Leman *et al*, Multiparametric Analysis of Colorectal Cancer Immune Responses

Reviewer 1:

Interesting review, well written, I have a few comments:

Comment 1: -Title should be adapted because this review is focused on T subsets rather than all immune cells (e.g. myeloid regulatory cells, stromal cells).

Response to Comment 1: The reviewer is correct that our focus is more on T cells than other immune cells. However, we do discuss immune cells such as macrophages so we feel that the current title is accurate. Instead we have added text to the first paragraph of the introduction to explain our T cell focus:

Comment 2: This is a lack of the manuscript. -Immune cell plasticity section: should also be expanded, few data on TAMs for instance

Response to Comment 2: We have added text on page 5 to include data on TAMs.

Comment 3: -page 7: the section starting with "The role of Tregs in CRC…" should not be in the section related to the multidimensional analysis

Response to Comment 3: Rather than move this section, we have re-written it to make it relevant to the discussion of multidimensional analysis.

Comment 4: -page 7: please cite the "exhaustion" markers

Response to Comment 4: We have listed the cited exhaustion markers and referenced these.

Comment 5: -page 8 and 10: 100 markers simultaneously analyzed by mass cytometry and IMC is very optimistic. For now 40-45 is more realistic.

Response to Comment 5: We have changed "100" to "40" in both these sections; and added text to postulate the use of 100 markers in the future.

Reviewer 2:

This is an interesting review which tries to show the complex heterogeneous inflammatory reaction associated to colorectal ccancer. The Authors analyze the currently available methods to determine the degree of iinflammatory cells, with the assumption of a probable correlation between inflammatory cells and efficacy of the immunological reaction. In this review they correctly found that different types of lymphocytes and macrophages are present in several cancer regions. Data are disocrdant about the prognostic value of the cancer infiltration by these types of cells. They analyzed several techniques to quantify this infiltartion of inflammatory-immunological mediators. Each method has its negative and positive sides.

Comment 6: The Authros should underline in this interesting review: -All reported methods present the limitation to be qualitative and not quantitative assessments -Today we are able only to give a phenotypic definition of the immunological response, based on number and tupe of cells which infiltrate the cancer. But what s their role and efficacyy -Major development in evaluating the efficacy of the immunological system will come from in vitro analysis of the behaviour of those infiltrating cells: which factors do they produce? which antibodies do thery produce? the substances they produce are effective in neutralizing the growth and diffusion of the cancer cells of that specific patient? Every patients has his/her own genotype; instead to work hard to define phenotypes and genotypes, we could just look at the immediate effect of infiltrating cells , and eventually lumphocytes in the lymph nodes, on cancer cells behaviour. In other words this is a very interesting review that show the cuurrent methods to analyze the immunological response to colorectakl cancer. The conclusion is that we are

llost in thye definition of mechanisms without looking at practical effects, and probably this is a major problem in basic research in all medical field. All this assumptions of mine should be somehow introduced in the coonclusions.

Response to Comment 6: We have added text to the conclusions to cover the points raised by the reviewer.

Comment 7: Finally, the paper will be more readable if picture related to the different techniiques will added to the paper.

Response to Comment 7: We have included a figure.

Please note, we have removed the funders statement since this is not a research article.