



Autologous CD34+ Cell Therapy for Ischemic Tissue Repair

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In 1997, the seminal manuscript by Asahara, Murohara, Isner et al outlined the evidence for the existence of circulating, bone marrow-derived cells capable of stimulating and contributing to the formation of new blood vessels. Consistent with the paradigm shift that this work represented, it triggered much scientific debate and controversy, some of which persists 2 decades later. In contrast, the clinical application of autologous CD34 cell therapy has been marked by a track record of consistent safety and clinical benefit in multiple ischemic conditions. In this review, we summarize the preclinical and clinical evidence from over 700 patients in clinical trials of CD34 cell therapy.

Key Words: Cell therapy; Critical limb ischemia; Endothelial progenitor cells; Ischemic tissue repair; Refractory angina

Discovery of CD34 Cells as Endothelial Progenitors

The CD34 cell surface antigen was first identified with monoclonal antibodies targeted to hematopoietic progenitor cells.¹ Early on, CD34+ cells were shown to effectively reconstitute the hematopoietic system of lethally irradiated baboons² and rhesus monkeys,³ providing evidence that CD34+ cells were indeed multipotent stem cells; however, the domain of their effect was thought to be restricted to the hematopoietic system.

That all changed in 1997 when Asahara, Murohara, and Isner et al published a ground-breaking report providing evidence that adult human CD34+ cells, well established as hematopoietic stem cells, also harbored the capability to differentiate into endothelial lineage cells.⁴ In vitro, CD34+ cells were shown to be null for expression of endothelial markers initially, but under specific culture conditions, typical markers of endothelial lineage were expressed. More notable was the in vivo evidence that human CD34 cells naturally migrated to areas of ischemic injury and preserved or restored microcirculatory integrity. Thus began the age of the endothelial progenitor cell, or EPC, as it is now well known in the cardiovascular lexicon. Subsequent work in multiple laboratories corroborated the existence of EPCs and further elucidated their function (reviewed in Rafi⁵). The most convincing evidence documenting the existence of adult vasculogenesis has come from animal and human studies in the setting of allogeneic bone marrow and organ transplantation, yielding irrefutable genetic verification of vascular endothelium of bone marrow origin.^{6–10}

Natural Human Evidence for the Role of CD34 Cells in Ischemic Repair

With documentation of the EPC contribution to the maintenance of vascular integrity provided by animal and human transplantation studies, a logical question is the role of progenitor cells in the setting of tissue injury and the specific identity of the precursors that effect repair. Shintani et al showed that CD34+ cell counts were significantly increased in patients following myocardial infarction,¹¹ and Werner et al extended those findings, showing that cardiovascular outcomes in patients following myocardial infarction were improved in patients who mobilized CD34 cells most efficiently.¹² These data suggested that the CD34+ cell was a naturally preprogrammed vascular repair cell, which ignited interest in the potential use of these cells in a therapeutic context.

Preclinical Evidence

Since the original report by Asahara et al, a large number of animal studies investigating the potential therapeutic utility of CD34 cell therapy for ischemic tissue repair have been published (summarized in **Tables 1–3**) (reviewed in Mackie and Losordo¹³) and we highlight a small percentage of the studies here. These studies document the natural ability of CD34 cells to replenish damaged microvasculature in ischemic tissue (**Figure 1**).

Hindlimb Ischemia

In 2000, Murohara et al showed that cord blood-derived CD34+ cells augmented perfusion and contributed to neovascularization in a murine hindlimb ischemia model,¹⁴ and the following year Kocher et al documented the

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Table 1. Preclinical Studies of CD34 Therapy for Ischemic Tissue Repair: Myocardial Infarction

