

Therapeutic Angiogenesis for Critical Limb Ischemia Microvascular Therapies Coming of Age

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Despite progressive insights into the pathologies underlying coronary, cerebral, and peripheral artery atherosclerosis, these conditions continue to cause critical tissue ischemia and disability on an epidemic scale. For the past several decades, research and therapeutic development have focused on preventing or reversing occlusive disease in conduit vessels. The ultimate failure of macrovessel-targeted therapies is never more evident than in peripheral arterial disease, in which progressive disease leads to amputation at rates that have not changed significantly in 30 years. Despite modern therapy, up to 8 million Americans with peripheral arterial disease are devastated by immobility, intractable ischemia, ulceration, impaired wound healing, or amputation,¹ and the lack of additional treatment options leaves many patients with little hope for relief.

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The concept of therapeutic angiogenesis evolved from pioneering work in the 1970s by Folkman,² who observed that the development and maintenance of an adequate microvascular supply is essential for the growth of neoplastic tissue. His hypothesis that the inhibition of “tumor angiogenic factors” would be effective against solid tumors was met with widespread skepticism, but 30 years of persistent research led to the development and approval of antiangiogenic treatments that now constitute a significant portion of the anticancer armamentarium. Soon after the identification of angiogenic growth factors, cardiovascular investigators began testing the hypothesis that stimulating angiogenesis could improve perfusion and function in ischemic tissues independent of macrovessel manipulation.³ Abundant preclinical data supported the safety and clinical potential of therapeutic angiogenesis that used growth factors or cellular-based strategies.^{4,5} Accordingly, given the grave prognosis and unrelenting disability associated with advanced peripheral arterial disease and the absence of predictive animal models, early-phase clinical

studies commenced more than a decade ago. The evidence accumulated during phase 1 and phase 2 studies supports the safety of these approaches in humans^{6–21} (Table 1) and also provides indications of bioactivity in patients with these dreaded conditions^{6–27} (Table 2). Even so, true breakthroughs have been elusive. Why?

In this issue of *Circulation*, Powell et al¹⁷ report on the safety and bioactivity profile of hepatocyte growth factor (HGF) plasmid injection for critical limb ischemia (CLI). In the double-blind, placebo-controlled, dose-escalating, multicenter HGF-STAT Trial, 104 patients with rest pain or tissue loss due to severe lower-extremity ischemia were assigned to receive injections of placebo or 1 of 3 dosing regimens of HGF plasmid into the ischemic leg muscle. A unique, prespecified analysis plan allowed the investigators to identify an increase in transcutaneous oxygen tension (TcPO₂) in the high-dose group that was not present in other treatment groups, thus providing objective evidence for bioactivity. Other end points, such as amputation, wound healing, and ankle/brachial or toe/brachial index, did not reveal differences between treatment groups.

The results of this study and another recent report²⁸ provide a degree of optimism for patients with CLI. They also illustrate the challenges inherent in implementing novel therapeutics in this patient population and can help guide the design of future studies. Patient enrollment is one major challenge in studies of gene therapy in patients with CLI, as evidenced by the fact that 2 of the largest randomized trials of angiogenic therapies to date required a total of 73 investigative sites and a cumulative span of ≈5 years to enroll 211 subjects. Thus, clinical trials are likely to be more expensive in patients with CLI than for many other conditions because of the need for large numbers of sites and the time required for enrollment of appropriately selected patients. A second key challenge for investigations in this patient population, which is nicely illustrated in the HGF-STAT study, is the fluctuating and somewhat unpredictable status of the CLI patient. CLI encompasses a range of disease severities and clinical trajectories, and even the most carefully selected patients will exhibit strikingly different clinical courses over short periods. Within a year of diagnosis, ≈20% of patients will die, 35% will require amputation, and the remainder will enter a more chronic state.^{29,30} Prospective definition of these subpopulations is problematic and often imprecise, and these difficulties must be addressed during trial design and data analysis. A similar challenge is encountered with variability within the more chronic group that tends to be represented in clinical trials. CLI challenges us to design studies in which a true signal of bioactivity will not be lost in the background noise of baseline variability.

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Table 1. Gene Therapy in Peripheral Arterial Disease: Safety Data

