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From Bench to Bedside: Review of Gene and Cell-Based Therapies and the Slow Advancement into Phase 3 Clinical Trials, with a Focus on Aastrom's Ixmyelocel-T

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Abstract There is a large body of preclinical research demonstrating the efficacy of gene and cellular therapy for the potential treatment of severe (limb-threatening) peripheral arterial disease (PAD), including evidence for growth and transcription factors, monocytes, and mesenchymal stem cells. While preclinical research has advanced into early phase clinical trials in patients, few late-phase clinical trials have been conducted. The reasons for the slow progression of these therapies from bench to bedside are as complicated as the fields of gene and cellular therapies. The variety of tissue sources of stem cells (embryonic, adult bone marrow, umbilical cord, placenta, adipose tissue, etc.); autologous versus allogeneic donation; types of cells (hematopoietic, mesenchymal stromal, progenitor, and mixed populations); confusion and stigmatism by the public and patients regarding gene, protein, and stem cell therapy; scaling of manufacturing; and the changing regulatory environment all contribute to the small number of late phase (Phase 3) clinical trials and the lack of Food and Drug Administration (FDA) approvals. This review article provides an overview of the progression of research from gene therapy to the cellular therapy field as it applies to peripheral arterial disease, as well as the position of Aastrom's cellular therapy, ixmyelocel-T, within this field.

Keywords Gene therapy · Stem cell · Cellular therapy · Ixmyelocel-T · Severe peripheral arterial disease · Limb-threatening ischemia · Critical limb ischemia · Mesenchymal stromal cell · CLI · PAD · Bone marrow

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Introduction

The exploration of cellular therapies and their application to various diseases and conditions is not new. Bone marrow stem cells have been studied since the 1950s. Accepted treatments are primarily for hematopoietic stem cells (HSCs) collected from the bone marrow or the peripheral blood for transplantation and treatment of specific types of bone marrow cancers (leukemia, lymphoma, and myeloma). Since the 1950s and 60s, the trajectory of cellular research has climbed steadily upward but few cellular therapies are Food and Drug Administration (FDA) approved [1]. The reasons for the slow progression from bench to bedside are as complicated as the field of cellular therapy itself. The variety of tissue sources of stem cells (embryonic, adult bone marrow, umbilical cord, placenta, adipose tissue, etc.); autologous versus allogeneic donation; types of cells (hematopoietic, mesenchymal stromal, progenitor, and mixed populations); confusion and stigmatism by the public and patients regarding gene, protein, and stem cell therapy; scaling of manufacturing; and the changing regulatory environment all contribute to the small number of Phase 3 trials and lack of approvals.

This review article provides an overview of the progression of research from gene therapy to the cellular therapy field as it applies to peripheral arterial disease, as well as the position of Aastrom's cellular therapy, ixmyelocel-T, within this field.

Historical Perspective of the Overlap Between Gene and Cell Therapies

Pioneering work by Folkman in the 1970s led to development and eventual approval of anti-angiogenic treatments for cancer [2]. The discovery of angiogenic growth factors

and signaling molecules that initiate and promote growth and survival of new blood vessels [3] captured the attention of cardiovascular investigators, who began testing the effects of growth factor stimulation on perfusion and function of ischemic tissues, independent of macrovessel surgery. Within the same timeframe, pioneering work was accomplished by British [4] and American scientists [5] in the isolation and culture of pluripotent embryonic stem cells (ESCs) from the inner cell mass of pre-embryos. During the 1980s and 1990s, stem cell research exploded; however, controversy over human cloning and embryo destruction led to policies that restricted ESC research. Only three Phase 1 clinical trials using ESCs have been conducted since 2010 [6]; the 3 studies were for indications of macular dystrophy, macular degeneration, and spinal cord injury.

Clinical research conducted using stem cells and stem cell-derived therapy from sources considered to be non-controversial have included research of adult multipotent stem cells from bone marrow, adipose tissue, umbilical cord, placenta, and endometrial tissue. Diseases studied have included graft versus host, Crohn's, osteogenesis imperfecta, Parkinson's, Alzheimer's, and diseases of the cardiovascular system such as myocardial infarction, heart failure, and peripheral arterial disease (PAD).

Pathophysiology of Severe Peripheral Arterial Disease

Severe PAD, or critical (limb-threatening) limb ischemia (CLI), occurs when arterial blood flow is restricted to such an extent that the nutritive requirements of tissue can't be met [7]. Ordinarily, compensatory mechanisms, including capillary sprouting as well as arteriogenesis [8], alleviate the effects of the deprivation, but in patients with CLI these mechanisms are exhausted. Inadequate blood flow to the skin and surrounding tissues leads to endothelial dysfunction, chronic inflammation [7], and muscle damage [9, 10]. The net effect of these changes is the occurrence of rest pain, chronic non-healing wounds, and gangrene. Treatment of severe PAD usually involves an attempt to address restricted blood flow through lower extremity revascularization using open bypass surgery or endovascular percutaneous intervention. While patients have benefits like wound healing from restoration of blood flow, simply reinstating flow on a macrovascular level does not reverse the damage of the alteration of structure and function of the endothelium and surrounding tissues that has occurred with severe PAD [8]. Limitations of the surgical or endovascular approach includes the increased mortality risk with open bypass procedures, restenosis, re-occlusion, re-intervention, and continued pain and expense of extensive wound care [11–13]. In addition, approximately 40 % of patients are not eligible for revascularization [14], leaving an opening for filling an unmet medical need in these very ill patients. The 5-year mortality rate for the most severe form of PAD is 60 % [15], exceeding that of prostate cancer (<1 %) [16], breast cancer (11 %) [17], acute myocardial infarction (20 %) [18], colorectal cancer (36 %) [19], and stroke (41 %) [20]. Cellular therapies may provide a treatment solution that has the potential to address multiple aspects of severe PAD including reduction of inflammation, tissue remodeling, and increased perfusion.

The sections below provide a summary of the preclinical evidence and the results of clinical trials in severe PAD for each of the gene and cellular therapy types. Table 1 provides a summary of ongoing or completed Phase 3 clinical trials by gene or cellular therapy type, Phase 2 clinical trials ongoing or completed and reported in the literature, and unique ongoing pilot studies.

Gene Therapy

Angiogenic Growth and Transcription Factors

The biological process for wound repair is initiated immediately after injury by release of various growth factors and cytokines. Therefore, angiogenic growth factors have been likely candidates for biological therapy and have been studied for the treatment of ischemic disease. Isner et al. published the first preclinical studies of therapeutic angiogenesis in the treatment of limb ischemia in 1995 [21, 22]. Several growth and transcription factors have advanced from basic research into vascular clinical trials for the treatment of PAD. These include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), hypoxia inducible factor-1α (HIF-1α), fibroblast growth factor (FGF), and stromal-derived factor (SDF-1). There is extensive preclinical research showing the angiogenic potential for each of these factors.

The **VEGF** family, comprised of seven major isoforms, is the most widely studied endothelial growth factor [23]. VEGF, a key promoter of angiogenesis, stimulates proliferation, migration, and vascular formation and mobilizes endothelial progenitor cells [24–28]. The therapeutic potential of VEGF has been shown in PAD models where bolus injection of VEGF increased blood perfusion and tissue oxygenation [29–31]. Clinical trials using various isoforms were primarily conducted during the early 2000s (Table 1). Only one of the placebo-controlled trials had a positive primary endpoint of vascularity as measured by digital subtraction angiography, but all secondary outcomes were negative relative to placebo [32]; all other trials were negative for the primary efficacy measures [23]. In addition, one of the clinical trials demonstrated dose-dependent peripheral edema in patients receiving VEGF [33].

FGF, consisting of 23 structurally related proteins, is a regulator of angiogenesis through potent mediation of

Table 1 Clinical trials by gene or cellular therapy type

Sponsor/cell type	Phase/NCT[a]/Status/Design	Primary Efficacy Outcomes/ Scientific Publications
Sponsors with PHASE 3 Studies		
Aastrom Biosciences	**Phase 3**/Active recruiting	AFS at 12 months
	NCT01483898	
Multicellular; autologous	594 patients	
(Ixmyelocel-T)	Randomized, DB, PBO-controlled	
	Phase 2/Completed	Time to first occurrence of treatment failure (TTF) was significantly longer for patients treated with ixmyelocel-T compared to control patients (p=0.0032, logrank test). TTF was defined as the earliest trial day on which any of the following treatment failure events occurred: major amputation of the injected leg, all-cause mortality (death), doubling of total wound surface area from baseline, and *de novo* gangrene. The survival curves diverged early and the difference between groups was maintained throughout the 12-month follow-up period. The Cox PH analysis gave a treatment HR=0.381, 95 % CI=(0.195, 0.744), conveying a significant reduction in the risk of treatment failure in the ixmyelocel-T treatment group of approximately 62 % (p=0.0047). The individual components of the treatment failure composite endpoint all trended in the same direction, favoring ixmyelocel-T treatment, with the exception of all-cause mortality that was the same in both treatment groups [91]
	NCT00468000	
	72 patients	
	Randomized, DB, PBO-controlled	
Harvest Technologies	**Phase 3**/Active recruiting	AFS at 6 months
	NCT01245335	
	210 patients	
(Device)	Randomized, DB, PBO-controlled	
BM-MNC; autologous	**Phase 2**/Ongoing	Time to amputation was longer in the BMAC group than in the placebo group (p=0.067). In patients with tissue loss, treatment with BMAC demonstrated a lower amputation rate than placebo (39.1 % vs 71.4 %; p=0.1337). Wound healing was not reported. Change in Rutherford Class (patients who improved at least one numeric category) showed that in Rutherford 4 patients, 81.8 % of the BMAC patients improved while 42.9 % of control patients improved (p=0.0874); there was only a small difference in improvement (~3 %) between treatment groups in Rutherford 5 patients [97]
	NCT00498069	
	48 patients	
	Randomized, DB, PBO-controlled	
Sanofi-Aventis	**Phase 3**/Completed (TAMARIS)	TAMARIS provided no evidence that NV1FGF is effective in reduction of amputation or death in patients with CLI [37].
Growth factor (FGF)	NCT00566657	
	526 patients	
	Randomized, DB, PBO-controlled	
	NV1FGF	Similar rates of ulcer healing occurred with NV1FGF (19.6 %) and PBO (14.3 %; P=0.514). The use of NV1FGF reduced by 2-fold the risk of all amputations [HR 0.498; P=0.015] and major amputations (HR 0.371; P=0.015) in the MITT study population (18 patients were excluded from efficacy analyses; the robustness of findings in relation to the occurrence of amputation, death, and AFS was confirmed in the total randomized population; however data were not shown). There was no statistically significant trend to suggest that NV1FGF reduced risk of death (HR 0.460; P=0.105) [36]
	Phase 2/Completed (TALISMAN 201)	
	NCT00368797	
	125 patients	
	DB, randomized, PBO-controlled	
Sponsors or Investigators with PHASE 2 Studies		
Makinen, 2002	**Phase 2**/Completed	Positive vascularity; negative restenosis rate, Rutherford class, and ABI [32]
VEGF-165	54 patients	
(adnovirus/plasmid)	DB, randomized, PBO-controlled	
RAVE	**Phase 2**/Completed	Negative for peak walking time, ABI, claudication onset time, and Quality of life. Treated arm associated with dose-dependent peripheral edema [33]
Rajagopalan, 2003	105 patients	
VEGF-121 (adenovirus)	DB, randomized, PBO-controlled	

Table 1 (continued)

