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Review

CD34$^+$ Stem Cells: Promising Roles in Cardiac Repair and Regeneration

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See editorial by Davis, pages 1278–1280 of this issue.

ABSTRACT

Cell therapy has received significant attention as a novel therapeutic approach to restore cardiac function after injury. CD34-positive (CD34$^+$) stem cells have been investigated for their ability to promote angiogenesis and contribute to the prevention of remodelling after infarct. However, there are significant differences between murine and human CD34$^+$ cells; understanding these differences might benefit the therapeutic use of these cells. Herein we discuss the function of the CD34 cell and highlight the similarities and differences between murine and human CD34 cell function, which might explain some of the differences between the animal and human evolutions. We also summarize the studies that report the application of murine and human CD34$^+$ cells in preclinical studies and clinical trials and current limitations with the application of cell therapy for cardiac repair. Finally, to overcome these limitations we discuss the application of novel humanized rodent models that can bridge the gap between preclinical and clinical studies as well as rejuvenation strategies for improving the quality of old CD34$^+$ cells for future clinical trials of autologous cell transplantation.

RÉSUMÉ

Le traitement par des cellules souches attire beaucoup d'attention comme démarche thérapeutique novatrice dans le rétablissement de la fonction cardiaque à la suite d'une lésion. Les cellules souches CD34 (CD34+) ont été étudiées en vue d'évaluer leur capacité à promouvoir l'angiogenèse et à contribuer à la prévention du remodelage cardiaque après un infarctus. Toutefois, les différences sont marquées entre les cellules CD34+ murines et humaines; comprendre ces différences pourrait être utile dans l'usage thérapeutique de ces cellules. Dans cet article, nous traitons de la fonction des cellules CD34 et mettons en évidence les similarités et les différences entre les fonctions des cellules CD34 murines et humaines, lesquelles pourraient expliquer certaines des variations entre les évolutions humaines et animales. Nous offrons aussi un résumé des études qui traitent de l'usage des cellules CD34+ humaines et murines pendant des études précliniques et des essais cliniques ainsi que de leurs limites actuelles dans la réparation des lésions cardiaques. Finalement, pour surmonter ces limites, nous discutons de l'application de nouveaux modèles humanisés de rongeurs qui pourraient combler l'écart entre les études précliniques et cliniques. Nous discutons également des stratégies de renouvellement permettant d'améliorer la qualité des cellules CD34+ vieillissantes en vue d'études ultérieures sur la transplantation de cellules souches autologues.

Ischemic heart disease is a major cause of global morbidity and mortality and it places a major economic burden on health care systems.[1] In Canada alone, approximately 70,000 patients suffer from myocardial infarction (MI) each year.[2] Many of these patients develop progressive heart failure as a result of ventricular dysfunction, which results in an annual 10% mortality.[2] After an MI, the damaged myocardium is replaced by fibrotic scar tissue because of the adult heart's minimal capacity to regenerate functional myocardial tissue. Chronic maladaptation to the loss of myocardium leads to progressive ventricular dilation, cardiac dysfunction, and ultimately heart failure.[3]

Treatment options for heart failure are relatively limited. Pharmacological therapy helps to manage symptoms and might slow the remodelling process.[4] Heart transplantation is the ideal therapy for most end stage patients. However, the demand for this approach greatly exceeds donor heart availability.[5] Mechanical circulatory support such as left ventricular assist devices are used as a bridge to cardiac transplantation, or for destination therapy. However, this option is not without complications.[6] Therefore, the increasing demand for preventative therapeutic interventions necessitates the development of new effective treatments that could complement existing therapeutic approaches.

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Over the past decade, cell therapy has received great attention for its potential applications in cardiovascular disease. Extensive preclinical and clinical studies have shown that cell therapy is safe and might be effective to reduce the progression of cardiac remodelling after an ischemic insult. Skeletal myoblasts, bone marrow hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs) have been shown to enhance endogenous repair mechanisms after ischemia.[7,8] However to date, stem cell-based therapies have not been sufficiently effective for widespread application and treatment of cardiovascular diseases. One such stem cell-based therapeutic approach has been the use of autologous CD34-positive ($CD34^+$) cells. Unfortunately, results obtained from clinical trials using these cells have been inconsistent. Factors such as the source of $CD34^+$ cells (ie, bone marrow, peripheral blood), the route of cell delivery (ie, intracoronary, intramuscular, transesophageal), or patient-specific factors (ie, age and comorbidities) introduce variabilities that contribute to the inconclusive results. Therefore, the application of cell therapy for cardiovascular disease has not shown sufficient results to allow clinical translation.

In this review we discuss human and murine $CD34^+$ cells by highlighting their differences in cell biology and function as well as preclinical and clinical applications. We further discuss the new directions for investigating the role of human $CD34^+$ stem cells in treating MI and summarize various rejuvenation techniques that might provide a guide to improve the quality of aged human $CD34^+$ stem cells.