Trial	Treatment(s)	Patients Verum/Placebo, n	Deaths, n	SAEs, n	Inflammatory Response, n	Side Effects, n	Retinopathy, n	Cancer, n	Reference
Phase I/II									
DELTA-1	Del-1 plasmid	52/53	1/2 Unrelated	11/9 Unrelated	Transient, mild local pain 22/23	None	None	Even 1/1	Grossman et al ⁶
PREVENT I	E2F decoy	16/8/17	N/A	N/A	N/A	None	N/A	N/A	Mann et al ⁷
—	FGF-1 plasmid	Safety 51/0; efficacy 15/0	2/0 Unrelated	29/0 Unrelated	Injection-site blood: FGF ↑	Peripheral edema, myasthenia, paresthesia	N/A	N/A	Comerota et al ⁸
PM202	FGF-1 plasmid	71	N/A	N/A	N/A	N/A	N/A	N/A	Henry et al ⁹
TALISMAN 201	FGF-1 plasmid	56/56	16/10 Unrelated	↓ in 27/42 possibly related (<i>P</i> =0.25)	N/A	None	Even 1/0	Even 3/3	Nikol et al ¹⁰
—	rh bFGF	16/8	1/0 Unrelated	N/A	None	Severe proteinuria, 4/16; GI disturbances, 6/16; hypertension, 2/16	N/A	N/A	Cooper et al ¹¹
—	rh bFGF	4–5–4/6	N/A	N/A	N/A	None	None	N/A	Lazarous et al ¹²
TRAFFIC	rh FGF-2	66–61/68	0–1/1 Unrelated	3–0/2 Unrelated	None	Severe proteinuria	None	None	Lederman et al ¹³
—	bFGF/gelatin hydrogel spheres	7/0	None	None	None	None	N/A	N/A	Marui et al ¹⁴
—	Ad5FGF-4	10/3	None	3/14 Events possibly related	None	Toe pain, myalgia	N/A	N/A	Matyas et al ¹⁵
—	HGF plasmid	6/0	None	None	None	None	None	None	Morishita et al ¹⁶
HGF-STAT	HGF plasmid	27–26–27/26	2–1–2/1 Unrelated	16–12–19/15 Unrelated	None	None	Even 0–0–0/1	Even 2–1–0/2	Powell et al ¹⁷
—	Ad2/HIF-1α/VP16	34/7	3/2 Unrelated	18/4 Unrelated	Mild local, 8/2; flu-like symptoms, 9/2	Edema 15/3	None	None	Rajagopalan et al ¹⁸
RAVE	AdVEGF ₁₂₁	32–40/33	0–0/1 Unrelated	N/A (AEs even)	None; blood: AdVEGF (high dose)	Edema (high dose)	Even, 2–0/1	Even, 0–2/1	Rajagopalan et al ¹⁹
—	VEGF ₁₆₅ plasmid	1/0	N/A	N/A	N/A	Edema, spider angioma	N/A	N/A	Isner et al ²⁰
—	VEGF ₁₆₅ plasmid	6 (7 Limbs)/0	None	N/A	Minimal local discomfort	Transient edema, 3/7	None	None	Isner et al ²¹
—	VEGF ₁₆₅ plasmid	9 (10)/–	None	N/A	Mild local blood: VEGF ↑	Transient edema	None	None	Baumgartner et al ²²
—	VEGF ₁₆₅ plasmid	21 (24)/–	None	N/A	Mild local blood: VEGF ↑	Transient edema	None	N/A	Shyu et al ²³
Groningen	VEGF ₁₆₅ plasmid	27/27	2/2 Unrelated	N/A (AEs even)	None; blood: VEGF ↑	None	Unchanged even	None	Kusumanto et al ²⁴
VEGF peripheral vascular disease	AdVEGF VEGF ₁₆₅ plasmid-liposome	18 (Ad)–17 (P/L)/19	1–1/1 Unrelated	0–2/0 Unrelated	None; blood: VEGF-Ab ↑ (11 Ad-group)	Fever (1–3/0)	None	None	Mäkinen et al ²⁵
—	Adβ-gal	8/2	None	None	None	N/A	N/A	N/A	Laitinen et al ²⁶
Phase III									
PREVENT III	E2F decoy	563/575	20/18 Unrelated	N/A (at 30 d)	N/A	None	N/A	N/A	Conte et al ²⁷

Overview on safety data of gene-therapy trials in the field of peripheral arterial disease. The studies are listed in alphabetic order of the therapeutic agent and year of publication; only published studies have been considered.

Ab, antibody; Ad, adenovirus; AEs, adverse events; bFGF, basic fibroblast growth factor; Del-1, developmentally regulated endothelial locus 1; DELTA-1, Design of the Del-1 for Therapeutic Angiogenesis; E2F, E2F transcription factor; even, evenly distributed; h, human; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; IA, intraarterial; IM, intramuscular; IV, intravenous; N/A, not available; PAD, peripheral arterial disease; PM202, study of different doses and schedules of administration of NV1FGF in patients with severe peripheral artery disease; PREVENT I, Project or ex Vivo Vein Graft Engineering via Transfection I study; RAVE, Regional Angiogenesis with Vascular Endothelial Growth Factor trial; rh, recombinant human; SAEs, serious adverse events; TALISMAN 201, Therapeutic Angiogenesis Leg Ischemia Study for the Management of Arteriopathy and Nonhealing Ulcers; TRAFFIC, Therapeutic Angiogenesis with FGF-2 for Intermittent Claudication Trial; VEGF, vascular endothelial growth factor.