Sponsor/cell type	Phase/NCT[a]/Status/Design	Primary Efficacy Outcomes/ Scientific Publications
GRONINGEN Kusumanto, 2006	**Phase 2**/Completed	Negative for amputation rates; improvement in ulcer healing and ABI [98]
VEGF-165 (plasmid)	54 diabetic patients DB, randomized, PBO-controlled	
Viromed Co, Ltd	**Phase 2**/Active recruiting NCT01064440	Difference in pain level between baseline and 9 month follow-up as determined by VAS
Growth factor HGF (2 isoforms HGF 728 and HGF 723)	50 patients Randomized, DB, PBO-controlled	
AnGes HGF and modified HGF	**Phase 2**/Completed NCT00189540 27 patients DB, Randomized, PBO-Controlled	Change in TBI significantly improved from baseline at 6 months in the HGF-treated group compared with placebo (0.05±0.05 vs -0.17±0.04; P=0.047). Change in VAS from baseline at 6 months was also significantly improved in the HGF-treated group compared with placebo. Complete ulcer healing at 12 months occurred in 31 % of the HGF group and 0 % of placebo (P=0.04). There was no difference in major amputation of the treated limb (HGF 29 % vs placebo 33 %) or mortality at 12 months (HGF 19 % vs placebo 17 %) [43].
	Phase 2/Completed (HGF-STAT) NCT00060892 104 patients DB, randomized, PBO-controlled	TcPO2 (mean SE) increased at 6 months in the high-dose group (24.0_4.2 mm Hg, 95 % CI 15.5 to 32.4 mmHg) compared with the placebo (9.4_4.2 mm Hg, 95 % CI 0.9 to 17.8), low-dose (11.1_3.7 mmHg, CI 3.7 to 18.7 mmHg), and middle-dose (7.3_4.8 mmHg, CI _2.2 to 17.0 mmHg) groups (ANCOVA P_0.0015). There was no difference between groups in secondary end points, including ankle brachial index, toe brachial index, pain relief, wound healing, or major amputation [44]
Genzyme WALK study	Ad2/HIF-1α/VP16 NCT00117650	No significant differences in claudication onset time, ABI, or quality of life measurements between placebo and each of 4 HIF-1α dose groups [99]
Transcription factor (HIF-1)	**Phase 2**/Completed 289 patients Intermittent claudication Randomized, DB, PBO-controlled	
Juventas Therapeutics, Inc	SDF1 **Phase 2**/Active recruiting	Tracking of major/minor amputations, overall survival, QoL, ulcer healing, and pressure assessments
Cytokine (SDF) in 1 trial and BM-MNC; autologous for 1 trial	NCT01410331	
UMC Utrecht for BM-MNC study	48 patients BM-MNC autologous **Phase 1–2**/Not enrolling NCT00371371 DB, randomized, PBO-controlled 109–160 patients	Major amputation (primary), number and extent of leg ulcers, resolution of rest pain, improvement of ABI, improvement TcPO2, QoL [100]
TACT study group BM-MNC and PB-MNC	Pilot study and Phase 2a NCT00145262 25 patients with unilateral disease in pilot study who were injected with BM-MNC, followed by 22 patients with bilateral disease who were randomly injected with BM-MNC in one leg and PB-MNC in the other leg as control.	At 4 weeks, ABI was significantly improved in legs injected with BM-MNC compared to PB-MNC. Similar improvements were seen for transcutaneous oxygen pressure and pain free walking time. These improvements were sustained at 24 weeks [101]

Table 1 (continued)

Sponsor/cell type	Phase/NCT[a]/Status/Design	Primary Efficacy Outcomes/ Scientific Publications
Johann Wolfgang Goethe University Hospitals (Germany) BM-MNC; autologous	**Phase 1–2**/Completed NCT00282646 40 patients DB, randomized, PBO-controlled	Intra-arterial administration of BM-MNC did not significantly increase ABI. Cell therapy was associated with significantly improved ulcer healing versus placebo, and reduced rest pain versus placebo within 3 months. Limb salvage and amputation-free survival rates did not differ between the groups [102]
Losordo, Douglas, M.D. Baxter Healthcare Corporation BM-MNC	**Phase 1–2**/Completed NCT00616980 28 patients Multicenter, DB, PBO-controlled	A single administration of unmodified, autologous CD34 cell therapy was associated with significantly reduced rates of amputation in subjects with Rutherford class 4 and 5 critical limb ischemia. Ongoing analysis will examine additional endpoints, and will determine sample size and suitability of this therapy for a phase III study in patients with critical limb ischemia [103]
Biomet Biologics, LLC **Device**	Phase not stated/Active recruiting NCT01049919 152 patients Randomized, DB, PBO-controlled	AFS at 1 year
BM-MNC	**Phase 1–2**/Completed 29 patients (30 limbs) OL, nonrandomized	AFS at 1 year was 86.3 %. There was a significant increase in FTP and TBI, and a trend in improvement in ABI. The VascuQol questionnaire demonstrated significant improvement in quality of life, and 3 of 9 ulcers (33 %) healed completely [104]
Washington University School of Medicine STEMPAD study G-CSF mobilized PB-MNC	Investigator Sponsored Study **Phase 3**/Ongoing; not recruiting NCT00797056 60 patients Randomized, DB, PBO-controlled	AFS at 1 year
Pluristem, Ltd MSCs; allogeneic	**Phase 2**/Not yet recruiting NCT01679990 150 patients DB, PBO-controlled	Primary outcome: Log ratio of week 52 maximal walking distance(MWD) to baseline MWD
Pilot Studies		
Medistem, Inc. (Device) Endometrial regenerative cells/allogeneic	**Phase 1–2**/Not yet recruiting in US NCT01558908 15 patients Open-label; no comparator Initiated in China (2 patients treated in early July 2012)	Improvements post-treatment in rest pain (VAS), toe pressure and ABI, transcutaneous oximetry and ulcer status (with picture) at 12 weeks
Investigator Trial (Northwestern University) Cord Blood injection IM	**Phase 1**/Active recruiting NCT01019681 25 patients OL, nonrandomized	ABI (15 % will be considered improvement), healing of ischemic ulcers, and decreased pain-follow-up at 1, 6, 12, and 24 months

[a] NCT (National Clinical Trial Number) if available

ABI ankle brachial index, *DB* double-blind, *PBO* placebo

endothelial cell migration, differentiation, and survival [34]. Ashara et al. was the first to show that FGF improved perfusion in an animal model of ischemia [35]. The results of 2 clinical trials have been published: a Phase 2 and a Phase 3 trial were completed using injections of NV1FGF, nonviral naked FGF plasmid DNA. Both studies were placebo-controlled. In the Phase 2 study there was a significant difference favoring NV1FGF treatment in the

secondary endpoint of amputation-free survival (AFS); however, none of the other endpoints were supportive of this finding including wound healing, rest pain, or death [36]. The large Phase 3 study conducted with 525 patients was negative for all endpoints (Table 1) [37].

HGF is a cytokine known to regulate cell growth, motility, morphogenesis and angiogenesis through activation of tyrosine kinase [38, 39]. In preclinical models, HGF has been shown to induce robust collateral formation [40, 41]. Expression of HGF was found to be strongly upregulated in cells of the wound epidermis during healing of excisional wounds in rats [42]. Two placebo-controlled clinical studies were conducted evaluating treatment of CLI patients with HGF. In the first study of 27 patients, change in toe brachial index was significantly improved from baseline at 6 months of follow-up in the HGF-treated group compared with placebo, and there was complete ulcer healing at 12 months in 31 % of HGF-treated compared to 0 % of placebo patients [43]. In the second study conducted by the same investigational group, 106 CLI patients were randomized to placebo (N=26) or to 1 of 4 doses of HGF (N=78). In all evaluable groups, measurement of blood flow by transcutaneous oxygen tension (TcPO2) at 6 months increased from baseline in HGF-treated relative to placebo patients; the highest increase was in the high-dose HGF group. All other endpoints were negative [44]. A Phase 3 study is planned to begin in 2012 in 560 CLI patients with rest pain or tissue loss [45].