Expression of CD34 in Mice and Humans

CD34 is a 90- to 170-kDa cell surface protein with O-glycosylated and sialylated serine-, threonine-, and proline-rich extracellular domain.[12] This protein contains a single transmembrane helix, as well as a highly conserved c-terminus that contains consensus phosphorylation sites and a post-synaptic density protein 95/discs large/zonula occludens-1 (PSD-95/Dlg/ZO-1)-domain-binding motif.[12] Comparisons of human and murine CD34 proteins show a high degree of amino acid sequence homology, with the strongest conservation at the carboxy end of the protein.[13] However, despite sequence homology, experimental studies have shown that *CD34* is differentially expressed between murine and human species. CD34 is regarded as a common progenitor cell marker and is expressed by a wide range of cell types such as HSCs, EPCs, and MSCs, which are discussed (Figs. 1 and 2).[14]

$CD34^+$ hematopoietic stem/progenitor cells

Bone marrow transplantation experiments revealed that human HSCs capable of long-term repopulation are found within the $CD34^+$/$CD38^-$/Lineage $(Lin)^-$ population.[15,16] In contrast, murine bone marrow HSCs capable of long-term reconstitution were shown to be found within the $CD34^-$/tyrosine-protein kinase *KIT* $(c\text{-}Kit)^+$/stem cells antigen-1 $(Sca\text{-}1)^+/Lin^-$ population.[17] To clarify whether transcriptional regulation of *CD34* differs between mice and humans Okuno and colleagues developed a transgenic mouse that expresses human *CD34* under the control of human 5′ and 3′ elements essential for human *CD34* expression.[18,19] Examination of human *CD34* expression in these transgenic mice revealed that HSCs capable of long-term repopulation were human $CD34^+$ and mouse $CD34^-$.[18,20,21] These studies support the notion that human and murine *CD34* are differentially expressed and regulated.

Although *CD34* expression differs with respect to long-term repopulating HSCs, *CD34* is commonly expressed by multipotent hematopoietic progenitor cells in mice and humans. For example, multipotent lymphoid progenitors[22] and common myeloid progenitors[23,24] in mice and humans are $CD34^+$. Moreover, additional similarities and differences in other cell surface markers exist between mouse and human hematopoietic stem/progenitor cells.[25] Because mouse $CD34^+$ progenitor cells are functionally distinct from human $CD34^+$ stem and multipotent progenitor cells, humanized mouse models have been developed to study the contribution of human $CD34^+$ cells to HSCs.[15,16] These models have been essential in studying and defining the function of human $CD34^+$ cells in vivo and are discussed in the *Human $CD34^+$ Xenograft Mouse Model* section.

$CD34^+$ endothelial progenitor cells

EPCs have been defined as circulating cells that express cell surface markers similar to those expressed by vascular endothelial cells, adhere to endothelium in zones of ischemia and vascular injury, and participate in neovascularization. Because of their angiogenic potential, EPCs have been studied as potential progenitor cells for the treatment of ischemic injury.

CD34 is a conserved marker of human and murine EPCs. Examination of cell surface markers expressed by human EPCs show that human EPCs are found within the $CD34^+$/$CD45^-$/vascular endothelial growth factor receptor (VEGFR) 2^+ population.[26] Isolation and characterization of $CD34^+$ cells from human peripheral blood showed that these cells are capable of endothelial cell colony-forming activity in vitro and incorporate into active sites of angiogenesis when injected in rodent models of hind limb ischemia.[27] CD34 is also considered a marker of EPCs in mice. Early studies in mice used Sca-1 as well as CD34 expression to identify EPCs capable of promoting blood vessel formation after endothelial or ischemic injury.[28,29] Recent studies have also shown that mouse bone marrow $CD34^+$ cells possess endothelial colony-forming ability and can incorporate into newly formed blood vessels after MI.[30] Therefore, although coexpressed cell surface markers might differ between EPCs in mice and humans, CD34 can be considered a conserved marker of these cells between the two species.

$CD34^+$ mesenchymal stem cells

Unlike EPCs, MSCs are nonhematopoietic, multipotent stromal stem cells with the capacity to differentiate into mesodermal, ectodermal, and endodermal lineages. MSCs do not directly participate in new blood vessel formation, but can reduce left ventricular remodelling after MI by stimulating angiogenesis and modulating the immune responses.[31]

Freshly isolated human and murine MSCs express the CD34 cell marker. However, human MSCs are commonly considered to be $CD34^-$ as stated by the International Society for Cellular Therapy.[32] Interestingly, examination of $CD34^+$ and $CD34^-$ cells from human bone marrow showed that although both populations contain MSCs, during long-term culture $CD34^+$ MSCs downregulated their CD34 surface marker and became $CD34^-$ cells in vitro.[33]