Table 2. Gene Therapy in Peripheral Arterial Disease: Bioactivity Data

Trial Design	Condition	Treatment(s) Dosing	Route	Patients Verum/ Placebo, n	Follow-Up	Parameters of Bioactivity	Trends for Bioactivity	Reference
Phase I/II								
DELTA-1 phase II double-blind, placebo-controlled, multicenter trial	PAD; claudication	Del-1 plasmid (VLTS-589)	IM	52/53	30–90–180 d	Δ Walking time (PE); Δ ABI; Δ COT; Δ QoL	None	Grossman et al ⁶
PREVENT I phase I double-blind trial	PAD/revascularization, CLI, vein bypass graft	E2F decoy (edifoligide)	Ex vivo	16/8/17	12 mo	Graft occlusion	↓ Graft occlusion, critical stenosis, revision	Mann et al ⁷
Phase I trial	PAD, CLI	FGF-1 plasmid (NV1FGF); 1× 500–16 000 μg, 2× 500–8000 μg	IM	Safety 51/0; efficacy 15/0	3–6 mo	TcPO ₂ , ABI, TBI, pain, ulcer healing	↑ TcPO ₂ (<i>P</i> =0.01), ↑ ABI (<i>P</i> =0.01), ↓ pain (<i>P</i> =0.001), ↓ ulcer size (<i>P</i> =0.01)	Comerota et al ⁸
PM202 phase II double-blind, placebo-controlled, multicenter trial	PAD, CLI	FGF-1 plasmid (NV1FGF), 2–16 mg	IM	71	N/A	Δ TcPO ₂ (PE), ABI, TBI, ulcer healing, amputation, death	↓ Death, amputation	Henry et al ⁹
TALISMAN 201 phase II double-blind, placebo-controlled, multicenter trial	PAD, CLI	FGF-1 plasmid (NV1FGF), 4× 4 mg	IM	56/56	25 wk	Complete ulcer healing (PE), ABI, amputation, death	↓ Risk of all amputations (<i>P</i> =0.015), ↓ risk of major amputations (<i>P</i> =0.015), ↓ risk of death	Nikol et al ¹⁰
Phase I dose-escalation, double-blind, placebo-controlled trial	PAD, claudication	rh bFGF, 1× 10, 1× 30, 2× 30 mg/kg	IA	4–5–4/6	6 mo	Calf flow (plethysmography)	↑ Blood flow (<i>P</i> =0.002)	Lazarous et al ¹²
Phase II dose-escalation, double-blind, placebo-controlled trial	PAD, claudication	rh bFGF, 6× 2 μg · kg ⁻¹ · wk ⁻¹	IV	16/8 (prematurely terminated)	4–8–12 wk	PWT (PE), QoL	None	Cooper et al ¹¹
TRAFFIC phase II, double-blind, placebo-controlled trial	PAD, claudication	rh FGF-2, 1× or 2× 30 μg/kg	IA	66–61/68	90–180 d	PWT (PE), Δ PWT, COT, Δ ABI, QoL	↑ Change in PWT (day 90, <i>P</i> =s), ↑ ABI (day 90, <i>P</i> =s)	Lederman et al ¹³
Phase I/IIa	PAD/Buerger's, CLI	bFGF/gelatin hydrogel sphere, 200 μg	IM	7/0	4–24 wk	6-min Walk, TcPO ₂ , rest pain, ABI, ulcer healing, perfusion (laser Doppler), thermography	↑ Distance for 6-min walk (<i>P</i> =0.023), ↑ TcPO ₂ (<i>P</i> =0.03), ↓ rest pain (<i>P</i> =0.022), improved ulcers (5/6), ↑ ABI (4 wk, <i>P</i> =0.024), ↑ limb perfusion (<i>P</i> =0.015), ↑ limb temperature (4 wk, <i>P</i> <0.05)	Marui et al ¹⁴
Phase I/II dose-escalation, double-blind, placebo-controlled, multicenter trial	PAD, CLI	Ad5FGF-4, 2.87×10 ⁸ –10 ¹⁰ VP	IM	10/3	12 wk	Rest pain, ABI, angiography (DSA), perfusion (MRI, scintigraphy)	↓ Rest pain (4/10), more/bigger vessels	Matyas et al ¹⁵

(Continued)

