Stem cells encode vascular endothelial growth factors (VEGFs), fibroblastic growth factors (FGFs), stem cell factor, stromal cell-derived factor, platelet growth factor and angiopoietin that can contribute to myocardial vascularization. VEGFs and FGFs are the most investigated growth factors. VEGFs regulate angiogenesis and vasculogenesis. FGFs stimulate vessel cell proliferation and differentiation and are regulators of endothelial cell migration, proliferation and survival. Clinical trials of VEGF or FGF for myocardial angiogenesis have produced disparate results. The efficacy of therapeutic angiogenesis can be improved by: (1) identifying the most optimal patients; (2) increased knowledge of angiogenic factor pharmacokinetics and proper dose; (3) prolonging contact of angiogenic factors with the myocardium; (4) increasing the efficiency of VEGF or FGF gene transduction; and (5) utilizing PET or MRI to measure myocardial perfusion and perfusion reserve.
Table 1. Bioactive paracrine factors.

<table>
<thead>
<tr>
<th>Paracrine bioactive factors</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFRP2, VEGF, HGF, SDF-1, TGF-β, IGF-1, bFGF</td>
<td>Myocyte survival</td>
</tr>
<tr>
<td>IGF-1, EGF</td>
<td>Proliferation, growth</td>
</tr>
<tr>
<td>VEGF, bFGF, HGF</td>
<td>Increase myocyte contractility</td>
</tr>
<tr>
<td>bFGF, VEGF, HGF, Ang-1, Ang-2, TGF-β, IGF-1, SDF-1, PLGF, MCP-1, PDGF-B</td>
<td>Neovascularization</td>
</tr>
<tr>
<td>VEGF, IGF-1, HGF, TNF-α, SCF, FGF-2, EGF</td>
<td>Cell differentiation</td>
</tr>
<tr>
<td>IL-10, MMP-2, MMP-9, MCP-1, TSP1, TGF-β, TIMP-1, -2, -9, HGF, NGF</td>
<td>Myocardial ventricular remodeling</td>
</tr>
</tbody>
</table>

Ang-1: Angiopoietin-1; Ang-2: Angiopoietin-2; bFGF: Basic fibroblastic growth factor; EGF: Epidermal growth factor; FGF-2: Fibroblastic growth factor-2; HGF: Hepatocyte growth factor; IGF-1: Insulin like growth factor-1; IL-10: Interleukin-10; MCP-1: Monocyte chemoattractant protein-1; MMP-2: matrix metalloproteinase-2; MMP-9: Matrix metalloproteinase-9; NGF: Nerve growth factor; PDGF-B: Platelet derived growth factor-B; PLGF: Placental growth factor; SCF: Stem cell factor; SDF-1: Stromal derived factor-1; SFRP2: Secreted frizzled related protein 2; TGF-β: Transforming growth factor-β; TIMP-1, -2, -9: Tissue inhibitors of metalloproteinase -1, -2, -9; TNF-α: Tumor necrosis factor-α; TSP-1: Thrombospondin 1; VEGF: Vascular endothelial growth factor.
investigations. In this regard, therapeutic angiogenesis involves the administration of stem cell growth factors or stem cells that are capable of expanding the myocardial microvascular network and increasing blood flow thereby limiting or preventing myocyte and vascular endothelial cell death. The use of stem cells in order to limit myocardial infarction size has been previously reviewed [5,6]. The purpose of this paper is to review the different stem cell bioactive growth factors that contribute to angiogenesis and postnatal vasculogenesis and their use in therapeutic angiogenesis.

Angiogenic growth factors
Oxygen tensions less than 5% in the myocardium activate hypoxia inducible factors (HIF-1 and HIF-2) in vascular endothelial cells and cardiac myocytes [17,18]. The hypoxia inducing factors serve as homing signals for the recruitment of stem cells and also directly activate the transcription of angiogenic genes in stem cells and also cardiac cells that encode stromal cell-derived factor 1a (SDF-1a), stem cell factor, platelet growth factor, vascular endothelial growth factor, and angiopoietin 1 and 2 for myocardial neovascularization [19]. In this manner, HIF-1 promotes blood vessel sprouting. HIF-1 expression also contributes to neovascularization by enhancing vascular endothelial cell proliferation [18,19]. HIF-2 mediates vascular maintenance [20]. However, aging and diabetes significantly impair ischemia-induced activation of the HIFs, the recruitment of vascular endothelial progenitor cells and the expression of angiogenic growth factors [21] (see Figure 1).

Vascular endothelial growth factors
To date, the most investigated growth factors for myocardial infarction (MI) angiogenesis are the VEGFs and FGFs.

Members of the VEGF family are major modulators of vascular biology and are secreted by stem cells and also rapidly induced in the ischemic heart in humans by hypoxia and free oxygen radicals [22]. The VEGF family regulates angiogenesis, in other words, the growth of new capillaries from pre-existing blood vessels by vascular sprouting or intussusception and also regulates vasculogenesis, in other words, the differentiation of vascular endothelial stem cells into endothelial cells and the de novo formation of a primitive vascular network. The VEGF family includes VEGF, also referred to as VEGF-A, and VEGF-B, VEGF-C, VEGF-D and placental growth factor. VEGF-A contributes to angiogenesis, vasculogenesis and vascular homeostasis. VEGF-B and VEGF-C guide newly formed blood vessels to maturity in ischemic myocardium, thereby providing long-term blood supply to cardiomyocytes and limiting adverse remodeling of the LV after myocardial infarction. Robust and uniform angiogenesis in MIs requires several different growth factors [23] (see Table 2).

In addition, VEGF-C and VEGF-D regulate lymphatogenesis. VEGFs act through three structurally related VEGF receptor tyrosine kinases, denoted VEGFR1 (Flt1), VEGFR2 (Flk1) and VEGFR3 [24]. The receptors have some overlap but also distinct expression patterns. In general, VEGFR1s are widely expressed and are involved in inhibition of angiogenesis and the negative regulation of VEGFR2 by binding VEGF [24]. However, VEGFR1s are also involved in fatty acid uptake in vascular endothelial cells. In addition, PLGF can preferentially bind to VEGFR1 and can amplify the recruitment of angiogenic progenitor cells to ischemic tissues [25]. VEGFR2 is the main VEGF receptor on endothelial cells and is essential for endothelial cell differentiation, proliferation, migration and formation of vascular tubes. In addition, VEGF-A by combining with VEGFR2 can cause increased vascular permeability and the extravasation of proteins from the intravascular to the interstitial space for vascular sprouting. Furthermore, VEGFR2 binds VEGF-C and prevents VEGF-C binding to VEGFR3 and therefore limits lymphatic cell proliferation [24]. VEGFR3s in combination with VEGF-C are instrumental in lymphatic development and VEGFR3 signal transduction is critical in regulation of lymphatic vessel function [24].

Fibroblastic growth factors
Fibroblastic growth factors are secreted by stem cells and also by damaged cardiac myocytes and vascular endothelial cells. Full comprehension of the angiogenic effects of the FGF family is limited by the redundancy in the FGF system and the fact that there are approximately 22 FGF ligands [22]. FGF-1 is unique among the FGF family in that it is the broadest-acting member of the FGF family and can bind to seven FGF-receptor subtypes. FGF-1 stimulates the proliferation and differentiation of all the cell types necessary for building an arterial vessel,
including endothelial cells and smooth muscle cells. Fibroblastic growth factor-2 (FGF-2) is an important pleotropic regulator of vascular endothelial cell migration, proliferation, and differentiation and the survival of blood vessel-associated cells. When FGF-2 signaling is inhibited, vascular endothelial cell junctions become compromised and blood vessel permeability is increased [22]. FGF-2 is less potent than FGF-1. The effect of FGF-2 is partially indirect because FGF-2 induces VEGF expression in endothelial cells and VEGF neutralization blocks the proangiogenic effects of FGF-2 [22]. In a porcine model of MI and in a model of hindlimb ischemia, co-administration of FGF-2 and PDGF increased angiogenesis, perfusion of ischemic myocardium and vascular stability [22].

The FGFs also stimulate endothelial cell synthesis of proteases, including plasminogen activator and metalloproteinases, which are important for extracellular matrix digestion during angiogenesis [20]. In addition to stimulating blood vessel growth, FGF-1 and FGF-2 are important in wound healing. These growth factors stimulate the proliferation of fibroblasts and endothelial cells that form granulation tissue in the healing of myocardial infarctions.

**The angiopoietin & TIE signaling system**
The human angiopoietin (ANG) family consists of ANG-1, ANG-2 and ANG-4 and two receptors, TIE-1 and TIE-2 [20]. ANG-1 functions as a TIE-2 agonist and maintains blood vessel membrane basement deposition, mural cell coverage and endothelial cell quiescence. ANG-2 functions as a competitive ANG-1 antagonist [20]. In the presence of angiogenic stimulators such as VEGF, sprouting endothelial
cells release ANG-2, which antagonizes ANG-1 and TIE-2 signaling and enhances mural cell detachment, vascular permeability and endothelial cell sprouting [20]. ANG-4 is currently thought to act like ANG-1 [20].

Additional angiogenic factors

Stromal derived factor-1alpha (SDF-1α) is activated and upregulated in acute myocardial infarction and helps to regulate bone marrow endothelial progenitor cell mobilization and recruitment to the infarcted myocardium for angiogenesis [26,27]. SDF-1 also protects ischemic cardiomyocytes from apoptosis through activation of the cell survival metabolic pathways ERK and Akt [28].

Hepatocyte growth factor (HGF) is an angiogenic growth factor expressed by stem cells, and also vascular endothelial and smooth muscle cells that can have direct motogenic or morphogenic effects on vascular endothelial cells or indirect effects by regulation of other angiogenic factors such as VEGF for blood vessel formation [29]. The hepatocyte growth factor can activate multiple signaling pathways that directly or indirectly stimulate endothelial cells. These pathways include the phosphoinositol 3 kinase/Akt (PI3K/Akt) pathway, which activates endothelial cell motility and cell survival, the p120/STAT3 pathway, which stimulates branching morphogenesis of endothelial cells, and the Ras/MEK pathway, which mediates HGF-induced proliferation and migration of vascular endothelial cells [29].

Platelet derived growth factor (PDGF-BB) contributes to blood vessel maturation and mural cell covering of blood vessels by chemotactically attracting pericytes. By contrast, PDGF or pericyte deficiency causes blood vessel leakage, tortuosity, microaneurysm formation and bleeding [20].

Insulin like growth factor-1 (IGF-1) reduces oxidative stress and inflammation and is a potent mitogen and antiapoptotic factor for vascular smooth muscle cells. Stimulation of IGF-1 also induces HIF-1α expression. In addition, IGF-1 plays a major role in vasodilation by regulating nitric oxide (NO) production in the vascular endothelium [30].

Thymosin beta4 (Tβ4) is a major actin regulating peptide found in heart cells and is not a growth factor. Tβ4 can induce the adult epicardium to contribute endothelial and smooth muscle cells for vascular repair. The peptide promotes maturation and migration of stem cells and the formation of blood vessels [31,32]. In addition, Tβ4 can regulate laminin-5, which affects cell migration and adhesion in the vascular basement membrane.

The endothelial cell nitric oxide synthase (eNOS) generates NO and is involved in the angiogenic response to myocardial ischemia and hypoxia, in part through HIF1-a and VEGF-dependent pathways. Endothelial cell NO dilates blood vessels and increases blood flow by stimulating in vascular smooth muscle cells soluble guanylyl cyclase and increasing cyclic guanosine monophosphate. This inhibits calcium entry into vascular smooth muscle cells, decreases intracellular calcium concentrations and activates potassium channels, which leads to hyperpolarization, and stimulates cyclic guanosine monophosphate-dependent protein kinase which leads to vascular smooth muscle relaxation.