HIF-1α, a transcription factor, regulates oxygen homeostasis and metabolism through adaptive responses to hypoxia at the cellular level [46–51], coordinating effort on multiple pathways that regulate angiogenesis, including VEGF, as well as pathways relevant to cell survival and metabolism [52–55]. In preclinical studies using rabbit hindlimb ischemia, administration of HIF-1α increased collateral blood vessels, capillary density and regional blood flow [56], and in a murine diabetic model of CLI enhanced neovascularization, mobilized progenitors cells from the bone marrow, and improved tissue perfusion [57]. Only one Phase 2 HIF-1α trial in patients with PAD has published results at the time of this manuscript (see Table 1; NCT00117650); in this trial HIF-1α was not shown to be an effective treatment for patients with intermittent claudication, a less severe form of PAD than CLI.

SDF-1 is a chemokine that is rapidly overexpressed in response to tissue injury. In the ischemic mouse hind limb, SDF-1 was shown to enhance angiogenesis [58]. SDF-1 has unique properties compared with the effects of angiogenic growth factors, including the absence of significant mitogenic actions which may prevent uncontrolled endothelial cell proliferation and subsequent formation of enlarged tortuous vessels, common in VEGF-induced angiogenesis [59]. Rapid inactivation of the chemokine in the protease-rich environment of the ischemic limb was addressed in the design by Segers et al. of recombinant SDF-1 proteins carrying mutations that provide resistance to protease cleavage [60]. Only one clinical trial is currently being conducted exploring the therapy value of SDF-1 (Table 1). The study is a Phase 2 trial that is currently ongoing.

Cellular Therapies

Mononuclear Cell Fraction/Endothelial Progenitor Cells

The exploration of both autologous bone marrow (BM) and peripheral blood (PB) mononuclear cells (MNCs) for the treatment of ischemic disease has been explored for more than 10 years. These mononuclear cells, also called endothelial progenitor cells (EPC), are obtained from the $CD34^+$ stem cell fraction of adult bone marrow and peripheral blood through cell separation techniques, and have the same lineage as hematopoietic stem cells with several shared surface antigens including KDR, Tie-2/Tek, and CD34 [61]. Early evidence showed postnatal neovascularization activities in response to ischemia including response of resident endothelial cells, but also the proliferation, differentiation, migration, and incorporation of BM-derived EPCs [62–65]. It was shown in experiments that EPCs specifically home to sites of ischemia and incorporate into capillaries and interstitial arteries in models of limb and myocardial ischemia [63, 66, 67]. EPCs have been shown in preclinical studies to improve capillary density in hindlimb models of ischemia [61, 68]. A review of the clinical study literature by Sprengers in 2008 found 25 published reports of clinical studies of BM-MNCs or PB-MNCs [69]. Most of the studies were case or patient series, 1 was a pilot, and 2 were randomized clinical trials. Results from the 2 randomized trials are presented in Table 1. In a review of stem and progenitor cell therapy by Lawall [70], the author concluded that despite the limitations of published BM-MNC clinical studies (small number of patients, lack of control group, and differing primary efficacy measurements), the outcomes were remarkably consistent. Clinical course (wound healing, walking distance) and perfusion parameters (ankle brachial index [ABI], TcPO2) were consistent and positive across trials. There are 2 studies using BM-MNC therapy actively recruiting patients (Table 1).

Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) were first identified in bone marrow 40 years ago [71]. MSCs are plastic-adherent cells that were shown to differentiate into osteoblasts, adipocytes, and chondrocytes in the 1980s [72, 73]. Caplan et al. showed that surrounding conditions are critical for inducing MSC differentiation [73]. In the 1990s, Pittenger et

al. demonstrated that individual adult human MSCs were capable of expanding to colonies while still retaining their multipotency [74]. An essential characteristic of MSCs is that they home to damaged tissues and have been shown to regulate immune and inflammatory responses at target sites [75–79]. Isolation of MSCs have been performed in tissues other than bone marrow, including peripheral blood [80], cord blood [81], adipose tissue [82], synovial membrane [83], and placenta tissue [84]. Baksh et al. showed that MSCs derived from different tissues show phenotype heterogeneity and different growth abilities but also show similarities, with the potential to differentiate into the classical mesenchymal lineages [85]. There are a broad number of indications under study for treatment with MSCs; at the time of this article there were 254 active clinical trials for MSCs listed on clinicaltrials.gov. Some of the studies use allogeneic sources of cells and some use autologous. Indications include graft versus host disease, heart failure, diabetes, Parkinson's, arthritis, aplastic anemia, Crohn's disease, and multiple sclerosis. Of the 254 studies, 13 are listed with an indication of CLI and 1 with an indication of intermittent claudication (IC). Almost all of these studies are being conducted outside of the United States (U.S.), primarily in India and China; only the IC study has sites listed for recruitment in the U.S. There are no published results in peer-reviewed journals for early clinical trials using MSC-only therapy for the treatment of PAD.