Table 2. Continued

Trial	Condition	Treatment(s) Dosing	Route	Patients Verum/ Placebo, n	Follow-Up	Parameters of Bioactivity	Trends for Bioactivity	Reference
Phase I/IIa open-label study	PAD/Buerger's, CLI	HGF plasmid, test dose 0.4 mg, therapeutic 2× 4 mg	IM	6/0	12 wk	Improvement of symptoms, ABI, TPI, TcPO ₂ , angiography (DSA, MRI, CT), rest pain, ulcer healing, QoL	↓ On pain scale (5/5), ↑ ABI (5/5), ↑ TPI, ↑ TcPO ₂ , ↓ long ulcer diameter (8/11)	Morishita et al ¹⁶
HGF-STAT phase I/II open-label, double-blind, placebo- controlled, multicenter trial	PAD, CLI	HGF plasmid, 3× 0.4, 2× 4, 3× 4 mg	IM	27–26– 27/26	6 mo (efficacy), 12 mo (safety)	TcPO ₂ (PE), No. of patients with TcPO ₂ >30, ABI, TBI, pain relief, wound healing, major amputation, death	↑ TcPO ₂ ($P=0.0015$), ↑ change in TcPO ₂ (high dose; $P=0.0018$), ↑ No. of patients with TcPO ₂ >30 (high dose); ↓ change in ulcer size	Powell et al ¹⁷
Phase I dose-escalation, double-blind, placebo- controlled trial; phase I extension, open label	PAD, CLI	Ad2/HIF-1 α /VP16 1×10 ⁸ –2×10 ¹¹ PU	IM	34/7 (study rollover)	1 y	Clinical response (PE)	↑ Rest pain resolution (14/32), ↑ complete ulcer healing (5/18), ↑ combined pain resolution/ulcer healing (5), no amputations (2 highest doses)	Rajagopalan et al ¹⁸
RAVE phase II double-blind, placebo- controlled trial	PAD, claudication	AdVEGF ₁₂₁ , 4×10 ⁹ PU, 4×10 ¹⁰ PU	IM	32–40/33	12/26 wk	PWT (PE), Δ PWT, ABI, COT, QoL	None	Rajagopalan et al ¹⁹
Phase I	Buerger's	VEGF ₁₆₅ plasmid 2000 μ g	IA balloon	1/0	12 wk	Feasibility, angiography (DSA), angiography (MRI), flow (Doppler)	Feasible, ↑ collateral vessels, ↑ flow, ↑ distal flow	Isner et al ²⁰
Phase I	Buerger's, CLI	rh VEGF ₁₆₅ 2× 2 mg	IM	6 (7 limbs)/0	14 mo	Distal flow (MRI angiography), collaterals (contrast angiography), ABI, ulcer healing, pain	↑ Distal flow (7/7), ↑ new collaterals (7/7), ↑ ABI (3/7), overall clinical improvement (5/7), ↑ ulcer healing (3/5), ↑ nocturnal pain (2/2), limb salvage (1)	Isner et al ²¹
Phase I	PAD, CLI	VEGF ₁₆₅ plasmid 2× 2000 μ g	IM	9 (10 limbs)/0	6 mo	Blood pressure, ABI, TBI, collateral formation (angiography), distal flow (MRI angiography), Rutherford class, ulcer healing, limb salvage, rest pain, pain-free WT, claudication-limited WT	↑ Blood pressure (8/9 limbs, $P=0.008$), ↑ ABI ($P=0.02$), ↑ TBI, ↑ collaterals (7/10 limbs), ↑ distal flow (8/10 limbs), ↓ Rutherford class, ↑ ulcer healing (4/7 limbs), ↑ limb salvage (3 limbs), ↓ rest pain ($P=0.043$), ↑ pain-free WT (5/5 limbs, $P=0.043$), ↑ claudication-limited WT ($P=0.018$)	Baumgartner et al ²²

(Continued)

Table 2. Continued

Trial	Condition	Treatment(s) Dosing	Route	Patients Verum/ Placebo, n	Follow-Up	Parameters of Bioactivity	Trends for Bioactivity	Reference
Phase I	PAD/Buerger's, CLI	VEGF ₁₆₅ plasmid 2× 400–2000 μg	IM	21 (24 limbs)/0	6 mo	ABI, distal flow (MR angiography), angiography (contrast), ulcer healing/ improvement, rest pain	↑ ABI ($P<0.001$), ↑ distal flow (19 limbs), ↑ angiographic score ($P<0.01$), ↑ healing/improved ulcers (75%), ↓ rest pain (83%)	Shyu et al ²³
Groningen phase I/II double-blind, placebo- controlled trial at 2 centers	PAD/diabetes, CLI	VEGF ₁₆₅ plasmid 2× 2000 μg	IM	27/27	100 d	Amputation rate (PE), ABI, TBI, clinical improvement, QoL	↓ Amputation rate (3/6), overall response (14/3, $P=0.003$), hemodynamic improvement (7/1, $P=0.05$), improved skin ulcers (7/0, $P=0.01$), ↓ rest pain (5/2)	Kusumanto et al ²⁴
VEGF Peripheral Vascular Disease phase II double-blind, placebo- controlled trial	PAD/PTA, claudication	AdVEGF ₁₆₅ 2×10 ¹⁰ PFU, VEGF ₁₆₅ plasmid-liposome 2000 μg	IA	18 Ad–17 P/L/19	3 mo	Vascularity (DSA, PE), restenosis, Rutherford class, ABI, amputation, rest pain	↑ Overall vascularity (Ad $P=0.03$, P/L $P=0.02$), ↑ ischemic zone vascularity (Ad $P=0.01$)	Mäkinen et al ²⁵
Phase I	PAD/amputation, CLI	Adβ-gal 1×10 ⁸ –4×10 ¹⁰ PFU	IA	8/2	1–2 d	Gene transfer feasibility	Successful transfer (6/8)	Laitinen et al ²⁶
Phase III								
PREVENT III phase III double-blind, placebo- controlled, multicenter trial	PAD/revascular- ization, CLI, vein bypass graft	E2F decoy (edifoligide)	Ex vivo	563/575	1 y	Time to graft failure (PE): reintervention, amputation; all-cause graft failure; clinically significant graft stenosis; amputation; reintervention-free survival; nontechnical primary graft patency	PE negative, ↑ secondary graft patency ($P=0.016$)	Conte et al ²⁷

Overview of bioactivity data from gene-therapy trials in the field of peripheral arterial disease. The studies are listed in alphabetic order of the therapeutic agent and year of publication; only published studies have been considered.

