Published in final edited form as:

Circ Res. 2014 November 7; 115(11): 904–907. doi:10.1161/CIRCRESAHA.114.305284.

Can Endothelial Progenitor Cells Treat Patients with Refractory Angina? Short Title: EPCs in Refractory Angina

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Keywords

stem cell; refractory angina

Refractory angina continues to pose a therapeutic challenge for patients and clinicians as treatment options including medication and surgery are limited¹. Therefore, the idea that cell-based therapy could offer a new therapeutic opportunity continues to raise enthusiasm². In this regard, circulating endothelial progenitor cells (EPCs) have gained significant attention due to their participation in neovascularization and re-endovascularization³. EPCs are a specific population of progenitor cells—expressing CD133, CD34, and KDR cell-surface markers—that differentiate into endothelial cells, thereby lining blood vessels and playing a critical role in angiogenesis and vasculogenesis³. EPCs incorporate into both new and injured vessels, and release pro-angiogenic and immunomodulatory factors³, making them an ideal candidate for treating patients with ischemic heart diseases like refractory angina⁴.

While cell-based therapy is exciting, it remains to be established which EPC cell population best targets refractory angina: CD34+ or CD133+ EPCs. Both CD34+ and CD133+ are endogenous cell surface markers expressed on EPCs that originate from bone marrow and are mobilized into peripheral blood in response to cytokine signaling and injury⁵. Over the past decade, it has become possible to purify EPCs by mobilizing them into peripheral blood using granulocyte colony-stimulating factor (G-CSF). Endogenous G-CSF is synthesized by the endothelium, macrophages, and immune cells, and it acts as a cytokine that stimulates bone marrow to produce granulocytes and stem cells and then release them into the bloodstream⁶. Therefore, G-CSF is used pharmacologically to enhance the number of EPCs obtained from patients undergoing cell therapy⁷. Specifically, patients are given 4–5 days of G-CSF treatment to mobilize EPCs, which is immediately followed by leukapheresis to separate the mononuclear layer from the peripheral blood⁷. After mobilization and separation, flow cytometry and magnetic sorting are used to purify the population by exploiting the expression of these cell surface markers⁸. Loss of this cell population is strongly implicated in atherosclerosis⁹ and endothelial dysfunction¹⁰.

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Conflict of Interest Disclosures: Dr. Hare reported having a patent for cardiac cell-based therapy, receiving research support from Biocardia, having equity interest and board membership in Vestion Inc,

Because CD34+ is found on both hematopoietic and endothelial progenitor cells, selection using this epitope results in a heterogeneous population of progenitor cells. CD34+ cells play a role in blocking cell differentiation while enhancing cell proliferation, and also in regulating cell to cell adhesion and cell to bone marrow, lymph nodes, and extracellular matrix adhesion¹¹. Although CD34 is an essential marker in identifying hematopoietic and endothelial stem cells, the exact biological function of the CD34 protein family remains elusive. CD34+ cell have demonstrated efficacy in patients with refractory angina. Losordo *et al.* showed improvements in exercise tolerance and reductions in angina frequency¹². Similarly, Wang *et al.* showed encouraging results for the intracoronary injection of CD34+ cells in intractable angina—reduction in frequency of angina episodes, increase in exercise time, improvement in CCS, and significant improvement in myocardial perfusion¹³.

CD133+ marks a subpopulation of CD34+ cells and is thought to represent a more robust marker of EPCs¹⁴. Previous clinical trials have demonstrated encouraging results with CD133+ injections delivered both by intracoronary and transmyocardial routes in patients with ischemic cardiomyopathy^{15–17}. Specifically, Bartunek *et al.* demonstrated that intracoronary injection of CD133+ cells enhances cardiac recovery—via increasing left ventricular ejection fraction, myocardial perfusion, and myocardial viability—after myocardial infarction. Likewise, Stamm *et al.* showed that intramyocardial delivery of CD133+ during coronary artery bypass grafting is safe and beneficial—improving LVEF. These promising results evident in ischemic cardiomyopathy prompted Jiminez-Quevado *et al.* to investigate the safety and efficacy of CD133+ EPCs in ischemic cardiomyopathy patients with refractory angina who were not candidates for coronary revascularization¹⁸.