Mixed Cellular Therapy

One hypothesis is that a mixture of regenerative cell types like MSCs ($CD90^+$) and alternatively activated or M2 macrophages ($CD14^+$ that express $CD206^+$ and/or $CD163^+$), rather than a single cell type, may be required to promote long-term tissue regeneration and repair [86, 87]. Aastrom Biosciences, Inc. manufactures ixmyelocel-T, an autologous multicellular therapy expanded from a patient's own bone marrow. Ixmyelocel-T is composed of a mixture of cell types that include those expected to be found in the BM-MNC population. These include myeloid cells (granulocytes, monocytes, and mixed myeloid progenitors) and lymphoid cells (T cells, B cells, and mixed lymphoid progenitors) that express CD45 on the cell surface, and $CD90^+$ MSCs/stromal cells, and $CD45^+CD14^+$ autofluorescent$^+$ ($CD14^+Auto^+$) macrophages. While the cell types are similar to those found in the BM-MNC population, the numbers of $CD90^+$ and $CD14^+Auto^+$ cells are significantly greater in ixmyelocel-T due to expansion during the manufacturing process. The manufacturing process and cell characterization of the product have been described previously [88, 89]. In in vitro studies it has been demonstrated that ixmyelocel-T produces anti-inflammatory cytokines which may aid in the healing process [90]. A Phase 2b clinical study (RESTORE-CLI) was successfully completed in 2011 [91], with results presented at the American Heart Association Scientific Sessions 2011 [92]. RESTORE-CLI was not powered to show statistical significance for efficacy endpoints; despite that limitation; however, there was a statistically significant difference in the time to first occurrence of treatment failure. The treatment failure composite, which consisted of major amputation of the index leg, all-cause mortality, doubling of wound total surface area from baseline and *de novo* gangrene can be considered a Phase 2 surrogate for the Phase 3 AFS (major amputation of the index leg, all-cause mortality) endpoint since tissue loss and gangrene are associated with higher rates of amputation and lower rates of survival [93]. Time to first occurrence of treatment failure is the earliest day at which any of the treatment failure events occurred. There was a 62 % risk reduction in treatment failure over the 12-month follow-up in the ixmyelocel-T group compared to the control group (hazard ratio 0.38, 95 % confidence interval=0.20 to 0.74). The individual components of the treatment failure composite endpoint all trended in the same direction, favoring ixmyelocel-T treatment, with the exception of all-cause mortality that was the same in both treatment groups. A pivotal Phase 3 clinical trial (REVIVE) is being conducted under a Special Protocol Assessment (SPA) approved by the FDA, and began screening patients in 2012.

Discussion

There is a large body of preclinical research demonstrating the efficacy of gene and cellular therapy in peripheral arterial disease including evidence for growth and transcription factors, monocytes, and mesenchymal stem cells. However, thus far, clinical investigations have remained trapped in earlier phase studies, with the exception of fibroblast growth factor which advanced to a large-scale Phase 3 clinical trial. The disappointing results of this trial as well as the mixed positive and negative results from early clinical trials in both gene and cellular therapy, the complexity of the stem cell field, and the changing regulatory landscape have contributed to both the perception and the reality of the slow progression of research into later phase clinical trials.

Among the complicating factors are differing composition and biologic activities within the field of candidate therapies in the gene and stem cell fields. Gene therapy, the delivery of a single gene to the ischemic tissue of interest, requires expression of resident cells at the right time and place for efficacy [94]. Promotion of a single gene expression may not address the complexity of the underlying disease. Cellular therapies with adult stem cells have either autologous or allogeneic sources of cells, as well as differences in cell types. Allogeneic

sources generally involve a single cell type such as MSCs. The expansion of the cells in vitro is essential for cost effectiveness and 'off the shelf' use. However, in vitro expansion may decrease the efficacy of the cells, causing dose to be an issue as well as engraftment potential of the cells. Repeat or multiple dosing may elicit an immune response. The requirements for follow-up of side effects and hurdles of regulatory oversight are more extensive for therapies using allogeneic cell sources. Autologous cell therapy can harvest single or multiple cell types. Using the patient's own cells has advantages for safety, the maintenance of potency, and the potential for long-term engraftment and clinical effects. Multiple cell types have the potential to deliver multiple mechanisms to address complex diseases. Autologous cell products have more complex manufacturing and logistical issues, but there is the advantage of strict quality control for manufactured products over point of care devices. Autologous cell therapy developed using bedside devices that concentrate a larger volume bone marrow aspirate for reinjection to ischemic tissue are not as strictly quality controlled, but as devices have lower regulatory hurdles compared with manufactured autologous cell therapies.

The traditional drug development pathway of pharmacokinetic and preclinical modeling does not always translate well for stem cell products. As a result, much of the traditional 'preclinical' work must be done and will continue to be done within the framework of clinical trials, while still demonstrating proof of concept with in vitro studies and applicable in vivo models. Therefore, it is vitally important to communicate and use standardized protocols for the evaluation of efficacy and safety in both preclinical and clinical evaluation of cellular products. Early clinical trials used measurements of blood flow as the primary efficacy measure (e.g., ABI or TcPO2); however, there is poor correlation between leg blood flow and functional disability in patients with PAD [95]. The influence of the requirement by FDA to use AFS as the primary efficacy measurement in later phase clinical trials for severe PAD is reflected in the primary efficacy measures listed on clinicaltrials.gov for device trials as well as trials being conducted outside the U.S.

The past 15 years of clinical trials in gene and cell therapy for PAD have provided important knowledge and insights in regenerative medicine for vascular disease. There has been critical movement towards standardized, quality controlled, good manufacturing processes and protocols for the isolation and reintroduction of cells [6]. There are currently only two Phase 3 trials currently recruiting patients for the treatment of severe PAD with sites in the U.S. [96], including Aastrom's trial with ixmyelocel-T, a multicellular expanded product, and a Harvest Technologies device trial using bedside concentration of BM-MNCs. Both of these trials use autologous bone marrow as the source of cells. In addition, a Phase 3 study is being planned for the evaluation of HGF [45]. The completion of these studies will add to the base of knowledge and provide new pieces of the regenerative medicine puzzle. Studies using allogeneic sources, including placental, cord blood, and endometrial tissue, remain in early-phase development.