Vascular endothelium-derived NO prevents vascular endothelial cell apoptosis induced by inflammatory cytokines, reactive oxygen species and angiotensin II (ATII) [30]. Endothelial nitric oxide synthase also recruits stem cells for neovascularization. Decreased NO bioavailability, which occurs in patients with ischemic heart diseases, results in impaired neovascularization due to a defect in progenitor cell mobilization. Moreover, oxidative stress during

<table>
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<tr>
<th>Table 2. Vascular endothelial growth factors.</th>
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<tr>
<td><strong>Growth factor</strong></td>
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<tr>
<td>VEGF (VEGF-A)</td>
</tr>
<tr>
<td>VEGF-B</td>
</tr>
<tr>
<td>VEGF-C (VEGF-2)</td>
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<tr>
<td>VEGF-D</td>
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<td>PLGF</td>
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Figure 2. Stimulation by VEGF (VEGF-A) causes the quiescent blood vessel to dilate (see facing page). An endothelial tip cell is selected (VEGFR, DLL4 and JAGGED1) to form a branch. Endothelial cell junctions loosen, the basement membrane degrades and pericytes detach. The interstitial matrix around the vessel is remodeled by proteases. Fibrinogen and fibronectin extravasate from the vessel lumen into the interstitial matrix and form a scaffold. Endothelial cells then migrate onto the scaffold and assemble as a solid cord. Stalk cells behind the tip cell proliferate, elongate into the scaffold and eventually form a lumen. Sprouts fuse to establish a perfused neovessel. Reproduced with permission from [20].
myocardial infarction can convert eNOS from a NO-producing enzyme to an enzyme that generates free oxygen radicals. This process is termed NOS uncoupling [22,33].

**Angiogenic factors, vascular endothelial cells & blood vessel formation in the myocardium**

Vascular endothelial cells in the normal myocardium have long half-lives because they are maintained and protected against damage by the actions of VEGF, FGFs, ANG-1 and NOTCH signaling proteins [20]. In addition, vascular endothelial cells are equipped with oxygen sensors and HIFs which allow the cells and vessels to adjust their shape to optimize blood flow.

In order for blood vessel formation to occur, vascular endothelial cells must integrate the different signals that arise from growth factors, cell to cell contacts and cell to matrix contacts. In this regard, blood vessel formation can occur by: sprouting angiogenesis; the recruitment of bone marrow-derived stem cells and/or vascular-wall-resident endothelial progenitor cells that differentiate into vascular endothelial cells; or by a process of vessel splitting known as intussusception.

**Blood vessel formation by vascular sprouting**

Low VEGF-A concentrations are required for the maintenance of blood vessel homeostasis, endothelial cell survival and production of NO. VEGF-A in high concentrations released by stem cells or hypoxic vascular endothelial cells activate vascular endothelial cells, loosen vascular junctions, and cause vessel dilation and the formation of transcellular gaps in blood vessels [34].

When a quiescent vessel senses an angiogenic signal, such as VEGF-A, VEGF-C, ANG-2 or FGFs, pericytes first detach from the vessel wall in response to ANG-2 and liberate themselves from the basement membrane by proteolytic degradation, which is mediated by matrix metalloproteinases [20]. Fibrinogen and fibronectin plasma proteins extravasate from the vessel into the extracellular matrix and form a primitive scaffold for migrating vascular endothelial cells. Endothelial cells then assemble as a solid cord in the scaffold. Vascular endothelial growth factor and FGF help model the scaffold for blood vessel formation (see Figure 2).

One vascular endothelial cell, known as ‘the Tip Cell,’ leads the other endothelial cells. Filopodia extend from the Tip Cell that scan the environment for angiogenic stimuli, such as VEGF, and guide the angiogenic sprout in the direction of the stimuli [35]. The endothelial cell neighbors of the tip cell assume positions as stalk cells, which proliferate and elongate the stalk in response to VEGF.

The differentiation of endothelial cells into tip cells or stalk cells is controlled by the NOTCH protein signaling pathway [36]. Notch-1, Notch-4 and three Notch ligands (JAG1, DLL1 and DLL4) are expressed in vascular endothelial cells, interact with VEGF and play an important role in the formation of tip and stalk cells and also arterial and venous differentiation [37]. The stalk cells form tubes and branches which elongate the stalk. Adhesion molecules, including integrins αβ and VE-cadherin, promote the adhesion of the endothelial cells (see Figure 2).

Stalk cells proliferate at a high rate and initiate the process of vacuolation for blood vessel lumen formation. During vacuolation, pinocytic vesicles coalesce to form large intracellular vacuoles. Subsequently, these vacuoles coalesce and lead to the formation of a blood vessel lumen [38]. Additional factors, such as Ang-1, PDGF and platelet-derived growth factor receptor (PDGFR), participate in the maturation of the blood vessel.

