**Reviewer’s report**

**Title:** Therapeutic potential of placental mesenchymal stem cells after transplantation through portal vein into Chinese miniature pigs with acute liver failure

**Version:** 1  **Date:** 25 October 2011

**Reviewer:** Tamara Vanhaecke

**Reviewer’s report:**

The general aim of the study is to determine the therapeutic potential of MSC from human placenta for D-galactosamine-induced acute liver failure in miniature pigs. The manuscript is well written and easy to understand. However, it needs some major revisions before publication (see below).

**Major compulsory revisions**

**Major remarks concerned with the methodologies used:**

- positive control cells should have been included for IC and PCR experiments with respect to osteogenic and adipocyte differentiation; so in Figure 4 both for the pictures and the PCR data results of human adipocytes and osteocytes should be shown as well

- in analogy to the previous point, in Figure 5 also the data for human hepatocytes should be included in the PCR data

- quantitative RT-PCR should have been used taking into account the MIQE guidelines

- an MTT assay measures cell viability, but not cell proliferation. So, to know the effect of irradiation on proliferation of hPMSCs, an assay specifically measuring DNA synthesis (e.g. thymidine incorporation, BrdU labeling) should have been used, or the authors have to talk about cell viability instead of proliferation in the text.

- based upon positive expression of ALB, AFP and CK18, the authors claim to be able to differentiate the hPMSC into ‘hepatocyte-like cells’. However, it would have been much more convincing if they could also show some expression of CYPs, and even better CYP activity (vs. human hepatocytes). In addition, as they also have expression of CK19, which is a marker for cholangiocytes, it is obvious that the obtained cells are in an early, hepatoblast state rather than being ‘hepatocyte-like cells’.

- with regard to osteogenic differentiation, it is better to use Alizarin Red S to stain for Ca2+-deposits (bone mineralization) instead of ALP expression as the latter is also present in cartilage (chondrocytes) and thus is not specific enough

**Major remarks concerned with the conclusion:**

- in the discussion part, 4 possible explanations are pointed out as why
non-irradiated hPMSCs transplantation is preferred over irradiated hPMSCs and no cell transplantations. Yet, real data are not included in the paper to support these 4 hypotheses. Moreover, in the ‘conclusion’ part, it is stated that the underlying mechanisms still need to be determined. Therefore, the authors should either clearly state in the discussion that it involves hypotheses for which no evidence is yet provided or provide evidence to support the hypotheses. Therefore, it is highly recommended to perform for example stainings of anti-human ALB, AFP and CK18 on liver slices obtained from sacrificed miniature pigs after transplantation (at least 1 month after successful engraftment) to show the successful in vivo conversion of hPMSCs into ‘hepatocyte-like cells’. Furthermore, the immunomodulating properties of engrafted hPMSCs can be evidenced by performing ELISA assays for immunomodulating agents such as LIF.

Minor essential revisions
- the list of abbreviations is not complete (e.g. MTT, Tri are missing)
- p7: are references 24, 25, 26 and 27 correct ?
- concerning the methods used: in general much more details need to be presented with respect to the isolation and cultivation of the hMPSCs (e.g. collagen concentration used, initial cell density, type of plates used,…)
- always specify what is meant by % when describing the methods: v/v, w/v?
- give the concentration of the antibodies used
- radiation should be replaced by irradiation
- p11: cells were ‘processed’: indicate how
- p14: 3000rpm should be converted into g force
- p<0.05: indicate what has been compared to what exactly
- Figure 9: indicate clearly on all figures the onset of the injury with D-galactosamine and the start of the transplantation with the hPMSCs

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests