

# Harvesting, ex vivo manipulation and clinical use of mononuclear and CD34+ cells in the treatment of myocardial ischemia

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The study objective was to recapitulate developing research frontiers in the mononuclear and stem cell (MNC and SC) biology and clinical use in the regenerative medicine. The preliminary results of the therapy of our patients with acute cardiac infarction (intracoronary infusion) and coronary artery bypass (intramyocardial application) – treated by intracoronary infusion or intramyocardial application of the steady state vs. activated (primed) autologous BM derived SCs – are also summarized.

Concisely, “autopoiesis” refers to ability of a biological system to reproduce and maintain itself. Within that, cytotogenesis or cytopoiesis involve the origin and development of different cells. This is a multipart cyclic event in which from a small quantity of toti/pluri/multipotent (immature) SCs – through proliferative and differentiative processes – a spectrum of mature cell populations is produced. The complex network of interactive cytokines and other mediators regulates SC survival and functions. Therefore, SCs – having well-balanced self-renewal, proliferative and differentiation potential – could be predictive of “steady-state homeostasis” in tissue-generating systems and BM repopulation or organ regeneration/repair after therapeutic use.

The SC compartment can be divided into embryonic and adult cells. Embryonic SCs are, reasonably, capable to differentiate into all cell types of human body. Immature adult SCs have also a potential to trans-differentiate into numerous somatic cell types (plasticity) – such as osteocytes, chondrocytes, hepatocytes, myocytes, cardiomyocytes and even endothelial cells (plasticity). Thus, they improve tissue/organ healing or repair due to their “regenerative-potential”. Thanks to this ability, immature SCs and mesenchymal progenitors are clinically applicable in the treatment of patients with myocardial, brain, vascular, liver, pancreas and some other tissue damages. The application of SCs has positive effects on myocardial ischemic damages, including prevention of post-infarction cardiac remodeling following acute myocardial infarction (AMI).

Immature SCs are applicable in the regenerative medicine, being capable of colonizing different tissues due to homing and lineage-plasticity potentials, despite the fact that the ideal type and source of cells have not yet been defined. In practice, SCs can be collected from BM or harvested from peripheral blood after mobilization (chemotherapy/rHuG-CSF). Peripheral blood transplant can be characterized by the absence of the risk of bone infections and anesthesia, with faster hematopoietic reconstruction. During the last decades, peripheral blood is used for more than 70-80% of allogeneic and almost for all autologous “conventional” transplants. On the contrary, BM is the most frequent source of cells used in the fields of regenerative medicine, especially for cardiac repair.

Our (pre)clinical study – is one decade practice by using of native SCs and progenitors, harvested from steady state vs. activated/primed BM – included cardiologic patients ( $\leq 71$  years) with a large first infarction of anterior wall (EF:  $\leq 41\%$ , but  $\geq 19\%$ ). The angioplasty and followed stent placement were performed using percutaneous coronary access. Cells from BM were harvested by multiple aspirations from the iliac crests – collections were performed under sterile conditions in the operation room, while the patents were in general anesthesia. For BM priming, rHuG-CSF was used (3–5  $\mu\text{g}/\text{kgbm}$ , subcutaneously) 24 hours before cell harvesting. The BM aspirate was initially filtered and then processed by Cobe-Spectra (TerumoBCT, USA) and resuspended in serum-free culture medium up to an optimized hematocrit level (Hct = 0.30). The total nucleated cells (TNCs) and MNCs were quantified by Advia-2120 (Bayer, Germany). Using triple staining, CD34 and CD45, as well as immature (CD34<sup>+</sup>/CD90<sup>+</sup>) vs. mature (CD34<sup>+</sup>/CD90<sup>-</sup>) CD34 subset antigens by EPICS-XL (Coulter, USA) were tested. The quantity of applied cells for MNCs and CD45<sup>+</sup>/CD34<sup>+</sup> cells was:  $8.4 \times 10^9 \pm 6.1 \times 10^9$  and  $10.4 \pm 7.54 \times 10^6$  per treatment, respectively. Results obtained for cell viability (determined by 7-aminoactinomycin D or 7-AAD testing) were: 7-AAD<sub>viability</sub> = 95.42%

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(for CD34<sup>+</sup> cells) and 7-AAD<sub>viability</sub> = 95.42% (for CD90<sup>+</sup> cells). Obtained cells (MNCs enriched with native SCs) were administered as an intermittent infusion into the affected artery across coronary catheter (cardiology) or directly into myocardium during coronary artery bypass grafting (cardiosurgery).

In this study, an effective protocol for cell harvesting and processing was verified. Results obtained indicate positive outcome of the clinical use of BM (especially rHuG-CSF primed) derived cells – such as improvement

of systolic function (evaluated 6 months after treatment in the 50% of patients): (a) ejection fraction (EF) improvement  $\geq 5\%$ ; (b) affected infarct region reduction  $\geq 5\%$ ; and (c) decreased left ventricular remodeling. Patients tolerated the use of cell-mediated treatment well, without any adverse effects. Although the mechanisms by which SCs can be trans-differentiated are still poorly understood, the presented results and facts suggest that appropriate populations of BM derived cells could be used as a way of recovery of damaged myocardium.