Reviewer's report

Title: Early stage transplantation of bone marrow cells markedly ameliorates copper metabolism and liver function in Wilson's disease mice

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Reviewer: Nicole Buck

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Major Compulsory Revisions

1. The writing of the manuscript needs to be edited for English language, currently it is difficult to understand, with several words used incorrectly.

2. The Authors only look in the liver for donor cell engraftment. Why not look at other sites as well to ensure that the liver is the major site of engraftment? The bone marrow, blood and spleen for example.

3. The Authors determine donor cell engraftment in the female recipient mice only. How many of these female mice were engrafted? What about the male mice?

4. The best method to determine cell engraftment is to use PCR of the WND gene on all the recipient mice. This method should be used on all the samples. The SRY gene could then be used as a secondary method to confirm the results.

5. What is the sensitivity of the SRY PCR? There are very nice bands in the gel photos, suggesting very high levels of liver engraftment, but how much is it? Could the Authors show a positive control, negative control and some mixing studies to give an estimate of the amount of cell engraftment and sensitivity of the method? Could they use real-time PCR?

6. Is it possible to look for CM-Dil fluorescence in BM cells isolated from the recipient mice by flow cytometry?

7. How many mice were in each group? The Authors say 40 mice/group with (3:2 female to male ratio), but there are no n values on the graphs or listed anywhere to know exactly how many mice were analysed for each test.

8. How many bone marrow cells were transplanted? The methods section does not mention lysis of the red blood cells, so how many bone marrow cells were transplanted and how many red blood cells? Final concentration in the methods sections states 6x10^7/ml, but then they transplant 0.2ml of 1.2x10^7 cells/mL = 2.4 x 10^7 cells transplanted, or should this be 1.2x10^7 cells?

9. Were the mice perfused prior to sample collection? Could the Authors comment on whether they think the cells may just be in the blood rather than in the liver and how they came to this conclusion?

10. Para 3 of the Background section suggests that bone marrow cells have been “proved to differentiate into hepatocytes”. Nothing can be proved. There discussion only mentions cell fusion as the mechanism of how the BM cells...
engraft in the liver...not differentiation. There is still a lot of controversy in the field as to the mechanism and thus the Authors should discuss both mechanisms and then suggest that their work did not attempt to discern between the two theories.

11. To test whether the donor cells are active they could look for WND gene mRNA. Given the recipient cells do not have the gene it would give direct evidence that the donor cells are active within the recipient mice.

12. Ref 14 is incorrect it should be Buck et al. 2007, Hepatol Int.

13. Please check all the references are correct. There are several incorrectly referenced. For example Ref # 23 does not mention Wilson’s disease mice.

14. What is in D-hanks?

15. The BM cells are transplanted in D-hanks, but the sham transplant control is saline. The authors need to explain why they did not use the D-hanks as the correct sham transplantation control. Or explain why the cells were not in saline.

16. Figure 1 shows some images of 4 weeks post transplant. Which group was this mouse from? How much engraftment did it have? Do the values and number of cells detected correlate?

17. If the error bars are overlapping, how can the results be significantly different?

18. Need to add n values to all the graphs.

19. Figure 2: A) has a final copper concentration of about 800, whilst B has an initial copper concentration of 1100...shouldn’t they be the same, or very similar?

Minor Essential Revisions

1. The abstract suggests that there is “significantly improved copper accumulation”, which is the opposite of what the results show and what the aim of the experiments were to do, could the authors re-word this sentence.

2. What sizes are the PCR products produced in the SRY assay?

3. Methods section: Statistical analysis provides a probability value rather than a possibility value, please fix wording.

4. What % viability did the cell preparation need to be for transplantation? Minimum of 90% viable cells?

5. What computer program was used to estimate the ratio of SRY and b-actin?

6. “Additional tissue from liver, brain and kidney were sorted”, should read “Additional tissue from liver, brain and kidney were stored”.

7. What sort of blood tubes were the blood samples collected in?

8. The Background section (para 1) says the sites of copper accumulation are ‘variable’. This is incorrect, I think the authors have used the wrong word, maybe a better word would be ‘various’?

9. Para 2 of the Background section should highlight that the recent evidence has been derived from rat experiments.
10. Figure legend 1 compares the amount of engraftment in the two groups to the saline group. The saline group would not be expected to have any cells, so the analysis is incorrect. I think this may be a typographical error rather than a data analysis problem.

11. Please provide the error values for the liver copper results in the text.

12. Figure 1 mentions green arrows…they are white.

**Level of interest:** An article of limited interest

**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