Dear Dr. Yang,

Thank you for considering our manuscript “Tumor-associated macrophages, multi-tasking cells in the cancer landscape” for publication in Cancer Research Frontiers.

We are grateful to the Reviewers for the positive comments and suggestions. We carefully considered all reviewers’ comments and revised several sections of our manuscript accordingly.

Here are listed the modifications we did based on the reviewers’ comments.

**Reviewer 1:**

According to the observation of this reviewer, although the majority of TAMs display an M2 phenotype, different studies demonstrated heterogeneity in the phenotype of TAM, having also an M1 phenotype and anti-tumor activity. For this reason, we added a paragraph (section “Origin and phenotype of tumor-associated macrophages (TAMs)”) describing the anti-tumor activity of TAM. In addition, we included the references indicated by the reviewer (Ref. 42, 43, 44).

**Reviewer 2:**

As requested by the reviewer:

- We changed the title of the second paragraph
- We moved the section on M1-M2 macrophages in the second paragraph discussing the phenotype of TAMs
- In the section “Origin and phenotype of tumor-associated macrophages (TAMs)” we better underlined how TAM localization within tumor tissues has a different impact on their phenotype and functions.

The paper of Casazza et al., Cancer cell 2013, which was by mistake deleted, has now been reintroduced in the list of references (Ref. 55).

**Reviewer 3:**

Reviewer 3 asked to include the original papers and not reviews when citing specific results. We have included several original references, though we feel it is almost impossible to cite all the pertinent papers in such a popular area of research. In the reply, we here below indicate the lines where reviewer 3 asked for modification. Line number has now slightly changed, as additional sentences have been included to respond to the reviewers. The line number indicated below thus refers to the original comments of reviewer 3.

- Line 23 : as requested we replaced “immune” with “leukocyte”
- Line 44-46: references were added (Ref. 3 and 4). Authors comment: the profound impact of the tumor microenvironment on macrophages is demonstrated by the different transcriptional profile of macrophages derived from normal and tumor tissues.
- Line 49: authors comment: the concept that tumors can be recognized by the immune system is well established. We now cite a more appropriate reference by Schreiber et al (Ref. 5). We would like to remind the Referee that though nude mice do not present increased tumor susceptibility, more immune-deficient mice (SCID, SCID NOD) do have higher incidence of spontaneous tumors, including non-haematological tumors.
Authors comment: regarding the similarity between wounds and tumors, we would like to raise the issue that wounds are not immunogenic, while cancer cells can express antigens.

Lines 63-67 and line 72: authors comment: in this paragraph we were discussing the M1/M2 differences in a general context, not in tumors. However, IL-4 can be present at tumor sites, produced by Th2 CD4 effector cells. For example: DeNardo et al, Cancer Cell. 2009. “CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing pro-tumor properties of macrophages” (Ref. 48) and: Wang et al, Cell cycle 2010: “Alternative activation of Tumor-Associated Macrophages by IL-4, priming for tumor functions” (Ref. 49); plus other papers. Some references have been added (26, 31 and 32).

We are surprised that the reviewer asks for demonstrating that IL-1 is an M1-associated product. IL-1 is stimulated by LPS, which is the canonical stimulus for M1 polarization.

- Line 73: the sentence has been modified and we added new references (Ref. 26 and 27) supporting our observations.
- Lines 118 and 120: we changed the original reference with other references supporting our observations (Ref. 34, 35 and 39).
- Line 122: see comment above, line 63-67
- Line 128: the sentence of double expression of M1 and M2 markers has been deleted
- Line 141-157 and line 215: we discuss about arginase in the section “Pro-tumoral function of TAM-Immunoregulation”
- Line 171: typo corrected
- Line 188: sentence has been modified and we added references supporting our observations (Ref. 86, 87 and 89).
- Line 285: typo corrected

Thanking you again for the opportunity to submit this manuscript to your Journal,

Best Regards

Marco Erreni