Format for ANSWERING REVIEWERS

October 1, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13418-review.docx).

Title: In vivo imaging of endogenous neural stem cells in the adult brain

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Name of Journal: *World Journal of Stem Cells*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 1:

1. “Endogenous neural stem cells (eNSCs) in the adult mammalian brain can be mobilized by e.g. pharmacological methods to facilitate regeneration and enhance functional recovery in neurological disease.” How can they propose to track down these endogenous eNSCs over a lifetime of the body? What’s minimum number of eNSCs monitored using the method? How did they use the non-invasive imaging of tumor cell proliferation with PET to locate if tumor cells cease the proliferation?

MR- and PET-imaging is non-invasive and can be applied repetitively over time, i.e., in a longitudinal fashion. This allows for tracking eNSC over any period of time. However,

while in vivo imaging monitors both the quantity and localization of cells, it is not adept to follow a specific single cell over time. As suggested by the reviewer, we now elaborate on this issue on p. 5 of the revised manuscript.

Using MRI as the imaging technique with the highest spatial resolution, single cells labeled with iron oxide particles can be detected migrating away from the in vivo injection site of the iron oxide. It must be noted that a distinction of cells is not possible at the injection site itself (NSC niche) due to image distortion. Bioluminescence imaging can be used to detect cell clusters of ~10^3 cells under ideal conditions, PET detects ~10^4 cells, depending on the quality of the scanner and on image reconstruction, while MRS has quite a low resolution, possibly detecting ~10^6 cells. As requested, we now include this information on pp. 6-7 of the manuscript.

The radiotracer [^{18}F]FLT labels all proliferating cells, and can therefore be used to detect proliferating tumor cells. Our own longitudinal studies on gliomas in mice and humans during antiproliferative therapies show that a reduction in tumor proliferation can be detected by [^{18}F]FLT-PET as soon as 3 days after initiation of the treatment. We now mention this in the revised manuscript on p. 7.

2. They should clearly provide the guideline to strike a balance of the specificity (signal versus noise ratio) and resolution acquired, such as Stem Cell Rev. 2010 Jun;6(2):317-33. doi: 10.1007/s12015-010-9130-9. (A biological global positioning system: considerations for tracking stem cell behaviors in the whole body.)

We thank the reviewer for drawing our attention to this highly relevant review on the topic of detecting transplanted, pre-labeled stem cells. We now cite this paper on p. 5 of the revised manuscript. As suggested, we now clearly highlight sensitivity and specificity of each imaging method on pp. 6-7.

3. Page 4: “More biomarkers were consecutively identified including Sox2, sonic hedgehog (Shh) pathway components, PDGF, EGFR, GFAP, Hes3, Hes5, Musashi, and CD133” – It’s a conundrum that these molecules are present in other cell types – how do the authors distinguished these different cells (somatic versus stem cells)?

It is indeed still difficult to distinguish endogenous neural stem cells immunohistochemically, since no single marker has been identified to label them

unambiguously. Therefore, co-staining is usually required to characterize the cells. We do not specialize in the immunohistochemical detection of NSCs ourselves, and the topic is also not in the scope of our review article. However, following the reviewer's suggestion, we now report this challenge on p. 4 of the revised manuscript.

4. Page 7: Stroke mediated focal cerebral ischemia induces pro-inflammatory cytokine production causing neuroinflammation, detrimental to normal tissues. However, neuroinflammation also induces prepare and fortify eNSCs after stroke. What's threshold or time course for such balance of pro-inflammatory cytokine production to achieve benefits but avoid detrimental? For example, Page 8 –" since immune cells proliferate in the ischemic brain just as eNSCs do, and [^{18}F]FLT-PET does not differentiate between stem cell- and immune cell-derived proliferation." How can you track eNSCs instead of immune cells? Or both? Or just one type of cells? How can they differentiate them?

Neuroinflammation has been characterized as a 'double-edged sword' with both beneficial and detrimental effects on the prevention of secondary tissue damage, regeneration and recovery. Destructive effects of neuroinflammation include the damage caused by reactive oxygen species and excessive production of proinflammatory cytokines by immune cells, beneficial aspects are the containment of necrotic tissue and the induction of a strong regenerative response including the recruitment of endogenous NSCs. Quality, extent and timing of neuroinflammatory processes determine whether manipulating that particular response after stroke will be deleterious or therapeutically beneficial. The activation of resident microglia can under some circumstances be neurotoxic, under others neuroprotective, depending on the specific activating conditions. Interestingly, differentially activated microglia also have opposing effects on NSC. As recommended by the reviewer, we now added this issue to the manuscript on p. 8.

On page 9, we highlight how eNSCs and immune cells – that are both visualized by the radiotracer [^{18}F]FLT – can be distinguished in vivo by the use of an additional radiotracer, [^{11}C]PK11195, that specifically visualizes post-ischemic cellular neuroinflammatory processes. Co-registering imaging data on [^{11}C]PK11195- and [^{18}F]FLT-accumulation then allows for the conclusive differentiation between cell

proliferation from eNSCs and immune cells, as illustrated in Figure 3. Ex vivo, NSCs and immune cells can be distinguished immunohistochemically, allowing a validation of the imaging data.

Reviewer 2:

1. It would be useful to add a comment on what is known about the potential toxicity of [^{18}F]FLT.

We thank the reviewer for this valuable comment. No evidence of toxicity or other complications have been reported following intravenous injection of the radiotracer [^{18}F]FLT, according to a study by Turcotte et al. (BMC Nucl Med. 2007). We now cite this study on p. 7 of the revised manuscript.

2. It would be helpful to detail more the legend of Figure 1 to help the reader interpreting each panel. In particular, what are the white circles pointing at?

As suggested by the reviewer, we expanded the legend to figure 1, explaining the images in more detail (p. 21).

Again, we are grateful for the reviewers' input that helped us to provide a comprehensive revision of our manuscript. I strongly hope that you and the reviewers now find this review article to be of high interest for the readers of the *World Journal of Stem Cells.*

Looking forward to hearing from you.

Yours sincerely,

Prof. Dr. M. Schroeter