Reference	Objective(s)	Treatment groups	Cell no.	ROA	Outcomes
Kocher et al ¹⁵	Examine effects of G-CSF-mobilized human CD34+ cells in a rat model of MI	CD34+ CD34– Saphenous vein ECs Saline	2×10 ⁶	IV	↑Neoangiogenesis in the infarct vascular bed ↓Myocyte apoptosis ↓Collagen deposition ↓Scar formation
Kawamoto et al ²²	Investigate whether catheter-based, IMC transplantation of CD34+ cells can enhance neovascularization in MI	CD34+ CD34– PBS	5×10 ⁵	IMC	↑Neovascularization ↓Myocardial fibrosis ↑LV function
Yeh et al ²¹	Investigate whether CD34+ cells can transdifferentiate into cardiomyocytes, endothelial and/or smooth muscle cells	CD34+	2.5×10 ⁶	IV or intraventricular	Confirmed transdifferentiation of CD34+ cells into cardiomyocytes, mature endothelial cells, and smooth muscle cells in vivo
Botta et al ⁴²	Compare CD34+KDR+ cells with CD34+KDR– cells in a mouse model of cardiac ischemia	CD34+ Unseparated MNCs PBS	2×10 ⁵ 2×10 ³	IMC	↑Cardiac function after MI with CD34+ cells ↑Cardiac hemodynamics with CD34+KDR+
Brenner et al ⁴³	Examine myocardial homing in infarcted and normal hearts	Infarcted or sham-operated	Not reported	Intracavitary-left ventricle	↑Homing of CD34+ cells to infarcted tissue with MI CD34+ cells are also found in lung, liver, kidneys, and spleen
Ott et al ⁴⁴	To investigate the therapeutic potential of CD34+ cells in nude rats with MI		1×10 ⁶	IMC	↑LV function
Yoshioka et al ²³	To track the fate of transplanted cells in a model of AMI	CD34+ PBS	1.2±0.73×10 ⁶	IC	↑Regional blood flow ↑Cardiac function
Iwasaki et al ⁴⁵	Examine multilineage capacity of CD34+ cells derived via mobilization and apheresis from human subjects with lower limb ischemia	Low CD34+ Medium CD34+ High CD34+ PBS	1×10 ³ 1×10 ⁵ 5×10 ⁵	IMC	Dose-dependent improvements: ↑Cardiomyocytes in ischemic myocardium ↑Vasculogenesis
Kawamoto et al ²²	Compare the therapeutic potential of CD34+ cells with MNCs for the preservation/recovery of myocardial tissue after MI	CD34+ Low-dose MNC High-dose MNC PBS	5×10 ⁵ 5×10 ⁵ With 5×10 ⁵ CD34+	IMC	↑Myocardial integrity ↑Function
Shintani et al ⁴⁶	Examine the effects of combining human CD34+ cell treatment with local VEGF2 gene therapy in MI	CD34–/empty vector CD34+ VEGF2 CD34+/VEGF2		Human CD34+ cells; IMC	↑Fractional shortening ↑Capillary density ↓Infarct size
Zhang et al ⁴⁷	Examine effect of CD34+ cells in a swine model of MI	CD34+ PBS	5×10 ⁷	ICN	↑Cardiac repairs after MI in swine with preexisting coronary collateral vessels
Iwasaki et al ⁴⁸	Examine effect of CD34+ cells on collateral microvessel development	CD34+ CD34– PBS	1×10 ⁵	IMC	↑Therapeutic benefits in MI
Wang et al ⁴⁹	Determine fate and function of CD34+ cells in SCID mice after experimental MI	CD34+	1×10 ⁶	IMC	↑Function resulting from angiogenesis and/or paracrine effect, but not myogenesis
Mackie et al ¹⁸	Explore whether the therapeutic efficacy of CD34+ cells is enhanced with SHH in NOD-SCID mice with MI	Low CD34+ High CD34+ Low CD34+/empty Low CD34+/SHH Low CD34+/SHH (200 ng) Saline	Low - 2.5×10 ⁴ High - 5×10 ⁴	IMC	↓Ventricular dilation ↓Cardiac function decline ↓Infarct size ↑Capillary density compared with unmodified CD34+ cells
Joladarashi et al ⁵⁰	Determine the role of microRNA on CD34+ cell function in cardiac remodeling	CD34+/miR-377 ctl CD34+/miR-377	5×10 ⁴	IMC	Silencing of miR-377 resulted in: ↑Angiogenesis ↓LV remodeling ↓Cardiac fibrosis

AMI, acute myocardial infarction; ECs, endothelial cells; IC, intracardiac; ICN, intracoronary; IMC, intramyocardial; IV, intravenous; LV, left ventricular; MI, myocardial infarction; MNCs, mononuclear cells; PBS, phosphate buffered saline; ROA, route of administration; SHH, sonic hedgehog; VEGF, vascular endothelial growth factor.

Table 2. Preclinical Studies of CD34 Therapy for Repair: Hindlimb Ischemia

Reference	Objective(s)	Treatment groups	Cell no.	ROA	Outcomes
Kanaya et al ¹⁷	Evaluate the possible effects of SHH on the vasculogenic effects of CD34+ cells in nude mice with HLI	Control CD34+ CD34+/SHH	1×10 ⁵	IM	↑Perfusion ratio with SHH
Du et al ⁵¹	Examine the effect of DFO on CD34+ cell targeting and neovascularization in nude mice with HLI	Control CD34+ CD34+/DFO	2×10 ⁵	IA	↑Vasculogenesis ↑Angiogenesis ↑Revascularization
Matsumura et al ⁵²	Determine effects of statins on angiogenesis	Atorvastatin Vehicle	—	—	↑Angiogenic cytokines ↑HO-1 ↑Number of CD34+ EPCs
Zhou et al ⁵³	Determine therapeutic efficacy of PBMNCs with and without CD34+ cell depletion in HLI	PBMNCs CD34-depleted PBMNCs PBS	1×10 ⁶	IM	↑Capillary density ↑Perfusion ↑VEGF Loss of effect with CD34 depletion
Madeddu et al ¹⁹	Examine the angiogenic effects of CD34+KDR+ cells vs. CD34+KDR− cells and placebo in SCID mice with HLI	CD34+KDR+ 1×10 ³ CD34+KDR+ 2×10 ³ CD34+KDR− 1×10 ⁴ Saline control	1×10 ³ 2×10 ³ 1×10 ⁴	IM	↑Limb salvage ↑Hemodynamic recovery Neovascularization induced by KDR+ cells was superior to that promoted by KDR− cells
Schatteman et al ¹⁶	Examine the ability of angioblast-containing CD34+ cells to restore blood flow in nondiabetic and diabetic mice with HLI	Vehicle control CD34+ CD34−	2.5–5.0×10 ⁵	IM	↑Blood flow in diabetic mice compared to nondiabetic mice

DFO, dextranferrioxamine; HLI, hindlimb ischemia; IA, intraarterial; IM, intramuscular; PBMNCs, peripheral blood mononuclear cells. Other abbreviations as in Table 1.

Table 3. Preclinical Studies of CD34 Therapy for Repair: Cerebral Ischemia/Stroke

Reference	Objective(s)	Treatment groups	Cell no.	ROA	Outcomes
Shyu et al ²⁵	Investigate effects of intracerebral administration of PB–CD34+ cells on chronic stroke in Sprague-Dawley rats	CD34+ Vehicle	2×10 ⁵	ICB	↑Neurological function
Taguchi et al ²⁴ (2004)	Determine whether CD34+ cells improve neovascularization of cerebral tissue following induction of stroke in SCID mice	Stroke model CD34+ CD34− Saline	5×10 ⁵	IV	↑Neovascularization of the ischemic zone

ICB, intracerebral; PB, peripheral blood. Other abbreviations as in Tables 1,2.

therapeutic efficacy of human CD34+ cells for treatment of myocardial infarction.¹⁵ Furthermore, treatment with CD34+ cells was able to restore blood flow in diabetic mice with hindlimb ischemia. Labeled CD34+ cells that had been transplanted were observed by histological analysis to have localized to the regenerating muscle.¹⁶

A more recent study by Kanaya et al evaluated the possible effects of sonic hedgehog (SHH) on the vasculogenic effects of CD34+-derived EPCs. SHH signaling regulates human CD34+ cell fate and function and may potentiate the therapeutic effect of granulocyte colony stimulating factor (G-CSF)-mobilized CD34+ cells on ischemic diseases. G-CSF-mobilized human peripheral blood CD34+ cells were injected intramuscularly to mice with severe hindlimb ischemia. Blood perfusion recovery, as well as capillary density, was significantly increased with administration of SHH-treated CD34+ cells compared with CD34+ cells alone, suggesting that SHH may be able to enhance the therapeutic potential of CD34+ cells.¹⁷ This corroborates an earlier report by Mackie et al.¹⁸

The use of subsets of CD34+ cells that are expressing a

combination of other endothelial markers has also been examined as a possible therapy for regenerating muscle. It has been shown that the concomitant expression of VEGF-receptor 2 (kinase domain receptor [KDR]) on CD34+ cells improved limb salvage and hemodynamic recovery, and that the neovascularization induced by KDR+ cells was superior to that promoted by KDR− cells.¹⁹

Taken together, CD34+ cell therapy effectively improves blood perfusion and angiogenesis in models of hindlimb ischemia, and optimization by combination gene therapy or subset selection of CD34+ cells may enhance the recovery response even further.

Myocardial Infarction

The first evaluation of therapeutic cardiac neovascularization with CD34+ cells used athymic nude rats to show that a single systemic injection of human CD34+ cells after induction of an acute myocardial infarction (AMI) resulted in increased capillary density, increased blood flow because of CD34+ cell-induced angiogenesis and vasculogenesis in the infarct zones. This led to better cardiac function and

CD34+ cells promote angiogenesis of the microvasculature

- CD34+ cells naturally reside in the bone marrow
- Induce capillary growth to regenerate damaged microcirculation

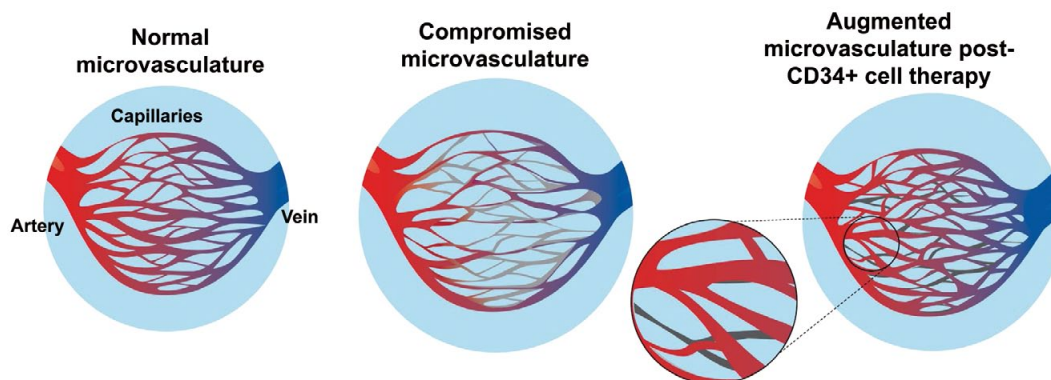


Figure 1. CD34+ cells promote angiogenesis of the microvasculature.

reduced infarct size, collagen deposition, and apoptosis of cardiomyocytes.¹⁵ The findings were specific to CD34+ cells, because neither the CD34- cell fraction nor fully differentiated endothelial cells was capable of salvaging the infarcted tissue to the same extent.

Kawamoto et al evaluated direct injection of human CD34+ cells into the ischemic zone in a nude-rat model of AMI and evaluated measurable factors associated with cardiac function.²⁰ Histologically, the results indicated that animals in receipt of CD34+ cells showed a marked increase in capillary density, accompanied by a substantial decrease in the amount of fibrosis associated with the infarct. Histological analysis also revealed that CD34+ cells had integrated into foci of neovascularization located within the peri-infarct zone where they expressed UAE-1 lectin, a marker of mature human endothelial cells, suggestive of differentiation into endothelial cells. This is in agreement with data from Yeh et al, who documented the transdifferentiation of human CD34+ cells into endothelial cells and also revealed that the migration and retention of CD34+ cells into myocardium occurred almost exclusively in the setting of ischemia.²¹

Furthermore, the therapeutic efficacy of mobilized, circulating human CD34+ cells has been compared with that of unselected total mononuclear cell fractions (MNCs) in a rat MI model.²² Three treatment groups were compared: (1) low-dose selected CD34+ cell group; (2) low-dose MNC cell group; and (3) high-dose MNC group, which contained the same absolute CD34+ cell dose as did group 1. Despite receiving the same absolute number of CD34+ cells, the high-MNC treatment group resulted in increased hemorrhagic MI as evaluated on postsurgical day 3. Tissue staining at that time point indicated an abundance of both hematopoietic and inflammatory cells derived from the xenotransplantation that were not found in the CD34+ cell group, which suggested that the total MNCs were responsible. The CD34+ cell group showed the greatest attenuation of structural changes attributable to the infarct, with the high-dose total MNC group showing an intermediate phenotype when compared with low-dose

total MNC or saline treatment.²²

The conclusions drawn from the studies described agree with those from a nonhuman primate study that also evaluated the therapeutic efficacy of locally injected human CD34+ cells after AMI.²³ Those authors showed that macaques that received intracardiac CD34+ cells showed improvements in regional blood flow and fractional shortening when compared with a saline-treated group.