ABI, ankle-brachial index; Ad, adenovirus; COT, claudication onset time; Del-1, developmentally regulated endothelial locus 1; DSA, digital subtraction angiography; E2F, E2F transcription factor; FGF, fibroblast growth factor; h, human; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; IA, intraarterial; IM, intramuscular; IV, intravenous; MRI, magnetic resonance imaging; N/A, not available; PE, primary endpoint; PAD, peripheral arterial disease; PU, particle units; PWT, peak walking time; r, recombinant; QoL, quality of life; TBI, toe-brachial index; TcPO₂, transcutaneous PO₂; VEGF, vascular endothelial growth factor; VP, viral particle; WT, walking time.

Whether the method used in the present study, preselection of a subpopulation of patients for TcPO₂ analysis, ultimately proves to be a useful surrogate that is predictive of clinical benefit remains to be seen, but documentation of bioactivity in a controlled trial is of paramount importance, and it is likely that the method applied by these investigators will be emulated and applied to other end points as well. In addition, the discordance between the surrogate and clinical end points observed in the study by Powell et al¹⁷ is prototypical of studies of angiogenic therapies. This is unsurprising when one considers the fact that all of the surrogate end points used in cardiovascular therapies have been validated for the detection and surveillance of large-vessel occlusive disease

rather than for the accurate measurement of changes induced by angiogenesis. Thus, new methods and strategies may be required to accurately assess the benefits of therapeutic angiogenesis.

Safety analyses present yet another challenge for studies of patients with CLI. In the subgroup of 93 patients who were monitored for safety over 12 months in HGF-STAT, ≈50% experienced serious adverse events. This high frequency of serious adverse events underscores the severe clinical condition of CLI patients and the labor intensity and associated costs both of their care and of the management of clinical trials in this population. Clinical care alone of patients with CLI has been estimated at \$43 000 per patient-year in 1990.³¹

Safety Aspects of Therapeutic Angiogenesis

Results from numerous randomized, controlled studies, including the study by Powell et al¹⁷ presented in this issue of *Circulation*, suggest that the transfer of proteins and genes to the human system is safe and feasible (Table 1). In the more than 1000 individuals who have been treated with gene therapy for therapeutic angiogenesis in phase I/II trials, adverse effects have generally been consistent with the baseline rate in the populations studied. However, until long-term safety data from large-scale investigations become available, these experimental therapies must be administered with scrupulous safety monitoring. A number of concerns need to be addressed, including the potential for angiogenesis-triggered malignancies, the impact of angiogenesis on physiological or pathological processes, and the specific adverse effects associated with each growth factor.

Because antiangiogenic therapies are effective for tumor treatment, it is logical to hypothesize that proangiogenic growth factors, in turn, might promote tumor development. Thus far, however, preclinical and clinical experiences with different growth factors have not identified an increased risk for malignancies, which appear to be related primarily to the older age of eligible patients. Stimulation of angiogenesis could also, in theory, induce or worsen retinopathy, as suggested by the high ocular fluid levels of vascular endothelial growth factor (VEGF) observed in patients with active proliferative diabetic retinopathy and by the successful treatment of this condition with anti-VEGF antibodies, but this problem has not materialized in clinical studies. There is concern that angiogenic factors may promote or destabilize atherosclerotic plaques by exerting angiogenic effects on the vasa vasorum; nevertheless, clinical studies have found no evidence of accelerated atherosclerosis in patients with advanced vascular/arteriosclerotic disease who were administered angiogenic cytokine therapy. Specific angiogenic factors have certain direct and predictable adverse effects. Some examples include hypotension after the administration of fibroblast growth factor-2 and VEGF proteins,^{32,33} which limited dosing in phase I trials; vascular leakage³⁴ and transient tissue edema³⁵ after VEGF and fibroblast growth factor gene transfer (although pedal edema usually responded well to diuretics³⁶); and renal insufficiency with fibroblast growth factor-2 treatment, likely caused by membranous nephropathy. The adverse effect profile of HGF has not been characterized, but its ability to stimulate multiple downstream factors, which is the source of much enthusiasm for this agent, indicates that ongoing surveillance for safety is particularly important.

