Reviewer’s report

**Title:** Effect of low frequency magnetic fields on melanoma: tumor inhibition and immune activation

**Version:** 2 **Date:** 15 June 2013

**Reviewer:** Robert M Lafrenie

**Reviewer’s report:**

Major revisions

The manuscript, “Effect of low frequency magnetic fields on melanoma: tumour inhibition and immune activation”, has been reviewed previously and revised. In my opinion, the current version of the manuscript is improved although several deficiencies remain. First there are significant issues with English grammar and style which sometimes obscure meaning. Another significant issue is the lack of description about how many times experiments were repeated which makes understanding the statistical analysis difficult.

One of the more obvious questions is regarding the flow cytometry data. It is stated that “the S-phase rate showed a significant decreasing, from 40.76% to 37.24%; and the G2/M phase-rate was significantly increased from 8.9% to 11.6%...”. These are extremely small differences which are unlikely to be statistically significant (and are likely not biologically significant). How was this done (the response to the previous review suggests 3 replicates)? I suspect the 40.76+~1% value was generated for the specific histogram and compared to the 37.24+~1% for a separate histogram (Figure 2F). This should be done by performing at least 3 independent experiments and determining the percent mean+SD for each condition and then using statistics to determine if they are different (as indicated in the response). In my experience, having a standard deviation in the percentage of positive cells of 5-10% is astonishingly good and would mean the reported differences are not statistical. Therefore, this data does not convincingly support the discussion and have to be supported by additional experiments (eg cyclin measurements, BrdU labelling, cell synchronization experiments) or the discussion altered.

How many independent experiments were performed in order to produce the figures? This should be easy to find. How many cells were used to calculate the % affected cells reported in Figure 3E and Figure 4D? How many microscope fields were examined for Figure 4B and C? It is still not clear to me whether the Ki-67 staining is measured in tumour tissue in the tumour-laden mice (and compared to normal lung tissue in the tumour-free mice). This would indicate that MF affects tumour proliferation but not normal lung proliferation. If the difference in tumour size is related to immune destruction, the proportion of apoptotic cells should increase in the tumours – and could be measured by TUNEL.
The cytokine array analysis in Figure 5 needs some more explanation. How many different arrays were used in the analysis? How many plasma samples from the mice were used to produce the graphs in Figure 5E and F. Were samples combined for each array (1 ml of plasma is very difficult to get from a mouse)? Was there any independent verification of the array data? The answers to such questions will determine the reliability of the array data.

I still have several issues with the flow cytometry data shown in Figures 6, Supp 1, and Supp3. How many individual mice were examined (3 different mice or the same mouse sample 3 times)? The standard errors reported are quite small and I wish to confirm how the data was generated. In figure 6A and B, the data seem straightforward and difference in FoxP+ T cells in spleen between tumour and no-tumour mice looks as expected on the plots. (I presume the difference between A and B reflects the graph in B being derived from 3 independent experiments.) The CD11+ cell number differences are still very small and the plots more difficult to be convincing – I would hesitate to make too much of these differences in the discussion.

The data for B16-BL6 apoptosis in Supp 2 still show the very small differences in annexin/PI positive cells and I think an additional type of experiment is needed to support the discussion about apoptosis (although this is appropriately discussed in the manuscript). Basically, the conclusion is that the effect is probably not apoptosis although the data really does not allow a conclusion. The data is not convincing one way or the other.

The data in Supp 3 shows very potent differences between T cells in the spleens of tumour and non-tumour mice. The differences between tumour-MF and tumour-Sham are less strong. The observation these numbers relate to cells in spleen may not be directly related to peripheral surveilling immune cells – could magnetic fields simply alter sequestration?

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

No competing interests.