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National multicentric M13 Stem Cell Trial reports negative outcome - Need to look at VSELs as an alternative to bone marrow MNCs for cardiac regeneration

Sir,

I read with great interest the article by Nair *et al*[1] on a multicentric autologous stem cell trial for patients with acute myocardial infarction (AMI). This was undertaken on the basis of promising results in a pilot ABCD (Autologous Bone marrow Cells in Dilated cardiomyopathy) study reported from All India Institute of Medical Sciences (AIIMS), New Delhi[2]. Though the outcome is negative, it is important for the scientific community as well as the public to be aware of it. The results were not surprising as similar outcome was reported by other groups also[3]. I was disappointed by the discussion which was expected to comprehend recent progress in the field since 2007, lessons learnt and the forward path for the translation of stem cells in the country. Notably, no published articles from 2013-2015 are cited except one of our paper published in 2013.

Simple logic for doing a pilot study is to gauge beneficial effect (if any) of a new concept and if no striking beneficial effect is observed, one should give up on the idea and look at alternative options rather than advocating or concluding by mentioning that similar study on larger number of patients is required. Stem Cell Programme at the National Institutes of Health (NIH), USA was recently closed down[4], and James Anderson, Director of the NIH's Division of Programme Coordination, Planning, and Strategic Initiatives, which administered the Centre for Regenerative Medicine mentioned that the field has progressed very fast and we need to brainstorm on how to move ahead. "To me that's just smart science," he said. "If something is not on track you don't keep spending money on it". The existing chaos and doubts in the field of stem cell therapy for the heart were discussed[5]. Nowbar *et al*[6] have recently compiled various discrepancies in autologous bone marrow (BM) stem cell trials and concluded that the effect of BM stem cell therapy on ejection fraction is zero.

As mentioned in the introduction of the article[1], the transplanted mononuclear cells may exert a paracrine effect, engraft and integrate with host myocardium to bring about regeneration, may help remodel extracellular matrix or improve neo-vascularization or recruit endogenous stem cells. There is some clarity on this now since 2007 when the present clinical trial was initiated by Nair *et al*[1]. Firstly, we need to accept the fact that bone marrow transplantation (BMT) has been successful over decades to cure haematological disorders and is a standard method of care because BM mononuclear cells harbour a sub-population of haematopoietic stem cells (HSCs) which are already committed to haematological fate and thus can regenerate and cure blood disorders[7]. Hoping that the same HSCs can trans-differentiate into cardiac or endothelial cells is probably not true. Expecting the transplanted BM mononuclear cells (MNCs) to exert a paracrine effect (rich source of growth factors and cytokines) may also not hold. Cells in the BM that may be a rich source of growth factors and cytokines are evidently the mesenchymal cells. If we are hoping that a paracrine action can regenerate a damaged cardiac infarct, it will be best to expand mesenchymal stem cells (MSCs) in culture for transplantation rather than concluding that we need to conduct similar study of transplanting BM MNCs (which contain very few MSCs) on larger number of patients. It may be possible that MSCs could differentiate into cardiac cells and integrate with host myocardium as suggested by the authors since both MSCs and cardiac cells are mesodermal in origin. But this needs to be demonstrated *in vitro* first. van Berlo *et al*[8] have shown using lineage tracing studies that c-Kit positive cardiac stem cells in the heart do not differentiate into cardiomyocytes but

this is still disputed[9]. Various groups have reviewed different kinds of stem cells and their potential for cardiac regeneration[10,11]. It becomes extremely crucial to select the best stem cell candidate in the clinical settings.

BMT in both male and female patients who undergo very aggressive oncotherapy and conditioning regimen often is accompanied with spontaneous pregnancies and live births[12]. It was postulated that possibly the BM is a source of germ cells[13]. Thus a study was undertaken wherein mice were treated with cyclophosphamide to ablate the gonads and later green fluorescent protein (GFP) labelled BM MNCs were transplanted. However, all the pups born were non-GFP[14] and belonged to the recipient germline.

Research being carried out in a handful of laboratories across the world including our laboratory suggests that the ideal stem cells for regenerative medicine are possibly the primitive and pluripotent endogenous stem cells termed the very small embryonic-like stem cells (VSELs) existing in various adult organs (including the heart) throughout life and give rise to tissue specific progenitor stem cells which further proliferate and differentiate to maintain homeostasis[15]. More clarity is needed to define stem cells and distinguish them from the progenitors in the body organs[16]. VSELs survive oncotherapy in mammalian testis[17], ovary[18] and also in the BM[19] and could possibly result in spontaneous pregnancies. Regenerative potential of VSELs has been reported in mice for lung epithelium[20]; cardiac tissue[21,22]; neurons[23]; pancreas[24,25]; liver[26]; and in humans for critical limb ischaemia[27]; cardiac tissue[28,29]; and diabetes[30].

It is not clear as to why Nair *et al*[1] did not use VSELs rather than bone marrow MNCs. Miyanishi *et al*[31] were unable to detect VSELs by flow cytometry despite using published protocols and the same equipment. It was Ratajczak's group which successfully characterized these stem cells and reported that the VSELs were equivalent to primordial germ cells (PGCs)[15]. Rather than migrating to the gonads during early development, PGCs possibly migrate and settle in all developing organs and persist throughout life as VSELs[15].

The reason why VSELs have remained elusive till date is their very small size and rare occurrence and these get unknowingly discarded while processing cells for various experiments. Density gradient centrifugation method is the standard method to isolate stem cells (from bone marrow or peripheral blood) wherein the mononuclear cells (MNCs) are enriched in the 'buffy coat' and the red blood cells at the bottom are discarded. However, it was found that VSELs invariably settle down with the RBCs[32,33] and thus have never been extensively studied.

It will be ideal for Nair *et al*[1] to modify their isolation protocols to include VSELs (for regeneration) and MSCs (as a source of growth factors and cytokines) in their trials in future rather than transplanting MNCs in more number of patients. HSCs do not trans-differentiate and regenerate the cardiac tissue. Moreover, injecting the stem cells into the margins of the infracted heart may be a better option than transplanting cells through coronary artery.

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