Clinical efforts to modulate angiogenesis in the adult: gene therapy versus conventional approaches

Gene Liau, Enming J. Su and Katharine D. Dixon

A gene therapy approach towards the modulation of neovascularization provides important advantages that could be crucial for the success of therapies that target blood vessels. These advantages include sustained local expression and the ability to supply multiple pro- or anti-angiogenic factors. There is potential near-term success in the application of this approach for the treatment of ischemic vascular diseases. Although there is convincing proof of concept in animal models that an anti-angiogenesis gene therapy approach can be used to treat cancer, this is a highly competitive field with small molecules, recombinant proteins and monoclonal antibodies already in clinical trials. The scientific rationale for the use of gene therapy is sound, but realization of its full potential for the treatment of a broad array of diseases will require several challenging technical hurdles to be overcome and safety concerns to be alleviated.

***Gene Liau**
Enming J. Su
Katherine D. Dixon
Genetic Therapy
A Novartis Company
938 Clopper Road
Gaithersburg, MD 20878, USA
tel: +1 301 258 4678
fax: +1 301 948 8034
*e-mail: gene.liau@pharma.novartis.com
***Gene Liau**
Dept of Anatomy and Cell Biology
The George Washington University Medical Center,
Washington, DC 20037, USA

▼ Gene therapy seeks to replace, supplement or alter a patient's cellular genetic makeup to restore, correct or enhance certain biological functions. The concept of engineering a patient's own cells to produce therapeutic proteins excites clinicians because it offers the possibility of curing otherwise intractable diseases and minimizing the side effects of potent biological molecules by delivering the drug specifically to where it is needed. However, since the first human gene therapy trial in 1990 (Ref. 1), a substantial number of clinical trials has failed to reach the anticipated end point primarily because of inadequacy of the technology. Recently, several successful findings have bolstered interests in the gene therapy arena. Avigen (Alameda, CA, USA) has reported positive results with its adenoviral associated virus (AAV) vector expressing the gene for coagulation factor IX (Coagulin-B) to treat hemophilia B patients[2]. Although the clinical trial size is small, the gene therapy procedure appears to be safe and well-tolerated, with evidence of persistent production of the factor IX protein and patient benefit. Avigen is co-developing Coagulin-B with Bayer (Leverkusen, Germany) and they anticipate filing a biologics license application in 2005. Transkaryotic Therapies (Cambridge, MA, USA), using a cell-based gene therapy technique, also reported positive preliminary results in a clinical trial for the treatment of hemophilia A (Ref. 3). Other recent successes include the treatment of severe combined immunodeficiency-X1 disease by transferring the γc cytokine gene to $CD34^+$ cells[4] and the use of an oncolytic adenoviral vector engineered to selectively replicate in, and kill, p53-deficient cancer cells[5]. These clinically positive outcomes, as well as the considerable progress made in enabling technologies including vector development and controlled gene expression, make significant gene therapy success probable within the next 10 years.

One of the more innovative and active uses of gene therapy technology is for the treatment of cardiovascular diseases (Fig. 1). Since December 2000, a total of 36 cardiovascular gene transfer protocols were registered with the National Institutes of Health Office of Biotechnology Activities. Of these, the overwhelming majority involve the delivery of vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF) to enhance

1359-6446/01/$ – see front matter PII: S1359-6446(01)01809-8

angiogenesis for the treatment of coronary artery and peripheral vascular diseases. This high interest is based on the large market potential, unmet medical need and preliminary reports of clinical success using this approach[6–10]. There is also intense interest in the inhibition of angiogenesis as a new modality to treat cancer. However, the major effort is focused on small molecules, recombinant proteins and monoclonal antibodies, and the use of gene therapy is less explored. The purpose of this article is to detail the rationale for a gene therapy approach to modulate the growth of new blood vessels, summarize the information to date and examine the potentials and hurdles for this approach.

Molecular mechanisms of adult neovascularization

The formation of new blood vessels in the adult is commonly believed to occur by sprouting from an existing vessel and is termed angiogenesis. This process requires local destabilization of the vessel with subsequent endothelial cell migration, proliferation, remodeling and the formation of tight attachments with the extracellular matrix and supporting mural cells[11]. New capillary invasion into previously avascular zones is stimulated by ischemic or hypoxic conditions. The lack of oxygen under these conditions leads to the upregulation of the transcription factor hypoxia inducible factor-1 (HIF-1). HIF-1 is the primary regulator of oxygen homeostasis in mammalian cells and, among its various activities, promotes the production of the key angiogenic molecule, VEGF (Ref. 12). VEGF acts through its cognate receptors to initiate a cascade of events that leads to the formation of a new capillary network[13]. The outline of this process is being worked out in remarkable detail and forms the molecular basis for angiogenic therapeutic strategies.

A distinct mechanism of blood vessel formation known to occur during early embryonic development is termed vasculogenesis. This early morphogenic process occurs via the differentiation of angioblasts (endothelial cell precursors) into blood islands that fuse to form the primitive vascular network[11]. There is now increasing evidence to suggest that angioblast-like endothelial cell precursors derived from the bone marrow might circulate in the adult vasculature and contribute to physiological and pathological neovascularization in the adult[14,15]. Although this concept remains highly controversial, it provides the possibility of intriguing alternative therapeutic options, as well as the potential to discover new targets for the effective modulation of neovascularization.