Cerebral Ischemia/Stroke

Vascularization, such as that induced by CD34+ cells, is necessary for recovery after stroke. Taguchi et al were the first to use CD34+ cells isolated from human umbilical cord blood (hUCB) to treat both young and aged mice subjected to permanent cerebral ischemia induced by ligation of the left middle cerebral artery. Purified CD34+ cells were administered intravenously 48 h after the induction of stroke. Results demonstrated that neovascularization occurred in the treated animals compared with the mice treated with CD34- cells, and that this blood vessel growth was required for endogenous neurogenesis. The induction of neuronal outgrowth after cerebral ischemia requires a supportive network of new vessels, and CD34+ cell therapy demonstrated a crucial role in recovery from cerebral ischemia.²⁴

In a separate rat model of chronic stroke, intracerebral injection of CD34+ cells derived from human peripheral blood induced angiogenesis and the injected cells expressed neuronal cell type markers, suggesting that the cells could differentiate to endothelial cells as well as playing an active role in neurogenesis. Functional benefits on neurological behavior were also observed between 14 and 28 days after treatment, including grip strength, body asymmetry, and locomotor activity.²⁵ Additional applications of CD34+ cell therapy for cerebral ischemia were explored by Tsuji et al in a report that showed beneficial effects of intravenous administration of hUCB-CD34+ cells for neonatal stroke, including improved blood flow to the infarcted area and reduced loss of ipsilateral hemispheric volume.²⁶

Table 4. Clinical Studies of CD34 Therapy for Ischemic Tissue Repair: Critical Limb Ischemia

Reference	Condition	Patients (n)	Study design	ROA	Cell no.	Follow-up (months)	Outcomes
Kawamoto et al ²⁸ Kinoshita et al ²⁹ (follow-up)	PAD CLI	5 12	Single blind, dose escalation trial	IM	1×10 ⁵ cells/kg 5×10 ⁵ cells/kg 1×10 ⁶ cells/kg	36–48	↑Toe brachial pressure index ↓Pain rating scale ↑Walking distance (Trends)
Losordo et al ³¹	CLI	7 9 12	Randomized, controlled trial	IM	Placebo 1×10 ⁵ cells/kg 1×10 ⁶ cells/kg	6–12	↓Amputation incidence
Fujita et al ³⁰	CLI	11	Open-label, uncontrolled	IM	6.3±5.1×10 ⁷	3–6	↓Rest pain scales ↑Physiological parameters ↑Functional parameters (TBPI, SPP, TcPO ₂ , TWD and PFWD) ↓Clinical severity of limb ischemia

CLI, critical limb ischemia; PAD, peripheral artery disease. Other abbreviations as in Tables 1,2.

Clinical Evidence

Following on from the robust preclinical evidence supporting the safety and efficacy of CD34 cells for treatment of ischemic limbs and myocardium, human clinical trials began in an attempt to leverage the innate capabilities of CD34+ cells in the setting of otherwise untreatable ischemic conditions. Here, we highlight the major milestones in the use of CD34 cell therapy in humans. A comprehensive summary of clinical studies performed for the treatment of ischemic tissue repair is presented in **Tables 4–6**. In all studies CD34 cells were mobilized with GCSF, collected via apheresis and purified using an antibody-coated magnetic bead sorting technique (**Figure 2**).

Critical Limb Ischemia

Inaba et al were the first to report selection and clinical usage of CD34+ cells from peripheral blood using magnetic separation. Five patients with ischemic limbs caused by atherosclerotic occlusion received intramuscular injections of autologous CD34+ cells, with a mean purity of 40.6%. The reported outcomes include amelioration of pain at 3 days post-injection and increased walking distance 1 week following treatment, benefits that were sustained for over 1 year.²⁷ This study indicates the utility of peripheral blood-derived CD34+ cells in angiogenesis.

A phase I/IIa clinical trial in 2009 by Kawamoto et al used autologous CD34+ cells to treat patients with CLI caused by peripheral artery disease or Buerger's disease. The results revealed improved walking distance, which was the primary endpoint, as well as improved pain rating, toe brachial pressure index, transcutaneous partial oxygen pressure, and decreased ulcer size at 12-weeks post-transplantation.²⁸ A follow-up study reported by Kinoshita et al in 2012 demonstrated a statistically significant improvement of these parameters at 12 months after treatment, with improvements to transcutaneous partial oxygen pressure and toe brachial pressure index that were sustained for 3 and 4 years, respectively.²⁹ Furthermore, over the course of 4 years, no major amputations occurred and the number of deaths was 1, 3, and 1 by years 2, 3, and 4, respectively. These clinical and physiological results indicated not only the safety and feasibility, but also the significant benefits of GCSF-mobilized CD34+ treatment in this patient population.

Expanding on this study, and to determine endpoint

selection and timing, Fujita et al performed a phase II clinical trial that included 11 no-option CLI patients.³⁰ Results revealed statistically significant improvements in rest pain scales, skin perfusion pressure, transcutaneous partial oxygen pressure, and pain-free walking distance just 2 weeks after treatment. Total walking distance and the toe brachial pressure index were significantly improved at 8 and 12 weeks, respectively. Taken together, these data indicate the safety of the cell therapy, as well as suitable endpoints for no-option CLI patients, and warrant additional later-phase clinical trials.

The first randomized, double-blind, placebo-controlled pilot study was performed in 2012, and followed 28 patients over the course of 12 months. Patients who were not suitable candidates for surgical options received low- or high-dose autologous CD34+ cells or placebo. A favorable and dose-dependent trend was observed in amputation-free survival at 6 months, and this trend was sustained at 12 months post-treatment. Furthermore, no adverse effects were observed after the administration of CD34+ cells.³¹

An additional study to assess the safety and potential bioactivity of CD34 cell therapy on neovascularization and symptom relief for patients with severe intermittent claudication has demonstrated consistent results.³² This double-blind, randomized, placebo-controlled pilot trial included 17 patients across 3 treatment groups: placebo, and low- and high-dose CD34+ cells. Initial results revealed improved quality of life and trends towards increased walking time, which are supportive of the safety of intramuscular autologous CD34 cell therapy for severe intermittent claudication.

Myocardial Ischemia

Refractory Angina CD34+ cell therapy has now been evaluated in multiple double-blind, randomized placebo-controlled clinical trials in “no option, refractory, disabling angina” (now referred to as NORDA³³) patients.

The 24 patients with NORDA were enrolled in a randomized, double-blind, placebo-controlled phase 1/2a clinical trial of intramyocardial, autologous, GCSF-mobilized CD34 cell therapy.³⁴ With the aid of a NOGA™ electro-mechanical mapping system, the CD34+ cells were locally injected intramuscularly into the ischemic myocardium. Observations indicated improvements in frequency of angina, use of nitroglycerin, exercise tolerance, and ranking

Table 5. Clinical Studies of CD34 Therapy for Ischemic Tissue Repair: Myocardial Ischemia/Infarction, RFA

Reference	Condition	Patients (n)	Study design	ROA	Cell no.	Follow-up (months)	Outcomes
Vrtovec et al ⁵⁴	DCM	28	Randomized, open-label study	IC	123±23×10 ⁶	12	↑LVEF ↑Walk test ↓NT-proBNP ↓1-year death or heart transplantation
Vrtovec et al ⁵⁵	DCM	20 20	Prospective, randomized study	IC IMC	103±27×10 ⁶ 105±31×10 ⁶	6	↑LVEF ↑Walk test distance ↓NT-proBNP in the transendocardial group compared with intracoronary
Vrtovec et al ⁵⁶	DCM	55	Prospective, randomized study	IC	113±26×10 ⁶	60	↑LVEF ↑Walk test ↓In NT-proBNP ↓Death
Lezaic et al ⁵⁷	DCM	21	Single-arm unblinded study	IC	123±53×10 ⁶ (range 54–284×10 ⁶)	6	↑Resting myocardial perfusion ↑LVEF ↑Walk test
Musialek et al ⁵⁸	MI	21 13	Randomized, controlled study	TC-perfusion TC-stop-flow	4.34×10 ⁶ (range 0.92×10 ⁶ –7.54×10 ⁶)	<1	Preferential CD34+ homing in the viable peri-infarct zone
Quyyumi et al ⁵⁹	STEMI		Randomized, controlled study	IA	Low - 5×10 ⁶ Mid - 10×10 ⁶ High - 15×10 ⁶	6	↑Infarct region perfusion ↑LVEF (trend) ↓Incidence of decreased LVEF
Quyyumi et al (2017) ³⁹	STEMI	78	Randomized, controlled study	IC	14.9±8×10 ⁶	18 (median)	↑Mean LVEF change (dose-dependent)
Wang et al ⁶⁰	Intractable angina	56	Double-blind, controlled study	IC	5.6±2.3×10 ⁷	3–6	↓Weekly angina episodes ↓Nitroglycerine usage ↑Exercise time ↑Myocardial perfusion
Losordo et al ³⁵	RFA	56 56	Prospective, double-blind, randomized, phase 2 study	IMC	Low - 1×10 ⁵ cells/kg High - 5×10 ⁵ cells/kg	12	↓Weekly angina ↑Exercise tolerance ↓Death at 12 months
Lee et al ⁶¹	CAD	18 18	Randomized, double-blind study	IC	Low - 1×10 ⁷ High - 3×10 ⁷	9–18.5	↑LVEF (both groups) ↑Neovascularization ↓Angina and heart failure No deaths

CAD, coronary artery disease; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; RFA, refractory angina; TC, transcatheter. Other abbreviations as in Tables 1,2.

on the Canadian Cardiovascular Society (CCS) angina classification scale. This first-in-human study provided initial evidence of safety and bioactivity of this approach and led to a subsequent 167-patient study.