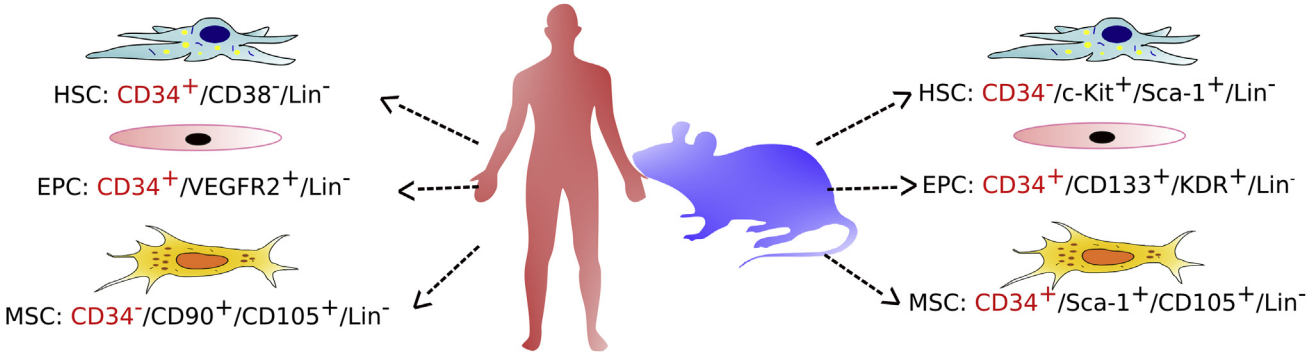

Figure 1. Comparing human vs murine cell surface markers for hematopoietic stem cells (HSC), endothelial progenitor cells (EPC), and mesenchymal stem cells (MSC). c-Kit, tyrosine-protein kinase *KIT*; KDR, kinase-insert domain-containing receptor; Lin, Lineage; Sca, stem cells antigen-1; VEGFR, vascular endothelial growth factor receptor.

Therefore, although freshly isolated MSCs appear to express CD34, cultured human MSCs are $CD34^{-}$. In comparison, mouse MSCs are considered to be $CD34^{+}$, even when cultured long-term.[34,35] In terms of functional implications, the presence of $CD34^{+}$ on mouse MSCs has been associated with increased proliferation and repair capacity compared with CD34 null[34] or $CD34^{-}$ cells.[36] Collectively, these studies suggest that human and mouse MSCs differentially express CD34 when subjected to long-term culture, and that the expression of CD34 has functional implications for cell therapy efficacy using MSCs. These difference might influence the trafficking of CD34 cells between the bone marrow and the infarcted myocardium.

Peripheral sources of $CD34^{+}$ cells

In humans, $CD34^{+}$ cells constitute 0.02%-1.43% of cord blood cells. These cells have a high regenerative capacity, are highly proliferative, and ideal for pediatric stem cell transplantation purposes.[37] In adults however, the main sources of $CD34^{+}$ cells are bone marrow (0.5%-5% of total cells) and peripheral blood (< 0.01% of total cells).[37] Adult bone

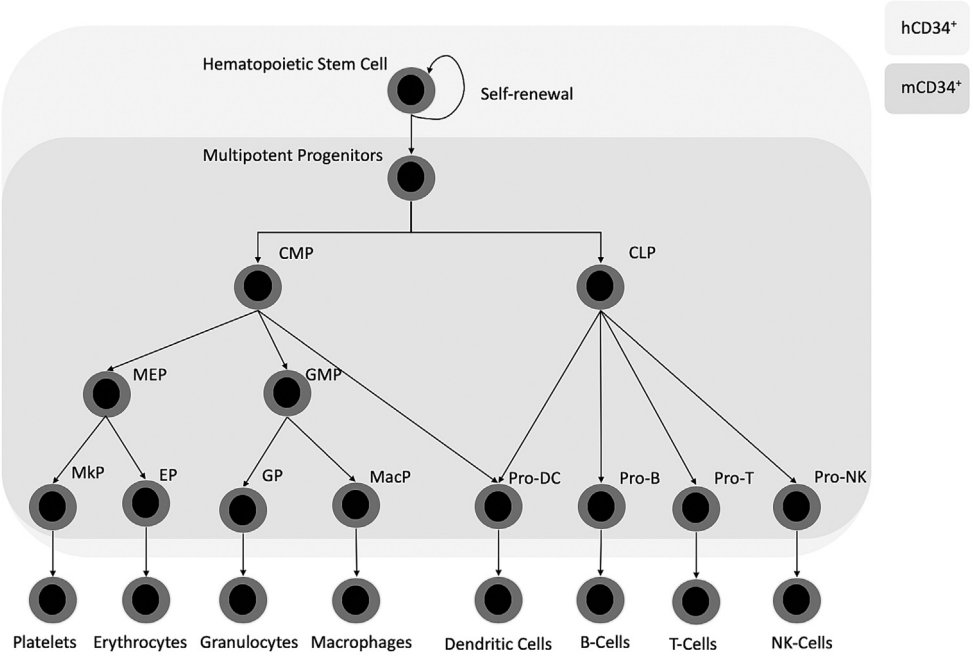

Figure 2. Schematic of hematopoietic development in humans and mice. CD34-positive ($CD34^{+}$) cells are indicated at **right** for human ($hCD34^{+}$) and mice ($mCD34^{+}$). Hematopoietic stem cells have long-term reconstitution and self-renewing ability; multipotent progenitors have limited self-renewal potential leading to transient but multilineage reconstitution. These cells give rise to specific lineages of mature hematopoietic cells. CMP, common myeloid progenitor; CLP, common lymphoid progenitor; EP, erythroid progenitor, GMP, granulocyte/macrophage progenitor; GP, granulocyte progenitor; MacP, macrophage progenitor; MEP, megakaryocyte/erythroid progenitor; MkP, megakaryocyte progenitor; NK, natural killer; Pro-B, B-cell progenitor; Pro-DC, dendritic cell progenitor; Pro-T, T-cell progenitor; Pro-NK, natural killer-cell progenitor.

