Ex-vivo Expanded Human Bone Marrow-Derived CD133$^+$ Cells Improve Cardiac Function in Rats With Acute Myocardial Infarction

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We have examined whether ex-vivo expanded human bone marrow (BM)-derived CD133$^+$ cells (EE-CD133c) in presence of the copper chelator tetraethylenepentamine (TEPA) may participate in post myocardial infarction (MI) healing. CD133$^+$ cells isolated from human BM, were expanded in the presence of IL-6, TPO, Flt-3 ligand, and SCF with or without TEPA for three weeks. After 3 weeks the total nuclear cell expanded by 210±27 fold. The increase in the CD34$^+$, CD133$^+$ and CD133$^+$/CD38$^-$cell populations was 28±8, 19±4 and 263±91 - fold, respectively. 3 weeks EE-CD133c expressed VEGF and VEGF receptor RNA as examined by RT-PCR and secreted VEGF at levels of 121±41 pg/10^6 cells as demonstrated by ELISA analysis. The biological activity of conditioned medium derived from EE-CD133c induced endothelial and vascular smooth muscle cell proliferation. CXCR4 was expressed on the surface of 40% of EE-CD133c as demonstrated by FACS analysis. An MI model was established in athymic nude rats. EE-CD133c (6x10^6) or saline (control) were injected into the infarcted myocardium 6 days

post MI. Echocardiographic studies compared 4 weeks post-treatment with those observed prior to treatment. EE-CD133c treatment, attenuated left ventricle (LV) systolic dilatation by 89% ($p=0.014$) and improved LV contractility by 72% as compared to control animals ($p=0.017$). Interestingly, injection of the unexpanded BM cell fraction not bound to the CD133 column upon isolation, alongside with EE-CD133c, greatly diminished the improving effect observed for EE-CD133c. Clinical trials examining the safety and feasibility of intra-corronary injection of autologous EE-CD133c to patients with ischemic heart disease are currently in preparation.

Conclusion: **A**. TEPA allows BM-derived $CD133^{+}$ cells to self renew by permitting their ex-vivo proliferation while hindering their differentiation. **B**. The resulting EE-CD133c express receptors (VEGF-R, CXCR4) involved in homing to hypoxic/inflammed zones and secret factors relevant to cardiac regeneration. **C.** Injection of EE-CD133c into infarcted myocardium improves left ventricular function.