During maturation, stalk cells transform into phalanx cells, which are so-called because they form an ordered monolayer of cells reminiscent of the military ‘phalanx formation’ [38]. Phalanx cells proliferate at a slower rate than stalk cells. They form the basement membrane and a tight barrier between the blood and the surrounding tissue. Tissue inhibitors of metalloproteinases and plasminogen activator inhibitor-1 (PAI-1) also contribute to the deposition of the basement membrane.

The last step of angiogenesis involves the recruitment of vascular supporting pericytes and smooth muscle cells. PDGF-BB stimulates migration and proliferation of pericytes and vascular smooth muscle cells for blood vessel stabilization [39]. This process is facilitated by HIF-2α, which is an enhancer of blood vessel stabilization (see Figure 1).

The new vascular sprout connects to neighboring sprouts or blood vessels in order to establish blood flow. Vascular endothelial cells then resume their quiescent state and the blood vessel becomes functional (see Figure 2). Vessels that are not perfused and functional tend to regress [18,20].
Blood flow in the new lumen shapes and remodels the vascular connections. Oxygen and nutrient delivery inactivate endothelial sensors and decrease VEGF expression thereby shifting endothelial cells into a quiescent phenotype \[40,41\]. In addition, blood flow shear stress activates the transcription factor Kruppel-like Factor 2, which promotes endothelial cell quiescence by upregulating endothelial NO synthase and thrombomodulin and downregulating VEGFR, which prevents tip cell formation \[42\]. Vascular quiescence is also maintained by the angiopoietin1 (Ang1)-Tie2 signaling pathway.

Proteinases terminate angiogenesis by liberating matrix bound antiangiogenic compounds such as thrombospondin-1, canstatin, tumstatin, endostatin, and platelet factor 4 and by inactivating SDF-1 \[35\].

- **Bone marrow endothelial cell recruitment in blood vessel formation**

  Endothelial progenitor cells (EPCs) contribute to angiogenesis and vasculogenesis in the adult myocardium. These progenitor cells from the bone marrow respond to chemoattractant signals from the ischemic myocardium, such as VEGF, FGF-2, placental growth factor and SDF-1 \[20\]. Due to chemoattractant signals from the heart the EPCs can home in on and invade sites of vascular remodeling/repair in the myocardium. Once the circulating EPCs have crossed the vascular endothelial monolayer, they migrate through the blood vessel basement membrane and the interstitium to arrive at specific niches where they proliferate and differentiate. VEGF and angiopoietin growth factors regulate EPC proliferation and survival (see Figure 3).

  The functional activity of EPCs depends on their differentiation into mature vascular endothelial cells, and their direct incorporation into neovessels, or more commonly the production of paracrine and/or juxtacrine bioactive factors that promote interactions with pre-existing vascular endothelial cells and other cell types that promote angiogenesis. In this regard, endothelial progenitor cells can produce VEGF, SDF-1, insulin-like growth factor 1, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1a and platelet-derived growth factor that can act on different cell types that promote angiogenesis.

- **Intussusception (splitting angiogenesis) in blood vessel formation**

  Preexisting blood vessels in the myocardium can split into two vessels by a process known as intussusception \[38,44–45\]. In this type of vessel formation, one side of a capillary wall extends into the vessel lumen to split a single vessel into two vessels. The two opposing vessel walls establish a zone of contact or ‘tissue pillar.’ Then, the endothelial cells and cell junctions are reorganized on each side of the tissue pillar and the pillar is perforated to allow growth factors, pericytes and myofibroblasts to penetrate into the pillar (see Figure 4). These cells lay collagen fibers that provide an extracellular matrix. The pillar grows and splits the blood vessel into two new blood vessels. In this manner, intussusception causes a reorganization of existing cells and permits a significant increase in the number of capillaries, venules and arterioles (see Figure 4).

  Three forms of intussusception angiogenesis are recognized: intussusceptive microvascular growth, intussusceptive arborization and intussusceptive branching remodeling \[38,44–45\]. Intussusceptive microvascular growth can expand an existing capillary network and produce a network of similarly sized capillaries. Intussusceptive arborization remodels an existing capillary into a vascular tree containing arterioles, venules and capillaries. Intussusceptive branching remodeling changes the branching pattern of blood vessels and prunes the vascular network of superfluous vessels in order to supply blood to the myocardium with the optimal number of blood vessels. Regulators of intussusception angiogenesis include HIF-2, VEGF, VEGF isoforms, angiopoietins, FGF, PDGF and erythropoietin. The vascularization of adult myocardium is primarily established through sprouting angiogenesis. Further remodeling is performed by intussusceptive arborization.

- **Therapeutic angiogenesis in patients with ischemic heart disease**

  Ten percent to 30% of patients with angina pectoris and obstructive coronary artery disease who undergo cardiac catheterization cannot be treated with either percutaneous coronary angioplasty and coronary stents or aorto-coronary bypass grafting because of diffuse obstructive coronary artery disease \[1\]. In addition, as many as 37% of patients who do undergo aorto-coronary bypass grafting have one or more coronary vessels that are not technically suitable for
bypass grafting [46–48]. Consequently, there is a substantial need for medical treatments that stimulate neovascularization of ischemic hearts in patients with diffuse coronary artery disease. The development and delivery of growth factors, genes or stem cells that consistently stimulate neovascularization of the heart is a major goal of cardiovascular research.

Clinical trials of angiogenesis have involved primarily bone marrow mononuclear cells, angiogenic growth factors or growth factor encoding DNA, which have been injected directly into the myocardium, the coronary arteries or intravenously in patients. The stem cell studies have been previously reviewed [5,6]. The most extensively studied growth factors for angiogenesis in patients after MI are the VEGF and FGF growth factors. The clinical trials have involved the injection of either VEGF [49] or alternatively FGF or their encoding DNA into the ischemic myocardium. In general, these trials demonstrate that VEGF and FGF can be safely administered and are well tolerated by patients. Despite positive neovascularization results with these angiogenic growth factors in animal studies, disparate therapeutic effects have been obtained in trials of VEGF and FGF for myocardial vascularization patients with significant ischemic heart disease.

● VEGF trials in ischemic heart disease

In the Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis (VIVA) trial, 178 patients with obstructive coronary artery disease, who were not candidates for myocardial revascularization, received an intracoronary infusion followed by intravenous injection of two different doses of recombinant human VEGF (rhVEGF-A165) protein or placebo [50]. In this trial there were no significant differences between the VEGF treated and the placebo treated patients in quality of life, exercise tolerance or myocardial perfusion when measured at 60 days after treatment. In the EUROINJECT-ONE Trial and The NORTHERN (NOGA

Figure 3. Postnatal vasculogenesis. Recruitment and incorporation of endothelial progenitor cells into angiogenic sites requires chemoattraction, bone marrow endothelial progenitor cell mobilization, migration, transmigration across vessel basement membranes, tissue invasion, in situ differentiation into mature endothelial cells and paracrine and/or juxtacrine factor production. Modified from [43].
Figure 4. Capillary intussusception. The opposite walls of the vessel migrate toward each other and form an intraluminal pillar. Endothelial cells and cell junctions are reorganized on each side of the tissue pillar and the pillar is perforated to allow growth factors, pericytes and myofibroblasts to penetrate into the pillar. The pillar grows and splits the blood vessel into two new blood vessels.

angiogenesis Revascularization Therapy: assessment by Radionuclide imaging] Trial [51,52], 80 and 93 patients with severe ischemic heart disease received either an intramyocardial injection of plasmid DNA expressing VEGF165 or placebo. In these trials, the treated patients did not experience a significant improvement in myocardial perfusion in comparison with the placebo treated patients at 3 and 3–6 months after treatment [51,52].

In contrast to the previous studies, 32 patients with intractable angina and no option for revascularization in the REVASC (Randomized Evaluation of VEGF for Angiogenesis) Trial, who were treated with intramyocardial adenoviral VEGF-A121 gene therapy by minithoracotomy, experienced significant improvements in exercise tolerance and quality of life in comparison with 37 patients treated with standard medical therapy without surgery in the control group [53]. However, a pseudo or placebo effect of minithoracotomy and intramyocardial injections in the VEGF treated patients cannot be entirely excluded in this study.