marrow contains most of the $CD34^+$ cells; however, the collection procedure is relatively difficult. Alternatively, $CD34^+$ cells can be obtained from peripheral blood less invasively but at a lower concentration. Both of these sources of adult $CD34^+$ stem cells have been used for cell therapy, hematopoietic reconstruction, and regenerative purposes.

CD34 Protein Function

The function of the CD34 protein in humans and rodents remains to be definitively ascertained, but experimental studies suggest that these include regulation of proliferation and maintenance of stemness,[38] interaction with cell surface adhesion molecules,[39] and trafficking hematopoietic cells to the bone marrow.[38] The CD34 protein is expressed on the cell surface of multipotent hematopoietic progenitors and is downregulated in mature cells. These functions suggest a potential role for maintenance of the undifferentiated stem/progenitor phenotype.[40] The CD34 knockout mice developed by Cheng et al. express significantly fewer progenitor cells derived from hematopoietic cells.[41] When CD34 proteins are expressed on the surface of specialized lymphoid vascular endothelia known as high endothelial venules, they are glycosylated for interaction with L-selectin and thereby provide ligands for proper migration and lymphocyte-endothelium adhesive interactions.[42-44]

One of the most notable functional defects observed in CD34-knockout mice is the profound migratory impairment of HSCs. HSCs lacking CD34 expression show reduced bone marrow repopulating potential because of their impaired ability to traffic to the bone marrow niche.[45] All together, these studies indicate a potential role for the CD34 cell surface protein in differentiation, cell migration, and adhesion.

CD34 Cell Function in Cardiac Repair and Regeneration

Aside from their widespread application in hematopoietic bone marrow transplantation in patients undergoing myeloablative therapy,[46,47] $CD34^+$ cells have a unique capability to improve repair after ischemia by stimulating angiogenesis. In this section we discuss the application of murine and human CD34 cells in cardiovascular therapy and the proposed mechanisms by which these cells confer beneficial effects.

Preclinical studies using murine $CD34^+$ cells

Because of the proangiogenic effects of $CD34^+$ cells, increasing the number of these stem and progenitor cells in the heart or peripheral organs has been proposed as a therapeutic strategy to improve tissue repair after ischemic injury. Two different strategies have been used; the first strategy is to increase the mobilization of endogenous $CD34^+$ cells to the site of injury. For example, administration of granulocyte colony-stimulating factor after MI increases the number of circulating $CD34^+$ cells and reduces cardiac remodelling.[48] Myocardial homing of $CD34^+$ cells has also been targeted through modulation of the stromal cell-derived factor 1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) pathway by reducing SDF-1 degradation, which increases $CD34^+$ cell mobilization to the heart, enhances myocardial neovascularization, and limits adverse remodelling after MI.[49]

The second strategy used to increase the number of $CD34^+$ cells in the heart is to supply a source of exogenously-derived $CD34^+$ cells. Injection of mouse bone marrow-derived $CD34^+$ cells into the heart 3 days after induction of MI was shown to increase myocardial angiogenesis and reduce infarct expansion by 28 days after injection.[30] Although preclinical models have shown that mouse $CD34^+$ cells are capable of increasing angiogenesis and improving infarct healing after MI, the mechanisms elucidated through these studies might not apply to human $CD34^+$ cells, because the population and function of cells might differ. Therefore, preclinical and clinical studies using human $CD34^+$ cells to study the therapeutic benefits of these cells in mice are of a higher translational value.

Preclinical studies using human $CD34^+$ cells

Madeddu et al. were the first to show that local injection of human $CD34^+$ cells leads to the formation of human $CD34^+CD31^+$ capillaries in a murine model of hind limb ischemia.[50] They observed that human $CD34^+$ cells differentiate into endothelial cells to improve angiogenesis. Later, a separate study validated these findings and showed that a specific dose-dependent response to the injection of human $CD34^+$ cells can improve the function of the ischemic limb.[51] They further showed that as few as 10^3-10^4 cells were sufficient for significant therapeutic improvements.

The therapeutic efficiency of human $CD34^+$ cells compared with total mononuclear cell (MNC) injection for vascular repair has also been studied. In a nude rat model of MI, treatment groups containing (1) human $CD34^+$ cells; (2) low-dose MNCs; and (3) high-dose MNCs with total count of human $CD34^+$ cells as group 1 were compared.[52] High-dose MNC and human $CD34^+$ groups both showed improved cardiac function. However, increased incidence of hemorrhagic MI 3 days after infarction was reported in the group that received high doses of MNCs. This showed a superior therapeutic potential of human $CD34^+$ cell therapy for cardiovascular repair.

