More importantly, the emerging understanding of defective angiogenic signalling in CAD points more towards post-receptor signalling defects, and less to insufficient growth factor expression, as a leading cause of insufficient endogenous collateral development.⁴²

Peripheral vascular diseases

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Despite advances in conventional therapy, one-fifth of patients with chronic critical limb ischaemia (CLI) cannot be treated with conventional methods. Currently, published phase II/III randomized, controlled trials have shown the following results.

- (1) The Kuopio Peripheral Angiogenesis Trial ($n = 54$) of catheter-mediated VEGF₁₆₅ gene delivery using plasmid-liposomes or adenoviruses, AdVEGF treatment improved vascularity on digital subtraction angiography at 3 months without differences in clinical outcomes.⁴³
- (2) The RAVE trial ($n = 105$) using intramuscular (im) injections of AdVEGF₁₂₁ showed no significant benefit in peak walking time at 3 months, or in ankle brachial pressure index and quality-of-life measures.⁴⁴
- (3) The Gröningen trial using i.m. injections of naked VEGF plasmid in diabetic patients with CLI ($n = 54$) showed no difference in the primary end point of amputation rate at 100 days, but significant improvements were achieved in secondary end points (ankle brachial index and clinical condition).⁴⁵
- (4) I.m. administered Del-1 plasmid, formulated with poloxamer 188, showed no efficacy in the peak walking time at 3 months in claudication patients ($n = 100$).
- (5) I.m. injected naked FGF-1 plasmid in CLI patients ($n = 107$) failed to achieve the primary end points of ulcer healing and increased transcutaneous pO₂, but resulted in a significant reduction in amputation rate.⁴⁶
- (6) For vein graft stenosis, despite early positive reports, an E2F transcription factor decoy was ineffective in preventing vein graft failure in patients undergoing peripheral bypass ($n = 3804$).⁴⁷

Currently, a phase II WALK trial is addressing the efficacy of AdHIF-1 α on peak walking time at 6 months in approximately 300 PAD patients. A HGF-STAT trial is ongoing to assess the efficacy of naked plasmid HGF on tissue perfusion in patients with non-reconstructable critical ischaemia. Finally, delivery of AdVEGF-D is also being addressed in the continuation of the Kuopio Peripheral Angiogenesis trial.

It seems pertinent to conclude that a major disappointing feature of the trials has been that while preclinical and all published uncontrolled phase I gene and protein pro-angiogenic trials have been positive, none of the randomized controlled phase II/III trials have shown clinically relevant positive primary endpoints. Therefore,

there are some key issues that need to be addressed to improve the design and outcome of therapy. One obvious confounding factor is a strong placebo effect, which is further complicated by the use of difficult end points, such as treadmill exercise tolerance test. More objective end points addressing perfusion, collateral flow and, ultimately, ulcer healing and amputation rate should be used. One likely explanation for the lack of efficacy in clinical pro-angiogenic trials is that growth factor concentration in target tissues has not reached sufficient levels and/or has not persisted long enough for triggering relevant vascular growth. The short half-life of growth factors, non-optimized dose, short time for gene expression and compromised delivery routes have likely contributed to the disappointing outcomes. Thus, it is likely that technical and pharmacological shortcomings in the current treatment approaches have contributed to the failures. Also, gene expression kinetics of gene delivery vectors are not very well known in humans and levels of therapeutic proteins in the target tissue (not only in plasma) should be measured quantitatively. Selection of the most suitable patient population for future trials will also be of significant importance since the most severe end-stage patients may no longer be able to respond to any kind of therapy. Also, new approaches, such as the use of therapeutic angiogenesis as an adjuvant therapy in combination with bypass surgery or angioplasty, rather than as sole therapy, should be tested.

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