Reviewer's report

Title: Anti-angiogenesis therapy based on the mesenchymal stem cells genetically engineered to express soluble Flt-1 in mouse tumor model

Version: 2 Date: 3 December 2007

Reviewer: Vincent Kindler

Reviewer's report:

Review of the work of Hu et al.

The work of Hu et al describes a series of experiments aimed at the modulation of tumor metastasis by MSC engineered to secrete large amounts of soluble Flt-1. The rationale is that soluble Flt-1 should saturate (and thus inactivate) the VEGF present within the tumor. This should in turn block local angiogenesis, and subsequently tumor growth and metastases. Murine MSC derived from male mice are injected into female and the fate of the recipients and/or their tumor is monitored. According to the data provided, VEGF sequestration by soluble Flt-1 is effective, as metastases and angiogenesis are significantly decreased in the animals treated with the engineered MSC, as long as these cells can reach the tumoral territory.

The work is in general well presented and the data are sound. However several improvements have to be done before releasing the manuscript for publication.

Major compulsory revisions:

- The correctness of the language has to be checked. Some sections of the results and the discussion are sometimes difficult to understand obviously because words are missing. Being myself not an english-speaking native (so please also forgive my mistakes) I know how it is difficult to be absolutely correct in the wording. The authors should have their text revised by a competent reader.

- Concerning the claim that authors work with MSC, I am not convinced that the analysis of 3 markers by FACS is sufficient to prove the point. MHC class I and class II markers should be evaluated, as well as other markers such as CD14, B220, CD86. Moreover, evidence of cell differentiation into mesenchymal lineages in vitro should also be provided. These data should be shown on a figure. Alternatively, the authors could choose to consider that their cells are simply "bone marrow-derived stromal cells" that can support an anti-angiogenic effect when transfected with Flt-1. In that case, the title and the discussion should be modified.

- Concerning figure 2, and the corresponding data. It seems clear that Flt-1-transfected MSC inhibit lung metastases, however, from the figure, one can see that unmanipulated stromal cells induce an increase of metastases. This should be discussed in details, especially taking in account the recent paper of

Minor essential revisions:

- **Introduction and discussion:** the backup information given by the authors quoting references 4-7 is correct, though their intermediate conclusion is a bit too optimistic. Human MSC are carefully and slowly reaching clinical trials, but the term "traditionally" is a misuse in this context. Moreover, according to an oral communication of Dr Horwitz (ref 5) the corrective effect is transient and infused MSC become rapidly undetectable in the hosts. It remains to be established if MSC are stem cells able to sustain long-term regeneration of tissue in human clinics.

Further down in the text: the role of tumor stroma in the modulation of tumor growth has been extensively explored. The reference (12) is valid, however the dynamic role of the stroma in the control of malignancies, as for instance described by Blankenstein et al in Current Opinion in Immunology 2005, 17:180â##186 should be taken in account and discussed in the discussion section.

- **Materials and methods:**
  Culture conditions of the MSC should be described. Temperature, humidity, CO2 level, as well as the cell passages used.

- **Results:**
  Observation of potential toxicity of infused MSC. I acknowledge the effort of the authors to scrutinize the organs of the animals for eventual pathologies provoked by MSC. However no data concerning MSC themselves are provided. What about the survival of the MSC injected in animals that were not co-injected with tumor cells? Did the authors detect MSC in these animals and can they at least discuss this point in the light that the long term-survival of MSC in the host may help the onset of spontaneous malignancies? Did they for instance run FISH analyses on tumor-free animals to follow up MSC survival?

- **Discussion:**
  Second paragraph: the statement of "transdifferentiation into stromal cells" has to be clarified.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**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.