Kocher et al. showed that systemic injection of human $CD34^+$ cells after acute MI in athymic nude rats were sufficient to preserve cardiac function and reduce the infarct size by increasing tissue perfusion.[51] However, it was later shown that systemic injection of human 111-oxine labelled $CD34^+$ cells after MI results in accumulation of these cells in the liver, spleen, and kidneys, and not the ischemic myocardium.[46,53,54] To circumvent this problem, others have developed methods of direct delivery of human $CD34^+$ cells to the infarcted myocardium. In these studies, the recipients of human $CD34^+$ cells showed improved cardiac function and increased capillary density.[55-57] Human $CD34^+$ cells were shown to integrate into the periinfarct zone and contributed to the neovascularization process for at least 52 weeks after infarction and improved left ventricular ejection fraction (LVEF) in the treatment group.[55] Although these short-term improvements do not necessarily translate to long-term functional benefits, they show that human $CD34^+$ cells might be valuable in preventing the expansion of the infarct region after MI. The perivascular stem cells not only contribute to neovascularization, but also limit matrix

remodelling and enhance the recruitment of beneficial bone marrow cells to the scar region after MI.

Clinical trials using human CD34+ cells

A recent meta-analysis of 21 randomized controlled trials (RCTs) that measuring the circulating levels of hematopoietic and progenitor cells in 4155 patients with high-risk cardiovascular phenotype indicated that reduced circulating CD34+ stem cells is associated with a twofold increase in risk of future cardiovascular events and death.[58] Other meta-analyses have shown a benefit with the use of human CD34+ cells for treatment of nonischemic cardiomyopathy[59] and MI.[60,61] A meta-analysis of 4 RCTs involving peripheral CD34+ therapy in 244 patients with nonischemic cardiomyopathy showed a 6.44% improvement in LVEF compared with the control group (95% confidence interval, 1.90%-10.99%; $P < 0.01$; $I^2 = 60\%$).[59] This finding is contrary to the conclusions from the Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) trial in which CD34+ cells were delivered to 200 patients with reduced cardiac function and did not result in any significant improvements of LVEF.[62] Zimmet et al. conducted a meta-analysis of RCTs of intracoronary bone marrow stem cell delivery within 9 days of reperfusion of ST-elevation MI patients. They included 29 RCTs that enrolled 1830 patients and reported a significant dose-response relationship between the quantity of CD34+ cells delivered and improvements in LVEF, concluding that increased CD34+ dose results in improved cardiac function after MI.[60] Furthermore, the LVEF functional improvements seem to persist for at least the 18-month follow-up as suggested in an earlier meta-analysis reported by Jian et al.[61] Table 1 summarizes the findings from the RCTs discussed in these meta-analyses and highlights the variability in design and different conclusions among them, showing that factors such as aging and presence of multiple comorbidities can affect the quality of cell therapy in clinical trials.

Current Limitations and Disparities in CD34+ Investigations

Together, the preclinical and clinical investigations of human CD34+ cells have generally shown safety and efficacy. However, the differences in trial design (ie, sources of CD34+ cells, routes of administration) and the inherent heterogeneity within the CD34+ cell population could explain why the reports have not been in complete agreement. Data from preclinical studies of mouse CD34+ cells might not explain the exact mechanism by which human CD34+ cells improve infarct healing because of the inherent differences in CD34+ cells between the 2 species, as discussed previously. Furthermore, even within the human source, CD34+ cells are a heterogeneous population that encompasses varying stem and progenitor cells depending on the source. CD34+ cells derived from bone marrow and peripheral blood could have varying HSC and EPC composition[63] and their quality could vary depending on whether they were used fresh from an autologous source or frozen via an allogenic donor.[64] Last, in autologous CD34+ cell therapy trials, patients' age and comorbidities could also affect the cell quality and hence the outcome of the trials.[65,66] Therefore, lack of proper understanding of the effect of these conditions on the quality and function of human CD34+ cells in the context of cardiac ischemia and the absence of standardization between trials potentially illuminate the inconclusive findings from these investigations.

The Efficacy and Safety of Targeted Intramyocardial Delivery of Auto CD34+ Stem Cells for Improving Exercise Capacity in Subjects With Refractory Angina (RENEW) trial, a phase III clinical trial on comparing the effect of autologous CD34+ cells with the current standard of care in patients with chronic myocardial ischemia was terminated early for strategic considerations.[67] This highlights the need for definitive preclinical investigations and in-depth understanding of the role of the previously mentioned conditions on the composition and quality of human CD34+ therapy to design the most effective clinical trials targeting populations who would benefit the most from this adjunct therapy. The early termination of this trial also highlights further challenges involving stem cell-based therapies because of the complexities inherent in the nature of cellular therapeutic agents, lack of standardization in techniques, varying cell sources and manufacturing practices, complexities embedded in the commercialization viability for stem cell cardiovascular therapy, and the lack of global regulatory practices for stem cell therapies.