Perhaps the least understood process of blood vessel formation is the generation of collateral vessels in the heart under ischemic stress. This process has been termed arteriogenesis and appears to involve remodeling of existing arterioles into large conductance arteries in the heart[16]. It is believed that dramatic changes in hemodynamic factors, such as shear stress, result in the activation of the endothelium, subsequent monocyte invasion and then the release of growth factors and cytokine, which contribute towards vessel enlargement. The mechanism underlying arteriogenesis appears to differ substantially from the angiogenesis process and might involve other growth factors and cytokines. The formation of large conductance arteries is presumably the most desired outcome for the relief of coronary heart disease. Unfortunately, in humans, this process does not occur naturally with sufficient frequency to alleviate ischemic diseases and, therefore, a better understanding of the arteriogenic process is warranted.

Disease applications in altering adult neovascularization

Neovascularization is an essential part of normal development but in the adult it usually occurs only as part of the female estrus cycle, during pregnancy and as part of tissue repair and remodeling (Table 1). Pro-angiogenesis therapy seeks to enhance the natural process of tissue repair and remodeling with primary emphasis on ischemic vascular diseases (Table 1). Pathological neovascularization has been implicated in several diseases including age-related macular degeneration, diabetic retinopathy and rheumatoid arthritis (Table 1). However, the major focus of anti-angiogenesis therapies today is on cancer. Continued tumor growth requires a concomitant increase in blood supply to nourish the cancer cells and the ability of these cells to switch to a pro-angiogenic phenotype is an early event in the progression of most cancers[17,18]. The tumor vasculature is an attractive and novel therapeutic target because endothelial cells should not be capable of developing resistance to anti-angiogenic agents. Furthermore, there is evidence to suggest that the tumor vasculature is sufficiently different from normal vessels so that strategies can be devised to target tumors specifically[18]. It is anticipated that once these therapies are proven safe and effective, they can be expanded to a large number of disease indications (Table 1).

Pro-angiogenic protein administration for the treatment of ischemic vascular diseases

The results of the first clinical trial of a drug designed to induce neovascularization were reported in 1998. This placebo-controlled trial demonstrated that injection of FGF protein into the heart resulted in the enhancement of capillary formation[19]. A three-year follow-up of this study, as well as other studies, has demonstrated that FGF and

VEGF proteins can be safely delivered and can potentially act to reduce angina and increase blood flow[20–23]. However, a subsequent placebo-controlled Phase II clinical trial of VEGF sponsored by Genentech (South San Francisco, CA, USA) was halted owing to lack of patient benefit[24]. Similarly, a Chiron (San Diego, CA, USA)-sponsored Phase II clinical trial with FGF-2 also did not achieve the primary end point of improved exercise capacity[25]. Taken as a whole, these early results in humans suggest that VEGF and FGF protein therapies are reasonably safe and can induce neovascularization but improvements in delivery of these therapeutic proteins and/or in the types of angiogenic proteins delivered will be necessary to develop an effective therapeutic drug.

Advantages of a gene therapy approach for enhancing neovascularization

The process of new vessel formation in the adult after vessel occlusion occurs over a period of 3–5 days[26,27] and it is possible that angiogenic molecules delivered to induce neovascularization will need to be supplied at least over this duration. The ability to deliver pro-angiogenic molecules in a sustained and localized manner is the key advantage of a gene therapy approach (Fig. 1 and Box 1). Thus, delivery of the vector to the appropriate site and subsequent local expression of the transgene for one to two weeks provide the ideal therapeutic scenario and can overcome the short half-life and side effects of systemic protein delivery[24,28,29]. Current gene therapy vectors allow expression of the introduced transgene for approximately one week[30–32], which is ideal for pro-angiogenesis therapy. However, there are issues with both of the most commonly used gene delivery systems: adenoviral vectors and plasmids. The limited duration of expression of adenoviral vectors might be because of an

Table 1. Neovascularization in physiology and pathology

Physiological neovascularization	Indications that require stimulation of neovascularization	Indications that require inhibition of neovascularization
Female estrus cycle	Coronary artery disease	Cancer
Pregnancy	Peripheral vascular disease	Age-related macular degeneration
Wound healing	Wound healing	Diabetic retinopathy
Collateral formation	Islet cell transplantation	Rheumatoid arthritis
Exercise-induced hypertrophy	Fracture and tendon repair	Psoriasis
–	Reconstructive surgery	Obesity
–	Tissue engineering	Hemangioma/AIDS-related Kaposi's sarcoma
–	Restenosis[a]	Atherosclerotic plaque rupture[a]

[a]Effect on disease progression needs clarification.

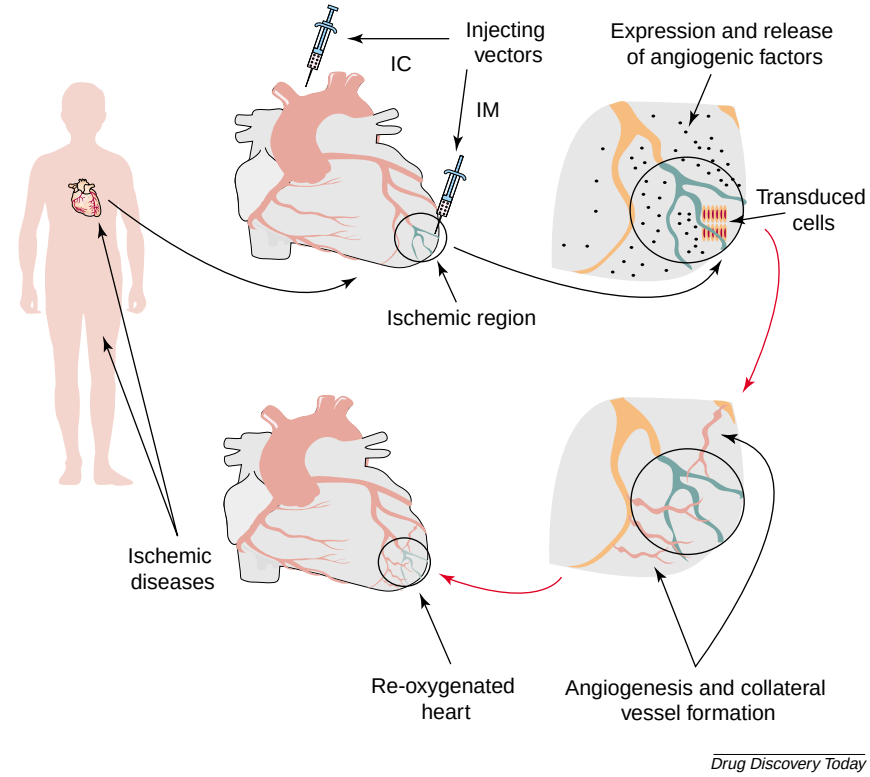

Figure 1. Cartoon of pro-angiogenesis gene therapy approach for the treatment of cardiovascular and peripheral vascular diseases. Abbreviations: IC, intracoronary; IM, intramuscular.

Box 1. Advantages and main concerns of using anti-angiogenic gene therapy approaches

Issue 1. Competition from small molecules & recombinant proteins

- In contrast to small molecules[a], recombinant protein and expressed genes can effectively target protein–protein interactions; offering improved specificity, less side effects and better efficacy.
- Potential to target several pathways with a single vector through the delivery of multiple genes[b].
- Enhanced delivery relative to recombinant protein that requires multiple intravenous administrations or delivery by pump[c].

Issue 2. Cancer is a systemic disease

- Current technology allows the delivery of vector to a primary site to establish a depot of anti-angiogenic protein.
  (a) Liver expresses high levels of therapeutic protein with adenoviral vector delivery but possible vector toxicity must be addressed.
  (b) Adenoviral vectors, adenoviral-associated vector and plasmid delivery to the muscle will require potent anti-angiogenic factor(s) because of lower expression levels.
- Vector can naturally target sites of metastatic disease (e.g. adenoviral vectors transduce hepatocytes efficiently to treat liver metastasis[d])
- Vector can potentially be engineered to specifically transduce or express in tumor cells or tumor endothelium.
- Systemic delivery of vectors is an important long-term goal. Systemic delivery of adenoviral vectors must overcome the hurdle of pre-existing neutralizing antibodies in 50% of the population.

Issue 3. Unknown effect of long-term expression of angiogenesis inhibitors

- Levels and persistence of expression of vectors cannot be directly controlled because vectors with regulatable promoters are not in clinical trials[c].
- An excisable tissue depot can be used to remove the source of anti-angiogenic protein.
- Tumor-targeted vectors will have less potential side effects.

References

a McCormick, F. (2000) Small-molecule inhibitors of cell signaling. *Curr. Opin. Biotechnol.* 11, 593–597

b Morsy, M.A. and Caskey, C.T. (1999) Expanded-capacity adenoviral vectors – the helper-dependent vectors. *Mol. Med. Today* 5, 18–24

c Morral, N. *et al*. (1999) Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12816–12821

d Chen, C.T. *et al*. (2000) Antiangiogenic gene therapy for cancer via systemic administration of adenoviral vectors expressing secretable endostatin. *Hum. Gene Ther.* 11, 1983–1996

inflammatory response to these vectors[33–35]. Plasmid-based gene therapy vectors remain attractive and the least complicated alternative[7,8,10,30], but whether such vectors can achieve a sufficiently robust expression to reach the appropriate therapeutic index is controversial. Although these vectors are not ideal, whether they will be sufficient to deliver positive clinical results remains to be seen.

An additional potential advantage of a gene therapy approach is the ease of delivering multiple angiogenic factors to obtain stable collateral vessels. This concept is based on the discovery that molecules such as angiopoietins and ephrins[11] as well as the G-protein coupled receptor-endothelial cell differentiation gene-1 (Ref. 36) have profound effects on the development and stabilization of the vasculature. Currently, it is unclear whether delivery of a single factor such as VEGF or FGF will be sufficient to generate appropriate vessels in the adult heart or ischemic limb. Gene therapy vectors will allow delivery of a combination of factors that might be ideal for the generation of new, stable blood vessels.

