Reviewer’s report

Title: MONITORING THE RESPONSIVENESS OF T AND ANTIGEN PRESENTING CELL COMPARTMENTS IN BREAST CANCER PATIENTS IS USEFUL TO PREDICT CLINICAL TUMOR RESPONSE TO NEOADJUVANT CHEMOTHERAPY

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Reviewer: Sylvain Ladoire

Reviewer’s report:

In the manuscript entitled "Monitoring the responsiveness of T and antigen presenting cell compartments in breast cancer patients is useful to predict clinical tumor response to neoadjuvant chemotherapy", Bernal-Estevez et al. reported their clinical experience of peripheral immunomonitoring before and after neoadjuvant anthracyclines-based chemotherapy in a small cohort of breast cancer patients.

The authors claimed in their conclusions that their model of immunomonitoring is suitable to predict tumor response, and to monitor the recovery of immune surveillance during chemotherapy.

The specific topic of the importance of immunogenic effects of some chemotherapies like anthracyclines is generally well described and the fact that these immune effects are poorly explored in human patients is also discussed. Thus, the manuscript appears to be one of the rare that is questioning the modulation of peripheral immune response during chemotherapy (especially during immunogenic chemo). Moreover, the setting of neoadjuvant chemotherapy leads to correlate many immunologic parameters with the response to chemotherapy (by pathological examination of surgical specimen after chemo).

However, many major experimental/methodological problems exist, disabling the results presented here:

First of all, some points need to be highlighted and/or clarified concerning the cohort of breast cancer patients:

1. How much patients were REALLY immunomonitored before AND after chemotherapy? (this number should appear in the abstract): 23 or 15? this should be précised for each experiments

2. How long before the 1st chemo et after the 3rd chemo were obtained the blood samples?

3. It is not clear in the present form, if blood samples have been collected prospectively, or if it is a retrospective analysis of previously biobanked frozen PBMCs? please clarify this point
4. Clinico-pathologic data (Table 1) are presented in a very unusual form: please use usual cutoff values concerning: age (>50 vs <50), axillary lymph node status (pN1 vs pN2 vs pN3, or cN1, cN2, cN3), ER (positive (eg >10%) vs negative) and same for PR expression, Ki67 (> or <14%), and HER2 (0,1,2 vs overexpressed (eg 3+ or 2+ and FISH+))

5. The cohort of breast cancer patients is very small and very heterogeneous: authors must precise the number of each molecular subtype (eg: luminal non HER2, HER2 overexpressing, and triple negative breast cancer). Moreover, can they explain why there is 4 stage IV patients according to TNM classification, and only 1 patient considered as metastatic ? please exclude metastatic patient(s) from all analyses.

6. Another clear limitation of this work is the absence of patients treated with neoadjuvant chemo, and non responding to AC schedule: such cohort of patients would allow the authors to really compare responding vs non responding patients (and not responding patients vs healthy donors)

Secondly, concerning the results reported here:

1. Concerning the quality of the response to chemotherapy: the gold standard to appreciate this is pathological complete response (pCR): however, pCR has not been observed in this cohort…nevertheless, the quality of response could not be evaluated on the median tumor shrinkage observed on 15-20 patients, which are not representative of the population of breast cancer patients. Authors could use other endpoints (reduction of > 50%, or better: the Residual Cancer Burden (RCB), which is the new gold standard to appreciate response to primary systemic chemo).

2. Is there a correlation between the level of Treg / MDSCs and the stage of disease ?

3. The absence of difference in level of Treg/MDSCs between cancer patients and HD can be also explained by lack of power (small cohort)…

4. Line 229-230: "together, these results suggest….that is partially recovered after 3 cycles of AC chemotherapy": this sentence should be removed, as results presented here in Fig 3 do not support this conclusion.

5. Concerning the last part of the manuscript: conclusions derived from a PCA conducted on 15-20 patients must be only considered as exploratory results, and many caution should be taken regarding such results (which must be in my opinion published only in supplementary data). Moreover, these preliminary data reached low statistical significance, and can only differentiate BC patients from HD (and not responder vs non responder patients to chemo: thus in my opinion, there is no clinical utility from these results).

6. In the last paragraph of results, by the same, a multivariate analysis conducted with more variable than patients should be regarded with caution…and the readout (tumor area
smaller than 2.4 cm2) is not clinically relevant: prefer the RCB, after working with a trained breast pathologist.

Finally the results presented here suffer from the small number and the heterogeneity of patients included in this cohort, and from high number of non-clinically relevant results to achieve the initial goal of this study: highlighting immunological differences in the profile of responding and non responding patients to immunogenic chemotherapy.

Additionally, other immunological parameters could be interesting to monitor in such cohort of patients, like immune checkpoints molecule expression by T cells or APCs. This could reinforce the results for further submission.

General comments-minor points:

1. The abstract must be re-written, because in the present form, parts of the results appear to be in the conclusion, and no real conclusion exists

2. Line 96 (patients and blood samples): the pathological diagnosis must be "ductal invasive carcinoma" (and not DCIS), because DCIS are never treated with chemotherapy (only surgery +/- radiation therapy)

3. Line 167: "there is no published evidence....": please remove this, as this type of data is published (for example Limagne et al, Cancer Res 2016)

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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