Potential Approaches for Overcoming the Limitations

To overcome the limitations already discussed, we believe that 2 approaches will be useful. The first is to better understand the mechanisms by which human CD34+ cells incorporate into the ischemic zones to modulate the healing process. The second is to overcome the effect patient comorbidities and age have on the efficacy of cell therapy. In this section we discuss potential strategies and developments that can help with these 2 approaches.

Human CD34+ xenograft mouse model

Because murine models of ischemia and mouse CD34+ cells are inherently different than of humans, using novel preclinical animal models that can more faithfully recapitulate the human biological system for investigating the role of factors such as comorbidities and aging on the quality of CD34+ cells is valuable.

Advancements in the development of immunodeficient mice bearing mutations in the interleukin-2 receptor common gamma chain (IL2rgnull), have allowed investigators to engraft mice with human HSCs that develop into functional human immune systems. Three strains of immunodeficient IL2rgnull mice are widely used today: NOD.Cg-PrkdcscidIl2rgtm1Wjl, NODShi.Cg-PrkdcscidIl2rgtm1Sug, and BALB/c-Rag2null IL2rgnull mice.[68] When engrafted with human CD34+ cells, these mice faithfully recapitulate the biological mechanisms seen in humans. This has led to major advances in studying human infectious diseases,[69] cancer,[70] allergies,[71] and autoimmune conditions.[72] However, to date, these humanized mice have been underutilized in studying the role of human CD34+ cells in cardiac regenerative medicine.

Application of these mice in preclinical investigations of human CD34+ cells can provide novel understanding of the

Table 1. Summary of key clinical trials of CD34 cell therapy for cardiovascular diseases

Reference	Design	Sample size	Patient condition	CD34 cell source	Delivery route	Follow-up months	Key findings	Clinical trial ID
Vrtovec et al.[100]	Phase II trial	110	DCM	PB	IC	60	Improved ventricular modelling, exercise tolerance, and survival	NCT01350310
Vrtovec et al.[101]	Phase II trial	40	DCM	PB	IC/TE	6	TE administration leads to higher myocardial retention rates and greater improvement in ventricular function, N-terminal pro-brain natriuretic peptide, and exercise capacity	NCT01350310
Henry et al.[102]	Phase II trial	167	Refractory angina	PB	IC	12	Significant improvements in angina frequency and exercise tolerance. No significant difference was observed between low- and high-dose injection	NCT00300053
Tendera et al.[62] (REGENT trial)	Prospective randomized trial	200	AMI with reduced < 40% LVEF	BM	IC	6	No significant improvement of LVEF. There was a trend in favour of cell therapy in patients with most severely impaired LVEF and longer delay between the symptoms	NCT00316381
Vrtovec et al.[100] (ACT34-CMI study)	Phase II trial	167	CCS class III-IV chronic refractory angina	PB	IM	24	Persistent improvement in angina and a trend for reduction in mortality	NCT00545610
Wang et al.[10]	Prospective double-blind, randomized trial	112	Triple-vessel disease and CCS class III-IV angina	BM	IC	6	Significant improvement in myocardial perfusion showed safety, efficacy, and feasibility of this therapy	N/A
Quyyumi et al.[103]	Phase I trial	31	STEMI	BM	IC	6	Dose-dependent effect on myocardial repair response. Dose of 10 million CD34-positive cells, associated with a significant improvement in perfusion that might limit deterioration in cardiac function	NCT00313339
Kawamoto et al.[53] (ACT34-CMI study)	Phase II trial	167	Refractory angina	PB	IM	12	Low-dose injections (10^5 cells/kg) resulted in significant improvements in angina frequency and exercise tolerance	NCT00300053
Povsic et al.[104] (RENEW trial)	Phase III trial	400	Refractory angina	PB	IM	24	Established efficacy of intramyocardially delivered autologous CD34-positive cells. Study was terminated early because of business considerations	NCT01508910
Lezaic et al.[9]	Phase II trial	21	Nonischemic DCM and LVEF < 40%	PB	IC	6	Improved myocardial perfusion. Patients with less severe myocardial perfusion defects at baseline might have an increased likelihood to respond favourably	NCT01350310
Quyyumi et al.[11] (PreSERVE-AMI)	Phase II trial	161	Patients undergoing stenting for STEMI and LVEF ≤ 48%	BM	IC	12	Showed safety and efficacy. No differences in myocardial perfusion or adverse events between the control and treatment groups, although increased perfusion was observed within each group from baseline to 6 months	NCT01495364
Losordo et al.[105]	Phase I/II trial	24	CCS class III-IV angina	PB	IM	12	Established feasibility, safety, and bioactivity. A larger phase IIb study is currently under way to further evaluate this therapy	NCT00081913
Poglajen et al.[106]	Phase I trial	33	ICM and NYHA class III and LVEF < 40%	PB	TE	6	Improved LV function, decreased N-terminal pro B-type natriuretic peptide levels, and better exercise capacity	NCT01350310
Vrtovec et al.[107]	Prospective trial	45	Nonischemic DCM and diabetes	PB	TE	6	CD34-positive cell therapy appears to be ineffective in DCM patients with diabetes	NCT02445534
Lee et al.[108]	Phase I trial	38	Severe diffuse coronary artery disease	PB	IC	18.5	Established safety and efficacy in improving heart function	N/A

