Reviewer’s report

**Title:** Results of multicenter double-blind placebo-controlled phase II clinical trial of preparation Panagen to evaluate its leukostimulatory activity and formation of the adaptive immunity response in patients with stage II-IV breast cancer

**Version:** 4  
**Date:** 2 December 2014

**Reviewer:** laura bracci

**Reviewer’s report:**

The manuscript describes the results of a multicenter, double-blind placebo controlled phase II clinical study in which breast cancer patients were treated with chemotherapy and a dsDNA-based preparation (Panagen). The results are interesting, but the clinical relevance of the data provided is not clearly addressed. The manuscript is clearly written, although lengthy. There are too many additional figures and text which make the reading cumbersome and hide the main message of the study. Some figures and figure legends are not exhaustive enough.

**Major Revisions**

1. Cytotoxicity of patients’ T cells has been assayed against the adenocarcinoma cell line MCF-7 and cytokine production by patients’ PBMCs has been measured either ex-vivo or following mitogenic stimulation. These are immunological readouts of immune competence but do not support the conclusion of the induction of anticancer immune responses by Panagen treatment (as stated in page 3, line 19-20). In my opinion, the induction of tumor specific immune-responses by Panagen treatment needs to be addressed.

2. The authors state: “This medication can also boost individual anticancer adaptive immunity resulting from the activation of dendritic cells by the circulating tumor cell debris.” (Page 3 lines 21-23). This sentence in mostly speculative as it is not supported by data within the study submitted for publication. It probably refers to previous data collected in mouse models and in vitro human settings but has not been demonstrated in the current study. As a matter of fact, the authors write that from the analysis of dendritic cells they failed to uncover pronounced different trends between the placebo and the Panagen groups (page 20, lines 18-21).

3. I do not see the clinical relevance of the cytokine profiles observed in Panagen vs placebo groups. It should be better supported or better clarified.

4. The clinical data reported in additional file 8 (long-term follow-up analysis) should be enriched with the tumor stage of each patient at the time of enrolment to evaluate the clinical benefit of the treatment with respect to cancer severity.

5. The authors disregard that similarly to Doxorubicin, in addition to depleting suppressive T lymphocyte subpopulations Cyclophosphamide has been shown
to induce immunogenic cell death (Schiavoni et al, Cancer Res 2011). Although this information will not change the overall message of the study, it needs to be included in the discussion and properly cited as it would reinforce the data provided and reflect a more comprehensive review of the literature in the field (page 10, lines 23-25, page 11, lines 1-14).

6. The authors often refer to the results of a phase I clinical study but it’s not clear whether the data are published or unpublished (e.g., pag.7, line 23; page 9, lines 19-22; page 17, line 9). Please specify and if data are published please provide reference.

7. It is not clear to me whether the cell counts reported in Figure 2 are mean values. If yes, it should be better specified in the figure legend and the number of samples analyzed per time point should be indicated in the figure of in the figure legend.

Minor Essential Revisions

1. Introduction is too long. I would write it more concisely.

2. The Materials and Methods section in largely incomplete. The DNA quantification methods should be briefly described besides referring to the paper (page 12, line 25). In the section “brief outline of phase II….etc”, the information on clinical trial approval and administrative issues should be separated from the information on patients’ recruitment and treatment in order to make the latter information easily accessible to the reader. The primary and secondary endpoints of the clinical study should be included in this section. There are too many details regarding the administrative and ethical compliances of the study. This part should be more concisely written.

3. References throughout Introduction section are missing

4. Reference n.74 is wrong. The journal is Cancer Biol Ther. 2014 instead of Cell Death Dis according to PubMed database

5. In figure 2, I would report the chemotherapeutic regimens as headings instead of the capital numbers “I”, “II”, “III”

6. Too many references are in Russian language which makes it difficult to access data reported therein

7. Page 14, line 8: stage number is differently written: 2 vs III-IV.

8. Figure 5 is not clear. Why are two graphs in the Panagen group and one in the Placebo group? Please clarify by adding more information either in the figure legend or in the plot.

9. The indication of the chemotherapeutic regimen (if FAC or AC) in Figure 6 and in figure legend are missing

10. There are too many additional files and figures. I would recommend including some of the data provided as “additional” into the main figures by creating multi-panel figures.

Discretionary Revisions
1. Given the fact that it is phase II study, the outcome of combined Chemo+Panagen treatment vs Chemo alone in terms of clinical efficacy is particularly relevant and must be better supported. The data reported in additional file 8 (long-term follow-up analysis) should not be provided as supplementary material but rather included in the manuscript (for example as table).

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests