Reviewer’s report

Title: Macrophage traits in cancer cells are induced by macrophage-cancer cell fusion and cannot be explained by cellular interaction

Version: 4 Date: 3 June 2015

Reviewer: Nathalie Scholler

Reviewer’s report:

The revised manuscript did not address my concerns, except for figure 2.

Major compulsory revisions remain needed for

Figure 3

The negative control for macrophage IF staining with CD163, that is the 2nd antibody alone (= goat anti-mouse IgG Alexa Fluor 546), needs to be shown. It is well known that staining needs to be calibrated for each cell type and that discrepancy between IF and flow cytometry analysis is common. The fact that MCF7 cells are not stained by anti-CD163 detected by goat anti-mouse IgG Alexa Fluor 546, does not demonstrate that goat anti-mouse IgG Alexa Fluor 546 antibody cannot directly bind to macrophages, for example through the Fc receptor. Since the entire manuscript aims to demonstrate the existence of cell fusion between GFP-MCF7 and macrophages, one must eliminate the non-specific IF staining of putative macrophage-cancer cell fusion with goat anti-mouse IgG Alexa Fluor 546 antibody.

Figure 4

The authors did not answer my question and did not edit the figure 4. The point of having 2 panels is to demonstrate that hybrids arise only after coculture (panel 4a) and that no hybrids are produced after transwell coculture (panel 4b). If there are no hybrids, then there are no cells looking like hybrids that can be isolate, called “hybrids”, and analyze as hybrids. Thus the panel 4b should be clarified or suppressed.

Figure 5

It is still not clear why the authors show 2 panels of IHC in figure 5a. Are these slides from 2 different patients? Why is the quality of the staining so different between the 2 panels? Furthermore, the text emphasizes the organization of CD163-positive cancer cells in a “growth pattern of clonal collections”, as marked by a red arrow, but it fails to reach a conclusion about the so-called "CD163-negative cancer cells". Finally, it is not clear at all to me that the cells marked with a blue arrow are actually cancer cells. They could be stroma cells and/or leukocytes. A pathologist should analyze the slides and provide a report to be included in the manuscript, and/or a positive staining demonstrating that all the cells of these slides are tumor cells should be added to the figure. In function
of these results, the figure 6 might have to be entirely replotted.

Likewise, the minor essential revision for Figure 1 remains the same: There are still exclamation points after macrophages, PET membrane, and MCF-7 cancer cells in the legend of the figure. The exclamation points should be removed.

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

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competitive interests.