References

1. FDA website accessed 03 October 2012: http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/default.htm.
2. Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *The New England Journal of Medicine, 285*, 1182–1186.
3. Dvorak, H., Orenstein, N., Carvallo, A., et al. (1979). Induction of a fibrin-gel investment: an early event in line 10 heptocarcinoma growth mediated by tumor-secreted products. *Journal of Immunology, 122*, 166–174.
4. Evans, M. J., & Kaufman, M. H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature, 292*, 154–156.
5. Martin, G. R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proceedings of the National Academy of Sciences, 78*, 7634–7638.
6. Brunt, K., Weisel, R., & Li, R.-K. (2012). Stem cells and regenerative medicine—future perspectives. *Canadian Journal of Physiology and Pharmacology, 90*, 327–335.
7. Norgren, L., Hiatt, W. R., Dormandy, J. A., Nehler, M. R., Harris, K. A., Fowkes, F. G., et al. (2007). Inter-society consensus for the management of peripheral arterial disease (TASC II). *European Journal of Vascular and Endovascular Surgery, 33*(Suppl 1), S1–S75.
8. Varu, V., Hogg, M., & Kibbe, M. (2010). Critical limb ischemia. *Journal of Vascular Surgery, 51*, 230–241.
9. Pipinos, I., Judge, A., Selsby, J., et al. (2008). The myopathy of peripheral arterial occlusive disease: part 1. Functional and histomorphological changes and evidence for mitochondrial dysfunction. *Vascular and Endovascular Surgery, 41*, 481–489.
10. Pipinos, I., Judge, A., Selsby, J., et al. (2008). The myopathy of peripheral arterial occlusive disease: part 2.Oxidative stress, neuropathy, and shift in muscle fiber type. *Vascular and Endovascular Surgery, 42*(2), 101–112.
11. Nicoloff, A. D., Taylor, L. M., Jr., McLafferty, R. B., Moneta, G. L., & Porter, J. M. (1998). Patient recovery after infrainguinal bypass grafting for limb salvage. *Journal of Vascular Surgery, 27*, 256–263.
12. Treiman, G. S., Copland, S., Yellin, A. E., et al. (2001). Wound infections involving infrainguinal autogenous vein grafts: a current evaluation of factors determining successful graft preservation. *Journal of Vascular Surgery, 33*, 948–954.
13. Tretinyak, A. S., Lee, E. S., Kuskowski, M. M., Caldwell, M. P., & Santilli, S. M. (2001). Revascularization and quality of life for patients with limb-threatening ischemia. *Annals of Vascular Surgery, 15*(1), 84–88.
14. Sprengers, R. W., Teraa, M., Moll, F. L., de Wit, G. A., van der Graaf, Y., Verhaar, M. C., et al. (2010). Quality of life in patients with no-option critical limb ischemia underlines the need for new effective treatment. *Journal of Vascular Surgery, 52*(4), 843–849.

15. Nehler, M. R., Hiatt, W. R., & Taylor, L. M. (2003). Is revascularization and limb salvage always the best treatment for critical limb ischemia? *Journal of Vascular Surgery, 37*(3), 704–708.
16. http://seer.cancer.gov/statfacts/html/prost.html (accessed 08 October 2012).
17. http://seer.cancer.gov/statfacts/html/breast.html (accessed 08 October 2012).
18. Kaul, P., Armstrong, P. W., Chang, W. C., et al. (2004). Long-term mortality of patients with acute myocardial infarction in the United States and Canada: comparison of patients enrolled in Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-I. *Circulation, 110*, 1754–1760.
19. http://seer.cancer.gov/statfacts/html/colorect.html (accessed 08 October 2012).
20. Hartmann, A., Rundek, T., Mast, H., et al. (2001). Mortality and causes of death after first ischemic stroke: the Northern Manhattan stroke study. *Neurology, 57*(11), 2000–2005.
21. Isner, J. M., Walsh, K., Symes, J., et al. (1995). Arterial gene therapy for therapeutic angiogenesis in patients with peripheral artery disease. *Circulation, 91*(11), 2687–2692.
22. Isner, J. M., Pieczek, A., Schainfeld, R., et al. (1996). Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet, 348*(9024), 370–374.
23. Ouma, G., Jonas, R., Haris, M., & Mohler, E. (2012). Targets and delivery methods for therapeutic angiogenesis in peripheral artery disease. *Vascular Medicine, 17*, 174–192.
24. Ferrara, N. (2005). VEGF as a therapeutic target in cancer. *Oncology, 69*(Suppl 3), 11–16.
25. Hudlicka, O., Milkiewicz, M., Cotter, M. A., & Brown, M. D. (2002). Hypoxia and expression of VEGF-A protein in relation to capillary growth in electrically stimulated rat and rabbit skeletal muscles. *Experimental Physiology, 87*, 373–381.
26. Shah, P. B., & Losordo, D. W. (2005). Non-viral vectors for gene therapy: clinical trials in cardiovascular disease. *Advances in Genetics, 54*, 339–361.
27. Shibuya, M., & Claesson-Welsh, L. (2006). Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Experimental Cell Research, 312*, 549–560.
28. van Wijngaarden, P., Coster, D. J., & Williams, K. A. (2005). Inhibitors of ocular neovascularization: promises and potential problems. *JAMA: The Journal of the American Medical Association, 293*, 1509–1513.
29. Ferrara, N., & Kerbel, R. S. (2005). Angiogenesis as a therapeutic target. *Nature, 438*, 967–974.
30. Hughes, G. C., & Annex, B. H. (2005). Angiogenic therapy for coronary artery and peripheral arterial disease. *Expert Review of Cardiovascular Therapy, 3*, 521–535.
31. Simons, M. (2005). Angiogenesis: where do we stand now? *Circulation, 111*, 1556–6.
32. Mäkinen, K., Manninen, H., Hedman, M., et al. (2002). 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. *Molecular Therapy, 6*, 127–133.
33. Rajagopalan, S., Mohler, E. R., 3rd, Lederman, R. J., et al. (2003). Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. *Circulation, 108*, 1933–1938.
34. Mathieu-Costello, O., Hoppeler, H., & Weibel, E. R. (1989). Capillary tortuosity in skeletal muscles of mammals depends on muscle contraction. *Journal of Applied Physiology, 66*, 1436–1442.
35. Lloyd, P. G., Prior, B. M., Yang, H. T., & Terjung, R. I. (2003). Angiogenic growth factor expression in rat skeletal muscle in response to exercise training. *American Journal of Physiology-Heart and Circulatory Physiology, 284*, H1668–H1678.
36. Nikol, S., Baumgartner, I., Van Belle, E., et al. (2008). Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critical limb ischemia. *Molecular Therapy, 16*, 972–978.
37. Belch, J., Hiatt, W., Baumgartner, I., et al. (2011). Effect of fibroblast growth factor NV1FGF on amputation and death: a randomized placebo-controlled trial of gene therapy in critical limb ischaemia. *Lancet, 377*, 1929–1937.
38. Mac Gabhann, F., Ji, J. W., & Popel, A. S. (2007). Multi-scale computational models of pro-angiogenic treatments in peripheral arterial disease. *Annals of Biomedical Engineering, 35*, 982–994.
39. Mac Gabhann, F., Ji, J. W., & Popel, A. S. (2007). VEGF gradients, receptor activation, and sprout guidance in resting and exercising skeletal muscle. *Journal of Applied Physiology, 102*, 722–734.
40. Morishita, R., Aoki, M., Yo, Y., & Ogihara, T. (2002). Hepatocyte growth factor as cardiovascular hormone: role of HGF in the pathogenesis of cardiovascular disease. *Endocrine Journal, 49*, 273–274.
41. Morishita, R., Nakamura, S., Hayashi, S., et al. (1999). Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. *Hypertension, 33*, 1379–1384.
42. Cowin, A. J., Kallincos, N., Hatzirodos, N., et al. (2001). Hepatocyte growth factor and macrophage-stimulating protein are upregulated during excisional wound repair in rats. *Cell and Tissue Research, 306*, 239–250.
43. Powell, R. J., Goodney, P., Mendelsohn, F. O., Moen, E. K., & Annex, B. H. (2010). HGF-0205 Trial Investigators. Safety and efficacy of patient specific intramuscular injection of HGF plasmid gene therapy on limb perfusion and wound healing in patients with ischemic lower extremity ulceration: results of the HGF-0205 trial. *Journal of Vascular Surgery, 52*(6), 1525–1530.
44. Powell, R. J., Simons, M., Mendelsohn, F. O., et al. (2008). 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, 118*(1), 58–65.
45. Powell, R. J. (2012). Update on clinical trials evaluating the effect of biologic therapy in patients with critical limb ischemia. *Journal of Vascular Surgery, 56*(1), 264–266.
46. Guillemin, K., & Krasnow, M. A. (1997). The hypoxic response: huffing and HIFing. *Cell, 89*, 9–12.
47. Wenger, R. H., & Gassmann, M. (1997). Oxygen(es) and the hypoxia-inducible factor-1. *Biological Chemistry, 378*, 609–616.
48. Wang, G. L., Jiang, B. H., Rue, E. A., & Semenza, G. L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proceedings of the National Academy of Sciences, 92*, 5510–5514.
49. Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J., & Whitelaw, M. L. (2002). Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science, 295*, 858–861.
50. Chang, E. I., Loh, S. A., Ceradini, D. J., et al. (2007). Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1alpha stabilization during ischemia. *Circulation, 116*, 2818–2829.
51. Rey, S., Lee, K., Wang, C. J., et al. (2009). Synergistic effect of HIF-1alpha gene therapy and HIF-1-activated bone marrow-derived angiogenic cells in a mouse model of limb ischemia. *Proceedings of the National Academy of Sciences, 106*, 20399–20404.
52. Huang, L. E., Arany, Z., Livingston, D. M., & Bunn, H. F. (1996). Activation of hypoxiainducible transcription factor depends

