INVITED COMMENTARY

Commentary on "Efficient Differentiation of Bone Marrow Mesenchymal Stem Cells into Endothelial Cells *in vitro*"

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Chengen *et al.* have published an interesting experimental study entitled "Efficient Differentiation of Bone Marrow Mesenchymal Stem Cells into Endothelial Cells *in vitro*".[1] They describe an improved method of differentiating human bone marrow mesenchymal stem cells (MSCs) into endothelial cells (ECs). Bone marrow cells from human donors were retrieved; MSCs were isolated by density gradient centrifugation and cell culture in Iscove's Modified Dulbecco's Medium supplemented with 10% fetal bovine serum. Adherent cells were grown in the same fresh medium until they displayed a marked spindle shape and the CD marker profile identified the cells as MSCs. A cocktail of growth factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, insulin-like growth factor, and epidermal growth factor was then added with ascorbic acid and heparin, to stimulate MSCs to differentiate into ECs. With this inducing medium, Chengen *et al.* were able to generate a larger proportion of ECs than in previous studies. They defined ECs by expression of CD31 and CD34 during immunostaining, and by von Willebrand factor, vascular endothelial cadherin and VEGF receptor 2 in quantitative reverse transcription polymerase chain reaction. They also show that the differentiated ECs were able to grow into tube like structures in Matrigel. The authors report a successful differentiation of up to 60% of MSCs into ECs after 14 days and conclude that these results provide a method to efficiently promote differentiation of MSCs into ECs *in vitro* for potential application in peripheral arterial disease (PAD).

In the last decade, cell based therapies have been explored as a treatment option for patients with PAD with critical limb ischaemia (CLI) with no surgical or endovascular option for revascularisation. Several clinical studies have been conducted to test the efficacy of autologous bone marrow derived cell therapies for the treatment of CLI, ranging from case reports, small series, uncontrolled trials, and randomized controlled trials. The Rejuvenating Endothelial Progenitor Cells via Transcutaneous Intra-arterial Supplementation (JUVENTAS) trial assessed the impact of a transfusion of autologous bone marrow mononuclear cells (BMMNCs) in severe non-revascularisable PAD.[2] After 6 months, there were no differences in the primary end points between those treated with BMMNCs and placebo regarding major amputation (19% vs. 13%; $p = .81$), minor amputation (11% vs. 13%; $p = .95$), or death (5% vs. 6%; $p = .74$). Along with the JUVENTAS results, the authors presented a meta-analysis of all placebo controlled trials using autologous BMMNCs. Regarding major amputation, BMMNC patients appeared to enjoy a significantly better outcome (risk ratio [RR] 0.66, 95% confidence interval [CI] 0.47—0.93; $p = .02$) compared with placebo; however, when the meta-analysis included only trials with a blinded design, this advantage disappeared (RR 0.95, 95% CI 0.64—1.39). These results have been confirmed by more recent meta-analyses,[3,4] which considered other end points besides amputation, such as survival, amputation free survival, and wound healing, and indicate that currently there is no clear evidence to support a therapy with autologous BMMNCs. New experimental strategies to improve BMMNC therapies should be explored and the results of the study by Chengen *et al.* may represent an interesting development. However, the authors should be encouraged to investigate their strategy in a high quality, placebo controlled clinical trial, with a mandatory double blinded design.

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