Summary of clinical trial results for pro-angiogenesis gene therapy

Early gene therapy clinical trials with VEGF and FGF to treat ischemic vascular diseases have yielded encouraging data and strongly support the conceptual framework underlying this line of therapy[7–10,37]. However, as in the protein therapy trials, claims of efficacy will need to be validated with larger, placebo-controlled clinical trials. In this context, it is important to note that Collateral Therapeutics (San Diego, CA, USA) has reported positive trends in angina class and treadmill results after completion of a double-blind, placebo-controlled Phase I and II study for FGF-4 (Ref. 38). Furthermore, interim results of a double blind, placebo-controlled Phase II clinical trial to treat peripheral vascular with VEGF by Valentis is also promising[39]. Genvec (Gaithersburg, MD, USA) is expected to complete two Phase II studies, and several other companies, including Aventis (Strasbourg, France), Vascular Genetics (Research Triangle Park, NC, USA), and Genzyme (Framingham, MA, USA) all have drugs at the early clinical trial stage. Overall, the clinical trial data indicate that pro-angiogenic gene therapy, as with the protein therapy approach, is reasonably safe and well-tolerated by the patients although efficacy of this first generation of novel drugs awaits the result of ongoing clinical trials.

Key issues for the success of pro-angiogenesis gene therapy in the next five years

The primary issue regarding near-term success of pro-angiogenesis gene therapy is whether the current

therapeutic:modality will be able to demonstrate an appropriate risk:benefit ratio. Although the small number of reported pro-angiogenic gene therapy clinical trials have demonstrated good safety results, the recent death of a patient that underwent a gene therapy-based ornithine transcarbamylase (OTC) deficiency treatment protocol has had an extraordinary impact on the practice of gene therapy and marks the transition to a more mature phase in the development of gene therapy-based medicine[40]. Given that many of the pro-angiogenic clinical trials are using modified adenoviral vectors similar to those used in the OTC protocol, the safety of such vectors for the treatment of cardiovascular diseases has been raised as an issue[41]. However, more than 3.8×10^{13} viral particles were infused into the patients in the OTC trial[42] whereas the highest vector dose used in pro-angiogenic trials has been 4×10^{10} particles[9]. The three-log difference in viral particle input, as well as differences in delivery regimen and patient population, make direct comparisons highly problematic. Only the ongoing pro-angiogenesis gene therapy trials can provide the essential safety information.

A second safety concern relates to possible side effects associated with the delivery of pro-angiogenic factors[41,43]. Both VEGF and FGF are hypotensive agents[29,44] and this can be dose limiting[24]. A gene therapy approach can minimize this side effect by directing localized expression of the pro-angiogenic protein. It is also possible that prolonged exposure to pro-angiogenic molecules might alter the growth or stability of atherosclerotic plaques, stimulate the growth and metastasis of cancer cells, and exacerbate proliferative retinopathy[41,43]. In this context, it is interesting to note that in addition to its lipid-lowering activities, the promotion of new blood vessel growth has recently been postulated to be one of the beneficial side effects of the statins, one of the safest classes of cardiovascular drugs[45]. Nevertheless, concerns for potential side effects of pro-angiogenic factors could limit the use of gene therapy vectors such as second generation adenoviral vectors, AAV vectors and lentiviral vectors that are designed to express for a longer duration[41,46]. The lack of proven controlled gene expression systems in AAV and lentiviral vectors suggests that further refinements will be required for applications other than the treatment of life-long diseases[2].

Data from current clinical trials suggest systemic angiogenic protein delivery is not an effective means of eliciting long-term efficacy, possibly because of the short half-life of the proteins in the circulation (Box 1). It is currently unclear whether a local depot of growth factors, sustained release formulations, a mixture of pro-angiogenic factors or alternative approaches such as transmyocardial revascularization[47] will prove effective. This is a crucial issue because, despite the successful use of percutaneous angioplasty, stent implantation, bypass surgery and thrombolytics to restore blood flow to the ischemic tissue, there is now a growing population of patients that are no longer candidates for these treatments because of the severity of their diseases[16]. A pro-angiogenesis gene therapy approach might provide the only alternative if novel treatment modalities, such as transmyocardial revascularization, prove ineffective.

Inhibition of neovascularization to treat cancer

Although the scientific conceptual framework for the inhibition and stimulation of neovascularization share many similarities, differences in the nature of disease pathologies dictate the use of distinct strategies to inhibit angiogenesis for the treatment of cancer. Alleviation of ischemic vascular diseases requires stimulation of the growth of blood vessels at a discrete location and this is facilitated by the use of mechanical devices such as catheters for localized delivery. By contrast, because anti-angiogenic therapy is designed to treat both tumor metastasis and growth, all therapeutic approaches to date have been systemic in nature. Furthermore, because small cancer lesions could remain viable for a prolonged period[17,18], effective treatment might require chronic administration of the anti-angiogenic drug. This is in contrast to the situation in pro-angiogenesis therapy in which treatment is needed for up to several weeks.

Advantages of an anti-angiogenesis gene therapy approach

Although several drugs based on small molecules, recombinant proteins and monoclonal antibodies have advanced to clinical trials[48], there are several potential advantages associated with a vector-mediated delivery of anti-angiogenic genes (Box 1). A key advantage of an anti-angiogenic gene therapy approach is similar to that for pro-angiogenesis: the potential for localized and sustained expression. However, the definition of localized expression is expanded to multiple tumor sites and expression up to several years is probably required. Another potential advantage of gene therapy is the ability of a vector to deliver more than one factor so that multiple angiogenic pathways can be blocked. There is also preclinical data to support the possibility that certain recombinant anti-angiogenic proteins have less side effects than anti-angiogenic small molecules[43,49] and this is consistent with the recent Phase I clinical trial safety results on endostatin[50,51]. This potential safety advantage of protein therapeutics over small molecules extends to a gene therapy approach. Finally, gene-therapy-mediated delivery of anti-angiogenic proteins might prove to be more feasible and economically viable

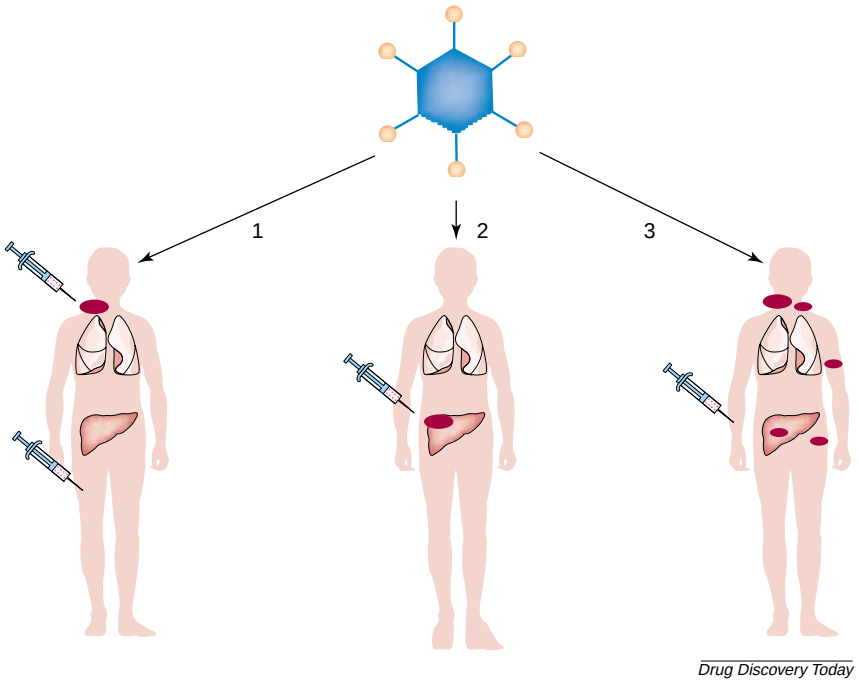

Figure 2. Cartoon of anti-angiogenesis gene therapy approach for the treatment of cancer. Scheme 1 represents direct intratumoral and intramuscular injection to treat primary tumors. Scheme 2 represents treatment of tumors at sites of vector localization (e.g. adenoviral vectors in the liver). Scheme 3 represents gene therapy vector delivered systemically and targeted directly to the tumor or tumor vasculature.

than intravenous delivery of recombinant proteins that are difficult and expensive to manufacture (Fig. 2 and Box 1).

Preclinical studies support the principle of anti-angiogenesis gene therapy

There is a substantial body of evidence in preclinical models to support the concept that an anti-angiogenic gene therapy approach can inhibit tumor growth[48]. Early studies demonstrated inhibition of tumor growth when vectors containing anti-angiogenic genes were injected directly into tumors or the surrounding tissue (Fig. 2). To achieve an effect throughout the tumor, the vectors used in these experiments coded for secreted factors, such as platelet factor 4 (Ref. 52), the soluble form of the VEGF receptor, flt-1 (Ref. 53) and plasminogen activator[54]. Clearly, the direct clinical application of this mode of vector delivery is limited. Investigators have also used gene therapy vectors to create a depot of secreted factor (Fig. 2). In most cases the vector was administered to mice by tail-vein injection, which resulted in substantial liver cell transduction and high levels of expression in the serum. Inhibition of tumor growth using this technique has been seen with soluble flt-1 (Ref. 55), soluble Tie2 receptor[56], plasminogen activator[57] and endostatin[58–60]. In some studies[57,60], the site of the neoplastic lesion was in the liver and probably coincided with the highest concentration of the inhibitors. These encouraging results underscore the potential use of a gene therapy approach in the clinic.

What we have learned from anti-angiogenesis clinical trials

Currently, there is intense activity in the clinical arena to test the safety and efficacy of a wide variety of anti-angiogenic therapies in the treatment of cancer[43]. Although a comprehensive review of these activites is beyond the scope of the current discussion, it is instructive to examine two of the largest classes of compounds with a specific molecular target. One class of anti-angiogenesis drugs in clinical trials is the matrix metalloproteinase inhibitors. Marimastat (British Biotech, Oxford, UK), Neovastat (AEterna, Quebec, Canada), and BAY129566 (Bayer) represent some of these that have progressed to Phase III trials. However, early results have been mixed and the lack of success has been attributed to the absence of specificity towards the relevant matrix metalloproteinases[61]. By contrast, several drugs that are designed to inhibit the VEGF pathway are moving forward rapidly. The small-molecule drug SU5416 (Ref. 62), when administered in combination with 5-fluorouracil (5FU) and leucovorin, was reported at the American Society of Clinical Oncology meeting in New Orleans (LA, USA, 20–23 May, 2000) to show preliminary evidence of activity in metastatic colorectal cancer in Phase II clinical trials. Both Pharmacia (NJ, USA; SU6668; Ref. 63) and Novartis (Bern, Switzerland; CGP79787; Ref. 64) have orally active VEGF-pathway inhibitors in Phase I and II clinical trials. A monoclonal antibody against VEGF has been reported to show activity in both non-small-cell lung cancer and metastatic colorectal cancer in Phase II clinical trials[65], and a monoclonal antibody against the VEGF receptor[66] is also in clinical trials. Thus, the preliminary indications are that targeting the VEGF pathway might be a clinically viable way to inhibit tumor growth and metastasis, whereas the use of matrix metalloproteinase inhibitors might require further refinement.

Although there are currently no gene therapy cancer treatments that target tumor angiogenesis, there are many gene therapy-based clinical trials for the treatment of cancer that are focused on immunotherapy, cancer vaccine, direct

tumor cell killing and the overexpression of genes such as those encoding p53 and BRCA1. Anti-angiogenic activity might account for some of the success that has been seen with adenoviral vectors that express p53 (Ref. 67). Expression of wild-type p53 in cell lines results in increased expression of the angiogenesis inhibitor thrombospondin-1 (Ref. 68) and decreased expression of VEGF (Ref. 69). In early clinical trials, tumor regressions were seen following intratumoral injection of p53-expressing adenoviral vectors even though distribution of the vector was limited[70]. One possible explanation for this *in vivo* bystander effect is the anti-angiogenic activity of p53. Another promising new area for gene therapy is in the use of oncolytic adenoviruses[5]. These modified viruses selectively produce lytic cycles of replication in transformed cells and thus have the potential to spread beyond an initial injection site. Interestingly, the viruses themselves might have intrinsic anti-angiogenic activity[5]. Adenoviral E1A can suppress angiogenesis *in vivo* and interfere with hypoxia-induced expression of VEGF by binding to the transcriptional adapters p300 and CREB-binding protein[71]. These results suggest that an appropriate anti-angiogenesis gene therapy approach can succeed in the clinic.

Key issues for the successful use of anti-angiogenesis gene therapy

Whether gene therapy will become a significant factor in anti-angiogenesis therapy depends to some extent on the results of the current wave of clinical trials. If some of the small-molecule and direct protein approaches prove both efficacious and widely applicable for a variety of cancers, then gene therapy will only be viable if it can demonstrate significant advantages over the prevailing therapy. However, it is more probable that the current therapies will meet with a more limited success. Under this scenario, a gene therapy approach will be competitive if it can overcome some of the technical hurdles that prevent realization of the theoretical advantages of this elegant approach. The properties of an ideal gene therapy vector are summarized in Box 1 and include the ability to maintain persistent expression, to regulate gene expression, to target the vector to the tumor, or tumor vasculature, and to deliver the vector systemically. The identification of potent angiogenesis inhibitors with a high safety profile could facilitate the near-term use of an anti-angiogenesis gene therapy approach because concerns associated with the potential risks of prolonged exposure to anti-angiogenic proteins would be diminished (Box 1).

Conclusions and future directions in angiogenesis gene therapy approaches

The outlook for a gene therapy approach to stimulate angiogenesis to treat ischemic diseases is bright, because both small and large pharmaceutical companies continue to invest heavily in this area. This is based on the clear conceptual advantages of the gene therapy approach that are supported by reports of efficacy in two Phase II clinical trials. The most crucial issue for the treatment of ischemic vascular diseases via gene therapy is the demonstration of safety and this data will be obtained from the ongoing clinical trials. Although the development of gene therapy approaches to deliver anti-angiogenic medicine for the treatment of cancer is behind more conventional anti-angiogenic therapy, the feasibility of this approach is supported by the results of several studies in preclinical models. The safety of gene therapy-based clinical trials to treat cancer will be less of an issue because there have been numerous gene therapy clinical trials for cancer and the lack of treatment options for critically ill patients justifies the use of more experimental therapies. In the final analysis, the possible near-term success of a gene therapy approach towards modulation of neovascularization rests on the ability of clinicians to design trials that are rigorous in oversight and patient protection and yet optimize the therapeutic potential of this revolutionary medicine.

The spectacular advances in our understanding of the neovascularization process and technological advances in gene therapy delivery systems suggest continued breakthroughs and a bright long-term future for the use of gene therapy for the treatment of neovascular diseases. However, important questions, such as the contribution of vasculogenesis and arteriogenesis in various disease states and the duration of expression of gene therapy vectors in humans, remain unanswered. It is probable that angiogenesis gene therapy will eventually become part of the armament in disease management. Possibilities include combination therapy with other approaches and the use of gene therapy medicine as surveillance or prophylactic tools to prevent ischemic vascular disorders, cancer and ocular diseases.

Acknowledgements

We thank Mike Kaleko for a thorough review of the manuscript and helpful comments.

References

1 Oldfield, E.H. *et al.* (1993) Gene therapy for the treatment of brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir. *Hum. Gene Ther.* 4, 39–69

2 Kay, M.A. *et al.* (2000) Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat. Genet.* 24, 257–261

3 Roth, D.A. and Seldon, R.H. (2000) Non-viral gene transfer of blood coagulation factor VIII in patients with severe hemophilia A. *American Society of Hematology 42nd Annual Meeting and Exposition*, 2–5 December, 2000, San Francisco, CA, USA (Abstract z2532)

4 Cavazzana-Calvo, M. *et al.* (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 288, 669–672

5 Khuri, F.R. *et al.* (2000) A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* 6, 879–885

6 Lee, J.S. and Feldman, A.M. (1998) Gene therapy for therapeutic myocardial angiogenesis: a promising synthesis of two emerging technologies. *Nat. Med.* 4, 739–742

7 Baumgartner, I. *et al.* (1998) Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 97, 1114–1123

8 Losordo, D.W. *et al.* (1998) Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 98, 2800–2804

9 Rosengart, T.K. *et al.* (1999) Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 100, 468–474

10 Symes, J.F. *et al.* (1999) Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. *Ann. Thorac. Surg.* 68, 830–836

11 Yancopoulos, G.D. *et al.* (2000) Vascular-specific growth factors and blood vessel formation. *Nature* 407, 242–248

12 Semenza, G.L. (1999) Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annu. Rev. Cell Dev. Biol.* 15, 551–578

13 Ferrara, N. (1999) Role of vascular endothelial growth factor in the regulation of angiogenesis. *Kidney Int.* 56, 794–814

14 Rafii, S. (2000) Circulating endothelial precursors: mystery, reality, and promise. *J. Clin. Invest.* 105, 17–19

15 Isner, J.M. and Asahara, T. (1999) Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. *J. Clin. Invest.* 103, 1231–1236

16 Buschmann, I. and Schaper, W. (2000) The pathophysiology of the collateral circulation (arteriogenesis). *J. Pathol.* 190, 338–342

17 Folkman, J. (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* 1, 27–31

18 Carmeliet, P. and Jain, R.K. (2000) Angiogenesis in cancer and other diseases. *Nature* 407, 249–257

19 Schumacher, B. *et al.* (1998) Induction of neoangiogenesis in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease. *Circulation* 97, 645–650

20 Sellke, F.W. *et al.* (1998) Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Ann. Thorac. Surg.* 65, 1540–1544

21 Hendel, R.C. *et al.* (2000) Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial perfusion: evidence for a dose-dependent effect. *Circulation* 101, 118–121

22 Lazarous, D.F. *et al.* (2000) Basic fibroblast growth factor in patients with intermittent claudication: results of a phase I trial. *J. Am. Coll. Cardiol.* 36, 1239–1244

23 Pecher, P. and Schumacher, B.A. (2000) Angiogenesis in ischemic human myocardium: clinical results after 3 years. *Ann. Thorac. Surg.* 69, 1414–1419

24 Henry, T.D. *et al.* (1999) Final results of the VIVA trial of rhVEGF for human therapeutic angiogenesis. *Circulation* 100, I-476

25 Bush, M.A. *et al.* (2001) Pharmacokinetics and pharmacodynamics of recombinant FGF-2 in a phase I trial in coronary artery disease. *J. Clin. Pharm.* 41, 378–385

26 Kumar, S. *et al.* (1983) Angiogenesis factor from human myocardial infarcts. *Lancet* 2, 364–368

27 Schaper, W. *et al.* (1990) Molecular biologic concepts of coronary anastomoses. *J. Am. Coll. Cardiol.* 15, 513–518

28 Mazue, G. *et al.* (1991) Preclinical and clinical studies with recombinant human basic fibroblast growth factor. *Ann. New York Acad. Sci.* 638, 329–340

29 Lopez, J.J. *et al.* (1997) Hemodynamic effects of intracoronary VEGF delivery: evidence of tachyphylaxis and NO dependence of response. *Am. J. Physiol.* 273, H1317–H1323

30 Tsurumi, Y. *et al.* (1996) Direct intramuscular gene transfer of naked DNA encoding vascular endothelial growth factor augments collateral development and tissue perfusion. *Circulation* 94, 3281–3290

31 Giordano, F.J. *et al.* (1996) Intracoronary gene transfer of fibroblast growth factor-5 increases blood flow and contractile function in an ischemic region of the heart. *Nat. Med.* 2, 534–539

32 Muhlhauser, J. *et al.* (1996) Safety and efficacy of *in vivo* gene transfer into the porcine heart with replication-deficient, recombinant adenovirus vectors. *Gene Ther.* 3, 145–153

33 Gilgenkrantz, H. *et al.* (1995) Transient expression of genes transferred *in vivo* into heart using first-generation adenoviral vectors: role of the immune response. *Hum. Gene Ther.* 6, 1265–1274

34 Newman, K.D. *et al.* (1995) Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. *J. Clin. Invest.* 96, 2955–2965

35 Channon, K.M. *et al.* (1998) Acute host-mediated endothelial injury after adenoviral gene transfer in normal rabbit arteries: impact on transgene expression and endothelial function. *Circ. Res.* 82, 1253–1262

36 Vale, P.R. *et al.* (1999) Direct myocardial injection of phVEGF165: results of complete patient cohort in phase 1/2 clinical trial. *Circulation* 100, I-477 (Abstract)

37 Liu, Y. *et al.* (2000) Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J. Clin. Invest.* 106, 951–961

38 Seachrist, L. (2000) Positive gene therapy results send collateral's tock soaring. *BioWorld Today* 11, 1 & 6

39 Makinen, K. *et al.* (1999) Catheter-mediated VEGF gene transfer to human lower limb arteries after PTA. *American Heart Association Meeting* I770, 7–10 November, 1999, Atlanta, GA, USA (Abstract 4064)