primarily upon redox-sensitive stabilization of its alpha subunit. *Journal of Biological Chemistry, 271*, 32253–32259.
53. Safran, M., & Kaelin, W. G., Jr. (2003). HIF hydroxylation and the mammalian oxygensensing pathway. *The Journal of Clinical Investigation, 111*, 779–783.
54. Pugh, C. W., & Ratcliffe, P. J. (2003). Regulation of angiogenesis by hypoxia: role of the HIF system. *Nature Medicine, 9*, 677–684.
55. Kaelin, W. G., Jr., & Ratcliffe, P. J. (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Molecular Cell, 30*, 393–402.
56. Vincent, K. A., Shyu, K. G., Luo, Y., et al. (2000). Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF- 1alpha/VP16 hybrid transcription factor. *Circulation, 102*, 2255–2261.
57. Sarkar, K., Fox-Talbot, K., Steenbergen, C., Bosch-Marce, M., & Semenza, G. L. (2009). Adenoviral transfer of HIF-1alpha enhances vascular responses to critical limb ischemia in diabetic mice. *Proceedings of the National Academy of Sciences, 106*, 18769–18774.
58. Hiasa, K., Ishibashi, M., Ohtani, K., et al. (2004). Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation, 109*(20), 2454–2461.
59. Segers, V. F., & Lee, R. T. (2010). Protein therapeutics for cardiac regeneration after myocardial infarction. *Journal of Cardiovascular Translational Research, 3*(5), 469–477.
60. Segers, V. F., Revin, V., Wu, W., et al. (2011). Protease-resistant stromal cell-derived factor-1 for the treatment of experimental peripheral artery disease. *Circulation, 123*(12), 1306–1315.
61. Murohara, T., Ikeda, H., Duan, J., et al. (2000). Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. *The Journal of Clinical Investigation, 105*, 1527–1536.
62. Crosby, J. R., Kaminski, W. E., Schatteman, G., et al. (2000). Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. *Circulation Research, 87*, 728–730.
63. Asahara, T., Masuda, H., Takahashi, T., et al. (1999). Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circulation Research, 85*, 221–228.
64. Capla, J. M., Ceradini, D. J., Tepper, O. M., et al. (2006). Skin graft vascularization involves precisely regulated regression and replacement of endothelial cells through both angiogenesis and vasculogenesis. *Plastic and Reconstructive Surgery, 117*, 836–844.
65. Shi, Q., Rafii, S., Wu, M. H., et al. (1998). Evidence for circulating bone marrow-derived endothelial cells. *Blood, 92*, 362–367.
66. Takahashi, T., Kalka, C., Masuda, H., et al. (1999). Ischemia-and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nature Medicine, 5*, 434–438.
67. Shintani, S., Murohara, T., Ikeda, H., et al. (2001). Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation, 103*, 897–903.
68. Kalka, C., Masuda, H., Takahashi, T., et al. (2000). Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proceedings of the National Academy of Sciences, 97*(7), 3422–3427.
69. Sprengers, R. W., Lips, D. J., Moll, F. L., & Verhaar, M. C. (2008). Progenitor cell therapy in patients with critical limb ischemia without surgical options. *Annals of Surgery, 247*, 411–420.
70. Lawall, H., Bramlage, P., & Amann, B. (2010). Stem cell and progenitor cell therapy in peripheral artery disease. *Thrombosis and Haemostasis, 103*, 696–709.
71. Friedenstein, A. J., Chailakhjan, R. K., & Lalykina, K. S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell and Tissue Kinetics, 3*, 393–403.
72. Piersma, A. H., Brockbank, K. G., Ploemacher, R. E., van Vliet, E., Brakel-van Peer, K. M., & Visser, P. J. (1985). Characterization of fibroblastic stromal cells from murine bone marrow. *Experimental Hematology, 13*, 237–243.
73. Caplan, A. I. (1986). Molecular and cellular differentiation of muscle, cartilage, and bone in the developing limb. *Progress in Clinical and Biological Research, 217B*, 307–318.
74. Pittenger, M. F., Mackay, A. M., Beck, S. C., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science, 284*, 143–147.
75. Honczarenko, M., Le, Y., Swierkowski, M., Ghiran, I., Glodek, A. M., & Silberstein, L. E. (2006). Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. *Stem Cells, 24*, 1030–1041.
76. Ji, J. F., He, B. P., Dheen, S. T., & Tay, S. S. (2004). Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells, 22*, 415–427.
77. Ponte, A. L., Marais, E., Gallay, N., et al. (2007). The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells, 25*, 1737–1745.
78. Ringe, J., Strassburg, S., Neumann, K., et al. (2007). Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2. *Journal of Cellular Biochemistry, 101*, 135–146.
79. Sordi, V., Malosio, M. L., Marchesi, F., et al. (2005). Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood, 106*, 419–427.
80. Zvaifler, N. J., Marinova-Mutafchieva, L., Adams, G., et al. (2000). Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Research, 2*(6), 477–488.
81. Erices, A., Conget, P., & Minguell, J. J. (2000). Mesenchymal progenitor cells in human umbilical cord blood. *British Journal of Haematology, 109*, 235–242.
82. Zuk, P. A., Zhu, M., Ashjian, P., et al. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cell, 13*(12), 4279–4295.
83. De Bari, C., Dell'Accio, F., Tylzanowski, P., & Luyten, F. P. (2001). Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis and Rheumatism, 44*, 1928–1942.
84. Barlow, S., Brooke, G., Chatterjee, K., et al. (2008). Comparison of human placenta-and bone marrow-derived multipotent mesenchymal stem cells. *Stem Cells and Development, 17*(6), 1095–1107.
85. Baksh, D., Yao, R., & Tuan, R. S. (2007). Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. *Stem Cells, 25*, 1384–1392.
86. van Weel, V., van Tongeren, R. B., van Hinsbergh, V. W., van Bockel, J. H., & Quax, P. H. (2008). Vascular growth in ischemic limbs: a review of mechanisms and possible therapeutic stimulation. *Annals of Vascular Surgery, 22*(4), 582–597.
87. Shireman, P. K. (2007). The chemokine system in arteriogenesis and hind limb ischemia. *Journal of Vascular Surgery, 45*(Suppl A), A48–A56.
88. Goltry, K, Hampson, B, Venturi, N, Bartel, R. (2009). Large-scale production of adult stem cells for clinical use. In: Lakshmipathy,