Aspects of Bioactivity in Therapeutic Angiogenesis

The elusiveness of a clinical breakthrough with angiogenic gene therapy may be explained by biological, technical, methodological, or disease-related factors (Table 2); for example, the poor efficacy obtained with intravascular administration of recombinant protein is likely caused by insufficient levels of growth factor in the targeted tissue. However, angiogenic gene therapy remains attractive because

of the convergence of several features and perceptions. First, gene therapy offers a biological solution to a biological problem. Second, single genes appear to activate potent angiogenic mechanisms. Third, the angiogenic effect may be multiplied by the administration of morphogens that activate specific targets and pathways. Fourth, treatments can be designed to counteract specific pathological mechanisms. Fifth, delivery of the therapeutic agent can be restricted to the disease locus, thereby (presumably) maximizing the potential benefit while minimizing the occurrence and severity of side effects. Lastly, the function of specific organs can be safely enhanced with targeted, short-term transgene expression.

In retrospect, the success of gene therapy in animal models of vascular disease may have raised expectations to unreasonable heights. Because clinical efforts are extrapolated from preclinical studies, species-specific variations are unavoidable. Furthermore, chronic disease has progressed for decades in most patients and is often polygenetic, so successful treatment with a 1-time administration of a single gene appears unrealistic. The clinical success or failure of vascular gene therapy is also determined by the gene administered, the delivery vector and method of administration, and the underlying illness. Currently, preclinical studies are under way to identify the potential for complementary or synergistic effects from combinations in hopes of identifying the most efficient and safe biological "cocktail." Viral vectors are generally effective for delivering genes, but immunogenic and pathogenic concerns have spurred efforts to find alternatives or novel virus serotypes. The transfection efficiency of nonviral vectors, on the other hand, is low and consequently presents a different set of challenges for in vivo applications that require a long-term effect. Novel formulations of DNA vectors may enable more efficient transfection, and methods that target nonviral vectors to specific tissues could increase efficiency and reduce adverse effects; however, targeted administration may be subject to technical limitations. For example, catheters are effective for local delivery to focal lesions in the vasculature or tissue of interest, but their usefulness depends on the target organ and its underlying pathology. Finally, a precise understanding of the mechanisms underlying neovascularization, including the time course and sequential roles of angiogenic and trophic factors, will enable researchers to better mimic the endogenous regenerative response.

Future Perspectives

Over the past 2 decades, angiogenic gene therapy has developed slowly but steadily as our mechanistic understanding of the factors involved has grown and as the selection of genes and vectors, the methods of administration, and clinical trial design continue to be refined. However, advancement from the safety-and-feasibility stage to routine clinical use will require carefully designed, adequately powered, large-scale, randomized, controlled phase II/III trials that incorporate end points that address methodological improvements, long-term safety, and bioactivity. Novel genetic targets will continue to be identified, and related factors capable of enhancing or attenuating the potency of gene therapy may be revealed.

Investigators may also begin to assess the application of gene therapy in other areas of cardiovascular medicine, such as the prevention of postinterventional vascular remodeling and bypass-graft failure, stabilization of vulnerable plaques and aneurysms, or the treatment of hypertension, hyperlipidemia, and thrombotic states. Implicit in the above is the need to acknowledge the accumulated evidence of safety and to begin the pursuit of gene therapy applications at earlier stages of disease, when the potential for observable benefit may be enhanced.

Although the beneficial mechanisms of cell therapy are not completely understood, the potential of leveraging both the native expression of certain factors via gene therapy and augmenting an innate cellular response is attractive as a means to overcome methodological and technical challenges and yield synergistic effects, permitting lower doses of each therapeutic agent and the potential for enhanced safety.³⁷ In theory, cell and gene therapy can be combined in 3 ways: (1) gene therapy supplemented with drug-induced stem cell or progenitor cell mobilization, (2) combined administration of both gene therapy and cell therapy, and (3) administration of genetically modified cells.

We believe that systematic efforts at the bench and the bedside will eventually lead to the routine clinical use of gene therapy for therapeutic angiogenesis. The development of gene therapy for other indications has progressed slowly; however, numerous targets for genetic modification can be envisioned.³⁸ As new genetic targets are characterized and as our understanding of angiogenic mechanisms becomes more sophisticated, combinations of factors and/or the inclusion of other emerging strategies, such as cell therapy and bionanotechnology, may enhance patient response by inducing complementary or synergistic effects. For now, we can continue to learn from each clinical trial, and the work by Powell et al¹⁷ has provided important lessons that will inform the approach to future studies.

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None.