ACT34-CMI, $CD34^+$ Cells for Reduction of Angina Episodes in Patients With Refractory Chronic Myocardial Ischemia; AMI, acute myocardial infarction; BM, bone marrow; CCS, Canadian Cardiovascular Society; DCM, dilated cardiomyopathy; IC, intracoronary; ICM, ischemic cardiomyopathy; IM, intramyocardial; LV, left ventricular; LVEF, left ventricular ejection fraction; N/A, not applicable; NYHA, New York Heart Association; PB, peripheral blood; PreSERVE-AMI, NBS10 Versus Placebo Post ST Segment Elevation Myocardial Infarction; REGENT, Myocardial **Reg**eneration by Intracoronary Infusion of Selected Population of St**e**m Cells in Acute Myocardial I**n**farc**t**ion; RENEW, Efficacy and Safety of Targeted Intramyocardial Delivery of Auto $CD34^+$ Stem Cells for Improving Exercise Capacity in Subjects With Refractory Angina; STEMI, ST-elevation myocardial infarction; TE, transesophageal.

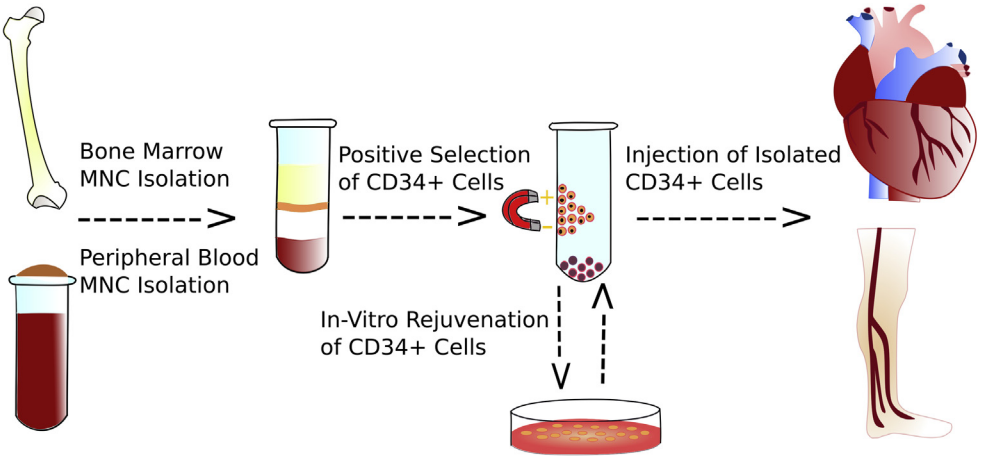

Figure 3. This illustration shows mononuclear cell (MNC) isolation from bone marrow and peripheral blood after treatment with cell mobilizing agents, isolation of CD34-positive ($CD34^+$) cells by immune-based magnetic cell sorting, and injection of the purified isolated cells into ischemic organs. Future studies should investigate in vitro rejuvenating techniques, which could increase the potency of $CD34^+$ cells for autologous stem cell transplantation.

role of human-specific $CD34^+$ cells in mediating the cardiac tissue healing process and the role of aging and comorbidities on the quality of these cells.

Rejuvenation to improve cardiac repair

Circulating levels of human $CD34^+$ cells have been shown to positively correlate with improved cardiovascular health while negatively correlating with advanced age and severity of disease states.[73-77] In the setting of ischemia, acute mobilization of endogenous human $CD34^+$ cells from bone marrow into the circulation promotes angiogenesis either through direct incorporation into new blood vessels or through secretion of proangiogenic cytokines necessary for neovascular endothelial development.[27,48,76,78-81] Therefore, identifying new ways to improve the function of endogenous $CD34^+$ in older individuals might have important therapeutic applications because decline in $CD34^+$ cell function can significantly alter the outcomes of preclinical and clinical studies using them for tissue regeneration.

Aging is associated with stem and progenitor cell damage as a result of diminished DNA damage response,[82,83] and increased reactive oxygen species[84] as well as epigenetic clonal selection factors. Aging-induced clonal skewing results in changes in the global hematopoietic stem and progenitor cell profile in mice and humans.[85-87] Furthermore, analysis of epigenetic markers has shown hypermethylation of genes leading to impaired self-renewal and stem cell differentiation ability in aged mice.[86,88] As discussed, human $CD34^+$ cells encompass HSCs, EPCs, and freshly isolated MSCs. Therefore, herein we discuss novel rejuvenating strategies conducted in these cell types. It is important to note however, that no study to date has investigated the effect of these modulatory rejuvenating techniques on the aging human $CD34^+$ cells but that could be a promising direction for future preclinical and clinical investigations involving $CD34^+$ cells to increase their efficacy in older patients (Fig. 3).