The prospective, double-blind, randomized, phase II ACT34-CMI study enrolled 168 NORDA patients³⁵. Subjects treated with an intramyocardial injection of autologous CD34+ cell therapy experienced greater decreases in the frequency of angina, and significant improvements in exercise tolerance after 6 months, which continued to improve at 12 months post-treatment. Further improvements that were observed in the low-dose group compared with controls included: time to onset of angina, nitroglycerine use, and assessment of quality of life and CCS classification. A second year of follow-up was conducted, providing evidence that a single administration of CD34 cells resulted in reduced angina frequency for at least 2 years, together with a significant reduction in deaths and favorable trends toward reduced major adverse cardiac events.³⁶ These findings, providing evidence for efficacy and enhanced safety in the severely ill NORDA patient population following intramyocardial injection of GCSF-mobilized autologous CD34+ cell therapy, indicated the need for further development to address the unmet need in

this patient population.

The randomized, double-blind, phase III RENEW study provided additional evidence for the improved safety and function in refractory angina patients treated with autologous CD34+ cell therapy.^{37,38} Although this study was terminated prematurely by the sponsor for financial reasons, the data from the partial study are consistent with prior studies showing reduced mortality rates and improved exercise tolerance in treated vs. control subjects.

Myocardial Infarction The PreSERVE-AMI was the largest clinical trial using CD34 cell therapy for left ventricular (LV) dysfunction post-ST-segment-elevation MI (STEMI). This randomized, double-blind, placebo-controlled phase II trial enrolled 161 patients, of whom 78 received autologous CD34+ cells and 83 received vehicle control treatment by intracoronary infusion.³⁹ After 12 months, treated subjects experienced 0% mortality vs. 3.6% of the control subjects. In fact, adjusting for total ischemic time resulted in a statistically significant increase in number of days alive and out of hospital when patients received CD34+ cell therapy. Similarly, whereas both the control and cell therapy groups demonstrated similar efficacy after 6 months, an adjustment for total ischemic time revealed a significant association between cell dose and change in

Table 6. Clinical Studies of CD34 Therapy for Ischemic Tissue Repair: Cerebral Ischemia/Stroke

Reference	Condition	Patients (n)	Study design	ROA	Cell no.	Follow-up (months)	Outcomes
Taguchi et al ⁶²	Stroke	6 - Low dose 6 - High dose	Non-randomized, open label	IV	Low: 2.5×10^8 High: 3.4×10^8 (mean) CD34+ 4.4×10^6	6	↑Neurological outcomes (trend/high dose) ↑Cerebral blood flow ↑O ₂ consumption rate
Banerjee et al ⁴⁰	Stroke	5	Non-randomized, open label	IA	$1.2\text{--}2.8 \times 10^6$	6	↑Clinical functional scores (modified Rankin Score and NIHSS score) ↓Lesion volume
Chen et al ⁴¹	Middle cerebral artery infarction	15	Single-blind, case controlled	IC	$3\text{--}8 \times 10^6$	12	↑Stroke scales (NIHSS, ESS, and EMS) ↑Function (modified Rankin Score) ↑Motor-evoked potential response ↓Fiber numbers asymmetry

EMS, ESS-Motor Subscale; ESS, European Stroke Scale; NIHSS, National Institutes of Health Stroke Scale. Other abbreviations as in Tables 1,2.