References

- 2007 Heart and Stroke Statistical Update. Dallas, Tex: American Heart Association; 2007.
- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182–1186.
- Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, Ferrara N, Symes JF, Isner JM. Therapeutic angiogenesis: a single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. *J Clin Invest*. 1994;93:662–670.
- Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease, part II: cell-based therapies. *Circulation*. 2004;109:2692–2697.
- Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease, part I: angiogenic cytokines. *Circulation*. 2004;109:2487–2491.
- Grossman PM, Mendelsohn F, Henry TD, Hermiller JB, Litt M, Saucedo JF, Weiss RJ, Kandzari DE, Kleiman N, Anderson RD, Gottlieb D, Karlsberg R, Snell J, Rocha-Singh K. Results from a phase II multicenter, double-blind placebo-controlled study of Del-1 (VLTS-589) for intermittent claudication in subjects with peripheral arterial disease. *Am Heart J*. 2007;153:874–880.
- Mann MJ, Whittemore AD, Donaldson MC, Belkin M, Conte MS, Polak JF, Orav EJ, Ehsan A, Dell'Acqua G, Dzau VJ. Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial. *Lancet*. 1999;354:1493–1498.
- Comerota AJ, Thom RC, Miller KA, Henry T, Chronos N, Laird J, Sequeira R, Kent CK, Bacchetta M, Goldman C, Salenius JP, Schmieder FA, Pilsudski R. Naked plasmid DNA encoding fibroblast growth factor type 1 for the treatment of end-stage unreconstructible lower extremity ischemia: preliminary results of a phase I trial. *J Vasc Surg*. 2002;35:930–936.
- Henry TD, Mendelsohn F, Comerota A, Pham E, Grek V, Coleman M. Dose and regimen effects of intramuscular NV1FGF in patients with critical limb ischemia: a randomized, double-blind, placebo-controlled study. *Eur Heart J*. 2006; 27(suppl 1):235. Abstract.
- Nikol S, Baumgartner I, Van Belle E, Diehm C, Visoná A, Capogrossi MC, Ferreira-Maldent N, Gallino A, Wyatt MG, Wijesinghe LD, Fusari M, Stephan D, Emmerich J, Pompilio G, Vermassen F, Pham E, Grek V, Coleman M, Meyer F; TALISMAN 201 Investigators. Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critical limb ischemia. *Mol Ther*. 2008;16:972–978.
- Cooper LT Jr, Hiatt WR, Creager MA, Regensteiner JG, Casscells W, Isner JM, Cooke JP, Hirsch AT. Proteinuria in a placebo-controlled study of basic fibroblast growth factor for intermittent claudication. *Vasc Med*. 2001;6:235–239.
- Lazarous DF, Unger EF, Epstein SE, Stine A, Arevalo JL, Chew EY, Quyyumi AA. Basic fibroblast growth factor in patients with intermittent claudication: results of a phase I trial. *J Am Coll Cardiol*. 2000;36:1239–1244.
- Lederman RJ, Mendelsohn FO, Anderson RD, Saucedo JF, Tenaglia AN, Hermiller JB, Hillegas WB, Rocha-Singh K, Moon TE, Whitehouse MJ, Annex BH; TRAFFIC Investigators. Therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (the TRAFFIC study): a randomised trial. *Lancet*. 2002;359:2053–2058.
- Marui A, Tabata Y, Kojima S, Yamamoto M, Tambara K, Nishina T, Saji Y, Inui K, Hashida T, Yokoyama S, Onodera R, Ikeda T, Fukushima M, Komeda M. A novel approach to therapeutic angiogenesis for patients with critical limb ischemia by sustained release of basic fibroblast growth factor using biodegradable gelatin hydrogel: an initial report of the phase I-IIa study. *Circ J*. 2007;71:1181–1186.
- Matyas L, Schulte KL, Dormandy JA, Norgren L, Sowade O, Grötzsch G, Palmer-Kazen U, Rubanyi GM, Wahlberg E. Arteriogenic gene therapy in patients with unreconstructible critical limb ischemia: a randomized, placebo-controlled clinical trial of adenovirus 5-delivered fibroblast growth factor-4. *Hum Gene Ther*. 2005;16:1202–1211.
- Morishita R, Aoki M, Hashiya N, Makino H, Yamasaki K, Azuma J, Sawa Y, Matsuda H, Kaneda Y, Ogihara T. Safety evaluation of clinical gene therapy using hepatocyte growth factor to treat peripheral arterial disease [published correction appears in *Hypertension*. 2006;48:e7]. *Hypertension*. 2004;44:203–209.
- Powell RJ, Simons M, Mendelsohn FO, Daniel G, Henry TD, Koga M, Morishita R, Annex BH. Results of a double-blind, placebo-controlled study to assess the safety of intramuscular injection of hepatocyte growth factor plasmid to improve limb perfusion in patients with critical limb ischemia. *Circulation*. 2008;118:58–65.
- Rajagopalan S, Olin J, Deitcher S, Pieczek A, Laird J, Grossman PM, Goldman CK, McEllin K, Kelly R, Chronos N. Use of a constitutively active hypoxia-inducible factor-1 α transgene as a therapeutic strategy in no-option critical limb ischemia patients: phase I dose-escalation experience. *Circulation*. 2007;115:1234–1243.
- Rajagopalan S, Mohler ER III, Lederman RJ, Mendelsohn FO, Saucedo JF, Goldman CK, Blebea J, Macko J, Kessler PD, Rasmussen HS, Annex BH. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, con-