The NOVA double-blinded, placebo-controlled study investigated the safety of intramyocardial injection and the efficacy of AdGVVEGF121 gene therapy in patients with severe refractory coronary artery disease [54]. In this study, VEGF121 did not improve exercise capacity or myocardial perfusion in 12 patients followed for 52 weeks. In contrast, the Kuopio Angiogenesis Trial showed that VEGF-A165 did significantly improve myocardial perfusion determined by SPECT but not exercise time in 37 patients at 6 months after treatment when the VEGF was delivered by intracoronary injection of an adenovirus carrying the VEGF gene in comparison with the placebo treated patients [55]. Finally, the Phase I Endocardial Vascular Endothelial Growth Factor D (VEGF-D) Gene Therapy for the Treatment of Severe Coronary Heart Disease (KAT301) Trial is currently evaluating the safety and efficacy of VEGF-D when delivered by adenovirus into the myocardium of patients with chronic
myocardial ischemia that are not candidates for either coronary angioplasty or coronary artery bypass surgery.

**FGF trials in ischemic heart disease**

FGF-2 and FGF-4 have been investigated for angiogenic therapy in patients after MI. The FIRST trial of recombinant FGF-2 protein involved 337 patients and demonstrated a decrease in patient angina frequency and severity but did not demonstrate any significant improvement in exercise tolerance or perfusion of ischemic myocardium in the treated patients at 180 days after treatment [56]. The efficacy of FGF-4 gene therapy was examined in the AGENT 3 and AGENT 4 Trials in patients with symptomatic angina pectoris who did not require immediate revascularization (AGENT 3) or were technically unsuitable for coronary revascularization (AGENT 4) [57]. These studies showed no statistically significant differences in exercise tolerance, angina frequency or angina severity in male patients treated with FGF-4 in comparison with placebo treated male patients at 12–27 weeks. Subgroup analyses, however, did demonstrate in women that FGF-4 produced a significant increase in total exercise tolerance time, time to 1 mm ECG ST-segment depression and time to angina pectoris during exercise, and an improvement in Canadian Cardiovascular Society class in comparison with women treated with placebo [57].

The ASPIRE Trial is currently enrolling coronary artery disease patients with angina pectoris and myocardial perfusion defects on SPECT who are randomized to treatment with either intracoronary AdSFGF-4 or standard antiangina medications. The goal of the study is to determine whether AdSFGF-4 decreases myocardial perfusion defect sizes at 8 weeks post treatment.

**Interpretation of the VEGF & FGF trials in patients with ischemic heart disease**

Despite reports of VEGF and FGF treatment in animal models that show evidence of myocardial neovascularization, investigations of VEGF and FGF therapy in patients with ischemic heart disease have thus far not shown definitive evidence of clinical efficacy.

The lack of consistent efficacy of angiogenic therapy in patients is due to multiple factors. The pathological changes that occur in elderly patients with ischemic heart disease are not completely reproduced in young research animals with coronary artery ligations and myocardial infarctions. The optimal patient population has not been identified and investigated. Most clinical studies of angiogenic therapy to date have utilized ‘end-stage’ patients that have failed previous myocardial revascularizations or are not candidates for myocardial revascularization because of severe obstructive three vessel coronary artery disease. These patients have demonstrated resistance to myocardial coronary collateral artery formation by endogenous angiogenic growth factors and are resistant to neovascularization. Consequently, they are not optimal candidates for angiogenic therapy.

In addition, advanced age, hypertension, diabetes and hyperlipidemia in these patients can impair therapeutically induced angiogenesis in ischemic myocardium.

A thorough understanding of the pharmacokinetics and proper dose of angiogenic drugs, genes or endothelial progenitor cells is lacking among many of the cardiovascular trials. To date, the most optimal angiogenic growth factor, gene or stem/progenitor cell has not been identified for patients with ischemic heart disease. Basic and clinical investigations have demonstrated that maintaining adequate concentrations of a VEGF or FGF recombinant protein for weeks in the ischemic myocardium for therapeutic angiogenesis is technically challenging and expensive. In addition, vascular leakage, tissue edema and hypotension can occur after VEGF and fibroblast growth factor protein administration, which limit the dosing of these growth factor proteins in patient trials.