U, Chesnut, J. D., Thyagarajan, B., (editors). Emerging technology platforms for stem cells. John Wiley & Sons, Inc. p 153–68.
89. Bartel, R. L., Cramer, C., Ledford, K., et al. (2012). The Aastrom experience. *Stem Cell Research & Therapy, 3*(4), 26.
90. Ledford, K. J., Murphy, E. N., Zeigler, F., Bartel, R. L. (2012). Evidence for the beneficial effects of ixmyelocel-T in the treatment of critical limb ischemia. Arteriosclerosis, Thrombosis, and Vascular Biology. April.
91. Powell, R. J., Marston, W. A., Berceli, S. A., et al. (2012). Cellular therapy with Ixmyelocel-T to treat critical limb ischemia: the randomized, double-blind, placebo-controlled RESTORE-CLI trial. *Molecular Therapy, 20*(6), 1280–1286.
92. http://circ.ahajournals.org/cgi/content/meeting_abstract/124/21_MeetingAbstracts/A8547?sid= ba1839f3-67aa-4e92-838e-e72a7da27a92 (accessed 08 October 2012).
93. CLI Performance Goals Work Group web site. Available from: http://www.criticallimb.org/ (accessed 08 October 2012).
94. Ruponen, M., Hyvonen, Z., Urtti, A., & Yia-Hertuala, S. (2005). Nonviral gene delivery methods in cardiovascular diseases. *Methods in Molecular Medicine, 108*, 315–328.
95. Pernow, B., & Zetterquist, S. (1968). Metabolic evaluation of the leg blood flow in claudicating patients with arterial obstructions at different levels. *Scandinavian Journal of Clinical & Laboratory Investigation, 21*(3), 277–287.
96. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 [08 October 2012]. Available from: http://clinicaltrials.gov/ct2/results?term=critical+limb+ischemia&recr=Open&no_unk=Y&rslt=&type=&cond=&intr=stem+cells&outc=&spons=&lead=&id=&state1=&cntry1=&state2=&cntry2=&state3=&cntry3=&locn=&gndr=&phase=2&rcv_s=&rcv_e=&lup_s=&lup_e=.
97. Benoit, E., O'Donnell, T. F. Jr., Iafrati, M. D., et al. (2011). The role of amputation as an outcome measure in cellular therapy for critical limb ischemia: implications for clinical trial design. *Journal of Translational Medicine,* 9, 165.
98. Kusumanto, Y. H., van Weel, V., Mulder, N. H., et al. (2006). 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. *Human Gene Therapy, 17*(6), 683–691.
99. Creager, M. A., Olin, J. W., Belch, J. J., et al. (2011). Effect of hypoxia-inducible factor-1alpha gene therapy on walking performance in patients with intermittent claudication. *Circulation, 124* (16), 1765–1773.
100. Sprengers, R. W., Moll, F. L., Teraa, M., Verhaar, M. C., JUVENTAS Study Group. (2010). Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with critical limb ischemia. *Journal of Vascular Surgery, 51*(6), 1564–1568.
101. Tateishi-Yuyama, E., Matsubara, H., Murohara, T., et al. (2002). Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet, 360* (9331), 427–435.
102. Walter, D. H., Krankenberg, H., Balzer, J. O., et al. (2011). Intra-arterial administration of bone marrow mononuclear cells in patients with critical limb ischemia: a randomized-start, placebo-controlled pilot trial (PROVASA). *Circulation: Cardiovascular Intervention, 4*(1), 26–37.
103. Losordo, D. (2010). Randomized double-blind, placebo controlled trial of autologous cd34+ cell therapy for critical limb ischemia: 1 year results. *Circulation, 122*, A16920.
104. Murphy, M. P., Lawson, J. H., Rapp, B. M., et al. (2011). Autologous bone marrow mononuclear cell therapy is safe and promotes amputation free survival in patients with critical limb ischemia. *Journal of Vascular Surgery, 53*(6), 1565–1574.