Simple, rapid, scalable and economical CD34 therapy process

- GCSF mobilizes CD34 cells from bone marrow
- Four days or less from donation to treatment

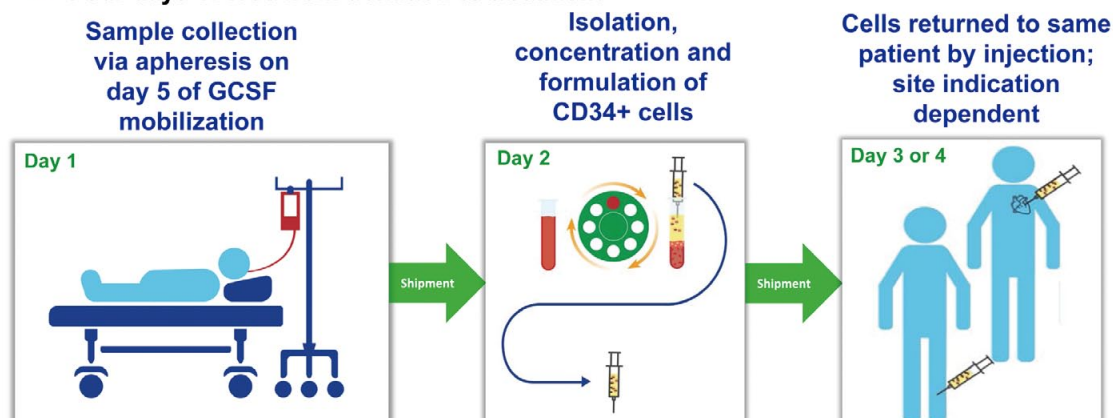


Figure 2. Simple, rapid, scalable and economical CD34 therapy process. GCSF, granulocyte colony stimulating factor.

LVEF as well as infarct size. To summarize, the PreSERVE-AMI study provides further evidence of the safety and potential for efficacy for intracoronary infusion of CD34+ cells after STEMI.

Stroke

Using enriched populations of CD34+ cells for treatment of stroke in humans is a more recent undertaking, with promising findings. The first report of using enriched CD34+ cells to treat stroke in humans was in patients with acute ischemic stroke in 2014.⁴⁰ CD34+ cells were administered within 7 days of stroke onset by intraarterial injection into the infarct territory; 5 patients were treated, and the treatment was well tolerated. All patients showed improvements in modified Rankin Score and National Institutes of Health Stroke Scale (NIHSS) score during the 6-month follow-up period. Also in 2014, a randomized, single-blind controlled trial was reported that included

patients with a middle cerebral artery infarct. CD34+ cells with 87–97% purity were administered by stereotactic implantation to 15 patients, ≥6 months after stroke onset. The treatment was well tolerated, and treated patients experienced significantly greater improvement in NIHSS, European Stroke Scale (ESS), and the ESS motor subscale compared with the control group 6 to 12 months after treatment.⁴¹

Conclusions

The CD34+ cell is a naturally occurring vascular repair cell. Natural history studies indicate that patients who mobilize CD34+ cells efficiently have improved outcomes after ischemic events, and human sex-mismatched transplant studies provide irrefutable evidence for the contribution of bone marrow in the formation of new endothelium. Preclinical studies in multiple species and ischemic models

provide further evidence of the ability of CD34+ cells to stimulation neovascularization and improve perfusion and function in tissues damaged by acute and chronic ischemia.

Most important is the consistent evidence of long-term reversal of ischemic conditions in patients after a single administration of CD34+ cells indicating a regenerative mechanism of action. An ongoing study in Japan, if successful, could lead to the first approval in the world of a cardiovascular regenerative medicine therapy.

Future Perspectives

The discovery in 1997 of the CD34+ cell as a naturally occurring microvascular repair cell suggested the attractive possibility that ischemic tissue damage could be repaired, resulting in the attenuation or reversal of declining function. Accumulating clinical evidence has validated that hypothesis. Indeed, to date, no study of CD34+ cell therapy for ischemic tissue repair has failed to provide evidence of safety and clinical effect. Notably, these studies have been performed in endstage patients who have exhausted all other available therapies and remain disabled with ischemic symptoms. Despite the targeting of these extremely ill patients single administrations of CD34+ cell therapy have generated clear evidence for the reversal of ischemia. Accordingly the evidence supports CD34+ cell therapy as a regenerative medicine.

At present a pivotal trial of CD34+ cell therapy for treatment of critical limb ischemia is underway in Japan. If successfully completed and approved this would represent the first approval in the world of a cardiovascular regenerative medicine.

Once approved it will be important to investigate strategies involving more than a single administration of CD34+ cells to determine if additive benefit can be achieved, as well as exploring other indications in which microvascular repair may be beneficial such as diabetic nephropathy.

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Disclosure

W.K.S., H.T. and D.W.L. are current employees of Caladrius Biosciences, Inc. and own stock in Caladrius Biosciences, Inc.

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