Few environmental, cellular, and systemic modifications have been examined for potential rejuvenation of human HSCs. Caloric restriction studies have shown that prolonged fasting has the ability to rejuvenate and reverse aged-induced myeloid skewing and improve aged HSC function as a result of the reduced insulin growth factor 1 signalling.[89] Interestingly, through a different pathway the same results were obtained by inhibiting the expression of a nutrient-sensing protein, mammalian target of rapamycin, using rapamycin, which improved reconstitution and differentiation potential of aged HSCs.[90] Aside from the caloric pathways, dominant noncanonical wingless-related integration site (*Wnt*) signalling through *Wnt5a* in aged HSCs has been shown to be correlated with the aging phenotype through the elevated activity of small Ras homolog gene family GTPase (RhoGTPase), cell division control protein 42 (Cdc42).[88,91] Inhibition of Cdc42 activity with Cdc42 inhibitor (CASIN) has shown rejuvenation of aged HSCs.[88] With regard to MSC rejuvenation strategies, ex vivo modification of the cells leading to overexpressing factors that decline in aged cells has been the most successful for improving their repair capacity. Our group has shown that overexpression of neuron-derived neurotrophic factor rejuvenates aged bone marrow MSC function, which helps these cells to more effectively stimulate cardiac repair compared with nonrejuvenated MSCs.[92] Overexpression of *ERBB4* also improves aged MSC function by stimulating increased protein kinase B (AKT) and extracellular receptor kinase (ERK) signalling and increasing the secretion of proangiogenic factors.[93] Another potential approach to rejuvenate aged MSCs is to transplant the cells within a biomaterial scaffold containing growth factors. For example, we showed that seeding aged MSCs on a collagen scaffold containing vascular endothelial growth factor and fibroblast growth factor improves the therapeutic efficacy of aged MSCs after MI.[94]

In contrast to MSCs and HSCs, fewer studies have investigated EPC rejuvenation for improving cardiac repair. However, some groups have shown that EPCs can be rejuvenated in aged individuals. Thum and colleagues showed that growth hormone administration to elderly male patients improves circulating EPC function through enhanced insulin growth factor 1 signalling.[95] It is important to note that these strategies require treatment before the onset of any adverse

cardiovascular event; strategies to enhance repair processes after onset of MI in aged individuals will also be important to investigate.

In addition to cellular-level rejuvenation strategies, cell replacement therapy can also rejuvenate the aged bone marrow and improve cardiac repair after MI. Our group and others have shown that reconstituting young bone marrow cells in aged mice improves cardiac repair and limits remodelling after MI.[96-99] Human $CD34^+$ stem cells have the potential to reconstitute the bone marrow and as such, bone marrow reconstitution with young healthy $CD34^+$ stem cells could restore progenitor cell function and improve repair after MI in aged individuals. Modification of the bone marrow $CD34^+$ cells offer significant advantages to direct injection of stem cells into the heart because the incorporation of the cells in the heart persists for at least 1 year and they appear to stimulate proliferation of bone marrow-derived stem cells after MI.[96,97] It is important to emphasize that these potential strategies might not be the only factors that could improve the efficacy of clinical trials. However, they are factors that could aid in the design of future trials by accounting for the inherent differences in the quality of patient-derived $CD34^+$ cells.

Conclusions and Future Directions

To fully harness the therapeutic potential of human $CD34^+$ cells, we must clearly delineate the mechanisms responsible for their beneficial effect. The differences between murine and human $CD34^+$ cells have limited our basic mechanistic understanding of the role of factors such as aging and comorbidities on the function of human $CD34^+$ cells in preclinical studies. Despite the fact that murine $CD34^+$ cells have also shown potential benefits for cardiovascular repair, the mechanisms of these cells are not directly translatable to human $CD34^+$ cell function because of the inherent differences between the cells from mouse and human. Clinical trials have shown safety and efficacy of local human $CD34^+$ cell therapy in ischemic myocardium; however, the benefits have been short-term. As such, there is a need for investigating the mechanism of improved cardiac function using human $CD34^+$ cells in preclinical studies and identifying more efficacious routes of human $CD34^+$ cell delivery for sustained long-term benefits. Improving bone marrow function by bone marrow transplantation is one potential treatment that might be beneficial. Furthermore, using an in vivo model of the human $CD34^+$ cells in the humanized mice could help illuminate novel findings on the role of human $CD34^+$ cells in the context of tissue ischemia and the effect aging and comorbidities have on their quality and function.

Lastly, aging is a critical factor implicated in the diminished responsiveness of reparative cells to ischemic-related injuries. As such, the next steps in human $CD34^+$ cell-based cardiac regenerative therapy should involve safe and efficacious rejuvenation of host stem cells for autologous cell transplantation. Future preclinical studies should target rejuvenating aged human repair processes by means of systemic influences, molecular pathways, or cell replacement therapy to improve the long-term efficacy of autologous human $CD34^+$ cell therapy. If these shortcomings of the current state of cardiovascular cell therapy using human $CD34^+$ stem cells are addressed and if the bioactivity of rejuvenated autologous cell therapies is verified in trials, delivery of $CD34^+$ cells could be a potential adjunct therapy for clinical management of patients for whom the current standard of care is inadequate to restore function.

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Disclosures

The authors have no conflicts of interest to disclose.

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