Response to Letter Regarding Article, “A Detailed Analysis of Bone Marrow From Patients with Ischemic Heart Disease and Left Ventricular Dysfunction: BM CD34, CD11b and Clonogenic Capacity as Biomarkers for Clinical Outcomes”

We thank Drs Gremmels, Papazova, Fledderus, and Verhaar for providing their perspective of our study.1

The primary question raised by these investigators whose research focus is on multipotent mesenchymal stromal cells (MSCs) is whether endothelial colony–forming cells (ECFCs) derived from bone marrow (BM) can originate from rare MSCs.

The answer is yes.

To answer more fully, it should be noted that there are no ECFCs in the human body. ECFCs are a synthetic representation of vasculogenic capacity, which varies depending on input cell source. We chose to use this laboratory assay in parallel with a cardiovascular cell therapy trial because the target organ for repair—the heart—suffered from ischemia and infarction. Until our report, all previous cardiovascular cell therapy trials used Methocult media (an assay measuring hematopoietic differentiation) as a biomarker for cell function. We chose to add the ECFC assay in addition to several other ancillary studies as a means to quantify the proangiogenic activity of BM mononuclear cells administered to patients.2

In our experience with hundreds of BM specimens, not just 6 as reported by Tura et al,3 when BM mononuclear cells are cultured in endothelial growth media 2 (EGM-2), endothelial-like colonies grow. BM-derived ECFC colonies (BM-ECFC) expressed endothelial surface proteins like CD31 (PECAM-1) and CD105 (Endoglin) and lacked hematopoietic expression (ie, CD45, CD14) and MSC expression (ie, CD146). Some endothelial adhesion molecules, such as VE-cadherin (CD144), were not present on ECFC colonies. However, BM-ECFCs functionally formed capillaries in Matrigel. The most that can be said about BM-ECFCs is that they are similar, not equivalent, to endothelial cells.

In a recent study, when we cultured BM from patients with acute myeloid leukemia—a stem cell malignancy—in EGM-2, endothelial-like colonies grew. BM malignancies that generate endothelial cells,5,6 which suggests neoplastic hemangioblast activity arising from within the BM. In a series of sex-mismatched BM transplant patients, BM-derived endothelia was found in skin and gastrointestinal tract, further supporting the notion that BM can be a source of vasculogenesis.7

Determining whether endothelial progenitor cells are intermediaries in BM-derived vasculogenesis has been hampered by the inability to define an endothelial progenitor cell. Yoder’s excellent endothelial progenitor cell review highlights the tribulations of fixing a definition on a dynamic cell subset.8 Some would argue that the term, endothelial progenitor cell, is actually an umbrella term encompassing a panoply of cell subsets, including hematopoietic and stromal stem/progenitor cells.

All of this being said, sorted MSCs cultured in EGM-2 grow endothelial-like colonies.8,9 However, sorting MSCs based on cell surface phenotype is perilous because of the promiscuity of protein expression on BM cells. For example, Stro-1 is expressed on MSCs and ECs, CD90 on MSCs and hematopoietic stem cells, and CD73 on both MSCs and activated lymphocytes.10 Moreover, MSC-derived ECFCs have been shown to promote angiogenesis in in vivo models, but have not shown vasculogenic capability.9 To rigorously evaluate MSC in EGM-2, single MSC cell proliferation studies and in vivo vasculogenesis modeling are required, neither of which have been performed convincingly nor were performed by our group.

Therefore, on the specific question of ECFC origin, the only firm answer is that EGM-2 media and its angiogenic cytokines are effective in pressuring input cells into endothelial-like differentiation.

To address the question of BM MSC activity and cardiovascular cell therapy clinical outcomes, we cultured BM specimens in CFU-F assay (Mesencult media), and these results are forthcoming in future publications.

Disclosures

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References


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