40 Zallen, D.T. (2000) US gene therapy in crisis. *Trends Genet.* 16, 272–275

41 Simons, M. *et al.* (2000) Clinical trials in coronary angiogenesis: issues, problems, consensus: an expert panel summary. *Circulation* 102, E73–E86

42 Verma, I.M. (2000) A tumultuous year for gene therapy. *Mol. Ther.* 2, 415–416

43 Thompson, W.D. *et al.* (2000) The clinical manipulation of angiogenesis: pathology, side effects, surprises, and opportunities with novel human therapies. *J. Pathol.* 190, 330–337

44 Cuevas, P. *et al.* (1991) Hypotensive activity of fibroblast growth factor. *Science* 254, 1208–1210

45 Kureishi, Y. *et al.* (2000) The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat. Med.* 6, 1004–1010

46 Lee, R.J. *et al.* (2000) VEGF gene delivery to myocardium: deleterious effects of unregulated expression. *Circulation* 102, 898–901

47 Lee, L.Y. and Rosengart, T.K. (1999) Transmyocardial laser revascularization and angiogenesis: the potential for therapeutic benefit. *Semin. Thorac. Cardiovasc. Surg.* 11, 29–35

48 Feldman, A.L. and Libutti, S.K. (2000) Progress in antiangiogenic gene therapy of cancer. *Cancer* 89, 1181–1194

49 Folkman, J. (1998) Antiangiogenic gene therapy. *Proc. Natl. Acad. Sci. U. S. A.* 95, 9064–9066

50 Herbst, R. *et al.* (2000) A phase I clinical trial of recombinant human endostatin (rHE) in patients (PTS) with solid tumors: pharmacokinetic (PK), safety and efficacy analysis. *Proceedings of the 11th NCI-EORTC-AACR Symposium* 95, Abstract 259

51 Thomas, J.P. *et al.* (2000) A phase I pharmacokinetic and pharmacodynamic study of recombinant human endostatin. *Proceedings of the 11th NCI-EORTC-AACR Symposium* 95, Abstract 260

52 Tanaka, T. *et al.* (1997) Viral vector-mediated transduction of a modified platelet factor 4 cDNA inhibits angiogenesis and tumor growth. *Nat. Med.* 3, 437–442

53 Kong, H.L. *et al.* (1998) Regional suppression of tumor growth by *in vivo* transfer of a cDNA encoding a secreted form of the extracellular domain of the flt-1 vascular endothelial growth factor receptor. *Hum. Gene Ther.* 9, 823–833

54 Li, H. *et al.* (1998) Adenovirus-mediated delivery of a uPA/uPAR antagonist suppresses angiogenesis-dependent tumor growth and dissemination in mice. *Gene Ther.* 5, 1105–1113

55 Takayama, K. *et al.* (2000) Suppression of tumor angiogenesis and growth by gene transfer of a soluble form of vascular endothelial growth factor receptor into a remote organ. *Cancer Res.* 60, 2169–2177

56 Lin, P. *et al.* (1998) Antiangiogenic gene therapy targeting the endothelium-specific receptor tyrosine kinase Tie2. *Proc. Natl. Acad. Sci. U. S. A.* 95, 8829–8834

57 Li, H. *et al.* (1999) Systemic delivery of antiangiogenic adenovirus AdmATF induces liver resistance to metastasis and prolongs survival of mice. *Hum. Gene Ther.* 10, 3045–3053

58 Sauter, B.V. *et al.* (2000) Adenovirus-mediated gene transfer of endostatin *in vivo* results in high level of transgene expression and inhibition of tumor growth and metastases. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4802–4807

59 Feldman, A.L. *et al.* (2000) Antiangiogenic gene therapy of cancer utilizing a recombinant adenovirus to elevate systemic endostatin levels in mice. *Cancer Res.* 60, 1503–1506

60 Chen, C.T. *et al.* (2000) Antiangiogenic gene therapy for cancer via systemic administration of adenoviral vectors expressing secretable endostatin. *Hum. Gene Ther.* 11, 1983–1996

61 Nelson, A.R. *et al.* (2000) Matrix metalloproteinases: biologic activity and clinical implications. *J. Clin. Oncol.* 18, 1135–1149

62 Fong, T.A. *et al.* (1999) SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res.* 59, 99–106

63 Laird, A.D. *et al.* (2000) SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res.* 60, 4152–4160

64 Wood, J.M. *et al.* (2000) PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res.* 60, 2178–2189

65 Ferrara, N. (2000) VEGF: an update on biological and therapeutic aspects. *Curr. Opin. Biotechnol.* 11, 617–624

66 Witte, L. *et al.* (1998) Monoclonal antibodies targeting the VEGF receptor-2 (Flk1/KDR) as an anti-angiogenic therapeutic strategy. *Cancer Metastasis Rev.* 17, 155–161

67 Ravi, R. *et al.* (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1-alpha. *Genes Dev.* 14, 34–44

68 Dameron, K.M. *et al.* (1994) Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. *Science* 265, 1582–1584

69 Mukhopadhyay, D. *et al.* (1995) Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. *Cancer Res.* 55, 6161–6165

70 Clayman, G.L. *et al.* (1998) Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. *J. Clin. Oncol.* 16, 2221–2232

71 Arany, Z. *et al.* (1996) An essential role for p300/CBP in the cellular response to hypoxia. *Proc. Natl. Acad. Sci. U. S. A.* 93, 12969–12973