- trolled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. *Circulation*. 2003;108:1933–1938.
20. Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, Rosenfield K, Razvi S, Walsh K, Symes JF. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet*. 1996;348:370–374.
 21. Isner JM, Baumgartner I, Rauh G, Schainfeld R, Blair R, Manor O, Razvi S, Symes JF. Treatment of thromboangiitis obliterans (Buerger's disease) by intramuscular gene transfer of vascular endothelial growth factor: preliminary clinical results. *J Vasc Surg*. 1998;28:964–973.
 22. Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, Isner JM. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation*. 1998;97:1114–1123.
 23. Shyu KG, Chang H, Wang BW, Kuan P. Intramuscular vascular endothelial growth factor gene therapy in patients with chronic critical leg ischemia. *Am J Med*. 2003;114:85–92.
 24. Kusumanto YH, van Weel V, Mulder NH, Smit AJ, van den Dungen JJ, Hooymans JM, Sluiter WJ, Tio RA, Quax PH, Gans RO, Dullaart RP, Hospers GA. Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: a double-blind randomized trial. *Hum Gene Ther*. 2006;17:683–691.
 25. Mäkinen K, Manninen H, Hedman M, Matsi P, Mussalo H, Alhava E, Ylä-Herttuala S. Increased vascularity detected by digital subtraction angiography after VEGF gene transfer to human lower limb artery: a randomized, placebo-controlled, double-blinded phase II study. *Mol Ther*. 2002;6:127–133.
 26. Laitinen M, Mäkinen K, Manninen H, Matsi P, Kossila M, Agrawal RS, Pakkanen T, Luoma JS, Viita H, Hartikainen J, Alhava E, Laakso M, Ylä-Herttuala S. Adenovirus-mediated gene transfer to lower limb artery of patients with chronic critical leg ischemia. *Hum Gene Ther*. 1998;9:1481–1486.
 27. Conte MS, Bandyk DF, Clowes AW, Moneta GL, Seely L, Lorenz TJ, Namini H, Hamdan AD, Roddy SP, Belkin M, Berceli SA, DeMasi RJ, Samson RH, Berman SS; PREVENT III Investigators. Results of PREVENT III: a multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery. *J Vasc Surg*. 2006;43:742–751.
 28. Nikol S, Baumgartner I, Van Belle E, Diehm C, Visona A, Capogrossi MC, Ferreira-Maldent N, Gallino A, Wyatt MG, Wijesinghe LD, Fusari M, Stephan D, Emmerich J, Pompilio G, Vermassen F, Pham E, Grek V, Coleman M, Meyer F. Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critical limb ischemia. *Mol Ther*. 2008;16:972–978.
 29. Dormandy JA, Loh A. Critical limb ischemia. In: Tooke JE, Lowe GDO, eds. *A Textbook of Vascular Medicine*. London, United Kingdom: Arnold; 1996:221–236.
 30. Marston WA, Davies SW, Armstrong B, Farber MA, Mendes RC, Fulton JJ, Keagy BA. Natural history of limbs with arterial insufficiency and chronic ulceration treated without revascularization. *J Vasc Surg*. 2006;44:108–114.
 31. Hunink MG, Wong JB, Donaldson MC, Meyerovitz MF, de Vries J, Harrington DP. Revascularization for femoropopliteal disease: a decision and cost-effectiveness analysis. *JAMA*. 1995;274:165–171.
 32. Cuevas P, Carceller F, Ortega S, Zazo M, Nieto I, Gimenez-Gallego G. Hypotensive activity of fibroblast growth factor. *Science*. 1991;254:1208–1210.
 33. Horowitz JR, Rivard A, van der Zee R, Hariawala M, Sheriff DD, Esakof DD, Chaudhry GM, Symes JF, Isner JM. Vascular endothelial growth factor/vascular permeability factor produces nitric oxide-dependent hypotension: evidence for a maintenance role in quiescent adult endothelium. *Arterioscler Thromb Vasc Biol*. 1997;17:2793–2800.
 34. Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, McDonald DM. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science*. 1999;286:2511–2514.
 35. Rissanen TT, Markkanen JE, Arve K, Rutanen J, Kettunen MI, Vajanto I, Jauhainen S, Cashion L, Gruchala M, Narvanen O, Taipale P, Kauppinen RA, Rubanyi GM, Ylä-Herttuala S. Fibroblast growth factor 4 induces vascular permeability, angiogenesis and arteriogenesis in a rabbit hindlimb ischemia model. *FASEB J*. 2003;17:100–102.
 36. Baumgartner I, Rauh G, Pieczek A, Wuensch D, Wagner M, Kearney M, Schainfeld R, Isner JM. Lower-extremity edema associated with gene transfer of naked DNA encoding vascular endothelial growth factor. *Ann Intern Med*. 2000;132:880–884.
 37. Shintani S, Kusano K, Ii M, Iwakura A, Heyd L, Curry C, Wecker A, Gavin M, Ma H, Kearney M, Silver M, Thorne T, Murohara T, Losordo DW. Synergistic effect of combined intramyocardial CD34(+) cells and VEGF2 gene therapy after MI. *Nat Clin Pract Cardiovasc Med*. 2006;3(suppl 1):S123–S128.
 38. Rissanen TT, Ylä-Herttuala S. Current status of cardiovascular gene therapy. *Mol Ther*. 2007;15:1233–1247.

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