Although angiogenic gene therapy has evolved in patients, this therapy is hampered by an inability to ensure that the gene therapy reaches and remains in close contact with the ischemic myocardium over prolonged periods. Currently, gene expression in the ischemic myocardium cannot be precisely quantified. The transduction efficiency of VEGF and FGF gene therapy by viral vectors is limited and the efficiency for gene transfer of nonviral vectors is low. Moreover, patients can develop neutralizing antibodies and cellular immune responses against viral vectors, which carry the angiogenic gene or protein, that limit the effectiveness of this therapy [58,59]. Consequently, vector failure can lead to incorrect conclusions about VEGF or FGF gene therapy in the ischemic myocardium of patients.

The most effective time for administration of an angiogenic growth factor, gene or stem/progenitor cell after myocardial infarction has not been
The cellular environment of a nascent myocardial infarction is vastly different from an established infarction with scar formation. Moreover, increased perfusion of an established infarction may reduce ventricular scar formation without actually increasing left ventricular contractility. Consequently, investigations of the most optimal time for administration of therapeutic angiogenesis are important in order to increase left ventricular perfusion and contractility.

To date, the most effective dose schedule and delivery method (intramyocardial, intracoronary or intravenous delivery) of angiogenic agents have not been established and require clarification. Intravenous delivery can lead to gene expression in organs other than the heart and intracoronary infusion can cause vector ‘washout’ from the heart due to coronary blood flow and cardiac contraction. Preclinical investigations in nonischemic animals suggest that angiogenesis may need to be induced for months before the newly formed capillaries mature and no longer require growth factor stimulation [60,61].

Most often myocardial perfusion in patients treated with angiogenic therapy has been determined with single photon emission computed tomography (SPECT), which measures the relative blood flow in different regions of the myocardium. However, positron emission tomography (PET) or MRI should be utilized in future clinical studies because these techniques provide superior myocardial image quality and the ability to assess...
absolute blood flow, subendocardial perfusion and myocardial perfusion reserve [35,61].

Conclusion & future perspective
The current limitations of angiogenesis therapy in patients can be overcome by continued and intensive investigations of the cellular and molecular mechanisms of angiogenic growth factors in myocardial vascularization, the determination of the optimal techniques for angiogenesis delivery and the utilization of PET or MRI for noninvasive monitoring of the effectiveness of angiogenic therapy in patients. Based on the clinical trials of angiogenesis therapy that have been performed to date, the delivery of a single growth factor or cell type into the coronary arteries or directly into the myocardium does not appear to promote sufficient angiogenesis for cardiac repair in patients with ischemic heart disease. Consequently, combination therapies should be investigated for therapeutic angiogenesis in patients with ischemic heart disease and may require the administration of several angiogenic genes or genes that activate multiple angiogenic pathways, angiogenic gene therapy plus stem cell therapy or the administration of stem cells that have been modified with angiogenic genes or fragments of double-stranded DNA that can replicate independently of chromosomal DNA, such as plasmids. In this manner, angiogenesis therapy will ultimately permit neovascularization of ischemic myocardium in patients with severe ischemic heart disease and will improve the quality and the quantity of patients’ lives.

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No writing assistance was utilized in the production of this manuscript.

References
Papers of special note have been highlighted as:
• of interest; ** of considerable interest. Space limitations prevent inclusion of all relevant publications.
** Review of clinical trials of stem cells in the treatment of patients with myocardial infarctions.
** Review of clinical trials of stem cells in the treatment of patients with myocardial infarctions.
11 Kinnaird T, Stabile E, Burnett MS et al. Marrow derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo angiogenesis through paracrine mechanisms. Circ. Res. 94, 678–685 (2004).
** Review of paracrine action of stem cells.
Henning


**Review of the mechanisms of angiogenesis.**


**Review of the mechanisms of angiogenesis.**


**Review of vasculogenesis and angiogenesis.**


Therapeutic angiogenesis: angiogenic growth factors for ischemic heart disease


