Sir

I read with interest the article by Bhansali et al where beneficial effects of transplanting mesenchymal stem cells (MSCs) by tail vein injection in streptozotocin (STZ) treated rats were reported. Similar beneficial effects have been earlier reported by the same group after autologous bone marrow transplantation in patients with type 2 diabetes mellitus (T2DM).

The authors are requested to observe carefully Figure 1 of their article copied below (Fig. 1). It clearly shows presence of a sub-population of very small embryonic-like stem cells (VSELs) in MSCs culture (circled) implying that a mix of MSCs and VSELs existed in their cultures. It was not clear from the information provided as to what passage cells were shown in the Figure and whether these cells were transplanted. The VSELs are observed very clearly in primary cultures and tend to get reduced in subsequent passages but these cells exist and also survive within the MSCs by a process termed ‘emperopolesis’. We have earlier discussed about the presence of VSELs as a sub-population among MSCs and how OCT-4 (octamer-binding transcription factor-4) staining can differentiate these from each other. We have shown presence of VSELs in Haematoxylin and Eosin (H&E) stained smears prepared by enzymatically digesting mouse pancreas (Fig. 2), characterized them and demonstrated how these are involved in regeneration of mice pancreas after more than 70 per cent of pancreas is surgically removed. Nuclear OCT-4 positive VSELs differentiated into cells (pancreas specific progenitors) with cytoplasmic OCT-4 which co-expressed pancreatic and duodenal homeobox-1 transcription factor (PDX-1). Our results are in agreement with earlier reports where Ratajczak’s group has reported a possible role for VSELs to improve pancreatic function after bone marrow transplantation (BMT) and also

**Fig. 1.** This is Figure 1 of the article by Bhansali et al showing VSELs (encircled) which have been missed out possibly because of the very small size. These cells have been ignored till now but are pluripotent stem cells that survive in various adult tissues throughout life. **Source:** Ref. 1 (Reproduced with permission).

**Fig. 2.** Haematoxylin & Eosin (H&E) stained smears of cells from normal mouse pancreas to appreciate the very small size of VSELs (arrow) at 10X, 20X and 40X. Cells marked in asterix are the pancreas specific progenitors. **Source:** Ref. 5 (Reproduced with permission).
demonstrated an increased mobilization of VSELs in patients with pancreatic cancer. Whenever the pancreas function gets compromised, e.g., by pancreatectomy or by STZ treatment, VSELs get mobilized from the bone marrow and rush to the damaged pancreas in order to regenerate it. We have observed 6-folds increase in numbers of VSELs in pancreas of STZ treated mouse compared to normal control mouse pancreas (unpublished observations). Similar mobilization and a role of VSELs during hepatic regeneration was recently reported by Chen et al. Two other groups have also reported presence of nuclear OCT-4 positive, very small sized cells in human pancreas (although they have not named them as VSELs).

The increase in numbers of islets in the transplanted group observed in the study is impressive but this may be due to differentiation of endogenous VSELs. It could be possible that STZ treatment mobilized VSELs into the rat pancreas which differentiated into islets. This was facilitated by the presence of healthy MSCs which were transplanted and acted as a source of growth factors. MSCs do not transdifferentiate into islets and this has been mentioned by the authors in their introduction. In a recent review we have discussed how VSELs have remained elusive till date and missed by various investigators because of their very small size and presence in very few numbers.

We agree that transplanting MSCs will have a crucial role for regenerative medicine but it is important to first understand basic stem cells biology and recognize presence of VSELs in various adult tissues. Direct inter-tubular injection of Sertoli/mesenchymal cells in chemoablated (25mg/kg busulphan) mouse testis restored spermatogenesis from VSELs that survived due to their relatively quiescent nature. This was confirmed by the observation that transplanting green fluorescent protein (GFP) positive Sertoli/mesenchymal cells yielded non-green sperm. Further, a direct transplantation of MSCs in a diabetic mouse pancreas may be a better option than tail vein injection used by the authors.

Conflicts of Interest: None.

References

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Authors’ response

We thank Deepa Bhartiya for her interest in our article and the opportunity to clarify a number of points from our work.
The Figure of mesenchymal stem cells (Fig. 1A)\(^2\), which we presented in our article was obtained at the time of adherence (P0) during the procedure of isolation/culturing of the mesenchymal stem cell. It is expected that some non-MSCs may also be present as these are derived from bone marrow. These cells were not characterized at P0 stage, as it has been previously reported that the expression of stemness marker and homogeneity of MSCs enhances with increasing passage number\(^3\). Therefore, the cultured cells were characterized after three passages for the expression of rat MSCs surface markers, and found high positivity of CD29 and CD54 (>99%) and absence of CD45 and CD34, indicating the negligible presence of non-MSCs cells. This shows that with prolonged culturing of MSCs, the numbers of non-specific cells are progressively reduced. Further, the isolated MSCs were shown to have high expression of CD29, which was absent in very small life embryonic (VSEL) stem cells\(^4\), substantiating that the cells used for transplantation were preferentially MSCs. We scrolled the images, which obtained at different days of MSCs culturing, but failed to find VSEL type cells as pointed out by the author\(^1\).

The mechanisms involved in improvement of glycaemic control after stem cells transplantation remains elusive. Existing data support the role of MSCs in regenerating the islet cells as well as facilitating the islet cell proliferation\(^5\). The role of VSELs in regeneration of β-cells as a part of procedure of MSCs culturing was unlikely in our study as we administered preferentially cell population of MSCs, though the possibility of STZ-induced mobilization of endogenous VSELs into the islets cannot be excluded\(^6\). Further, it was difficult to conclude in our study whether trans-differentiation or fusion of labelled stem cells with β-cells resulted in improved glycaemia due to experimental limitations.

Your suggestion of transplantation of stem cells directly into the pancreas rather than through rat-tail vein is impressive. This may be because direct transplantation of stem cells into the pancreas may be more effective to control hyperglycaemia than through peripheral route\(^7,8\).

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References