In this issue of *Circulation Research*, Jiminez-Quevado *et al.* present their results from the first study done in man where CD133+ cells were injected transendocardially using the Myostar injection catheter in patients with refractory angina (figure 1). This was a small (n=28) Phase I/II, multicenter, single-blinded, randomized study with the primary endpoint being safety and the prespecified secondary endpoint being efficacy measured as the change in myocardial perfusion defect (SPECT) at baseline versus 6 months, 12 months, and 24 months follow-up. The trial successfully demonstrated that transendocardial CD133+ injections are safe in this patient population, consistent with other studies using transendocardial injections^{19, 20} and CD133+ cells^{21, 22}. Their initial secondary endpoint demonstrated no efficacy after cell treatment, therefore Jiminez-Quevado *et al.* addressed other exploratory endpoints (treadmill test, Canadian Cardiovascular Society (CCS) class, number of angina episodes/month, nitroglycerin consumption/month, and quality of life via Seattle questionnaire) 6 months post CD133+ treatment. In doing so, they suggest that there is evidence of efficacy via an increase in local linear shortening (LLS)—although these results did not coincide with the results from wall motion index—an improvement in CCS class, a reduction in angina episodes per month and number of nitroglycerin-tablet consumption, and an increase in certain questions on the Seattle Angina Questionnaire. While potentially encouraging, we must remain aware that exploratory endpoints are hypothesis generating, and require confirmation in other studies.

In vitro, the authors illustrated that these clearly labeled CD133+ cells were angiogenic when co-cultured with human umbilical vein endothelial cells (HUVECS), and that after 14

days in culture, the expression of endothelial markers increased. Unfortunately, the authors did not distinguish between HUVECs and CD133+ cells in their matrigel assays, nor did they show direct evidence for these cells promoting angiogenesis, limiting the ability to conclude that CD133+ cells promote angiogenesis in patients.

Given these preliminary findings, do EPCs hold promise as a future therapy for refractory angina or should other stem cells such as MSCs hold more promise (Figure 2)? Similar to EPCs, MSCs are pro-angiogenic²³ and anti-inflammatory²⁴; however unlike EPCs, MSCs are immunoprivileged, allowing their allogeneic usage^{25, 26}. By virtue of not eliciting an immune response, the use of healthy allogeneic donor cells over unhealthy autologous cells is feasible for treating refractory angina. Haack-Sørensen *et al.* showed that intramyocardial MSC injections in patients with refractory angina resulted in a significant increase in exercise capacity via metabolic exercise training (MET)²⁷, a result that was not shown by Jiminez-Quevado *et al.* using CD133+ cells. More strikingly, they demonstrated a sustained effect 24 months after MSC injection in patients with severe coronary artery disease and refractory angina—increase in exercise time, MET, CCS class, all parameters of the Seattle Angina Questionnaire and a decrease in weekly number of angina attacks and use of nitroglycerine²⁸. Although allogeneic MSCs have not yet been used for refractory angina, our group has demonstrated both efficacy and safety in patients with ischemic cardiomyopathy¹⁹. Accordingly, allogeneic MSCs may have a role in refractory angina and therefore can avoid the need to stimulate EPC release in these patients.

Ultimately, the article by Jiminez-Quevado *et al.* in this issue of *Circulation Research* is an insightful contribution to the limited available literature for the use of CD133+ cells in patients with refractory angina. However, the efficacy results are exploratory and represent a first step compared to that of MSCs and CD34+ cells, suggesting that other cell types should be further explored to truly identify the selected cells for treating patients with refractory angina. This is an important indication for cell therapy and further larger studies are warranted. Specifically, comparison studies should be performed designed to identify the optimal cell type for use, both from a feasibility and efficacy perspective.

Acknowledgments

Funding/Support: Dr. Hare is supported by National Institutes of Health (NIH) grants RO1 HL094849, P20 HL101443, RO1 HL084275, RO1 HL107110, RO1 HL110737, and UM1HL113460, and the Starr Foundation.

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Figure 1.

Schematic of the PROGENITOR randomized trial and results. 28 patients with refractory angina were treated with either CD133+ cells (n=19) or placebo (n=9). Abbreviations are as follows: MACCE= Major Adverse Cardiac and Cerebrovascular Event, CCS= Canadian Cardiovascular Society, SPECT=single photon emission computed tomography, LVEF= left ventricular ejection fraction, UV= unipolar voltage, LLS= linear local shortening.

Figure 2.

Chart illustrating various cell types^{5,11,14,23} used in clinical trials for refractory angina. Blue arrows indicate the reported results from cell trials^{12,13,17,18,27,28} with red wording indicating results reported by Jiminez-Quevado *et al.*