Author's response to reviews

Title: A word of caution: Do not wake sleeping dogs. Micrometastases of melanoma suddenly grew after progesterone treatment.

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Editor

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Dear Editor,

We are pleased to submit a revised version of our manuscript entitled “A word of caution: Do not wake sleeping dogs. Micrometastases of melanoma suddenly grew after progesterone treatment” by José Mordoh et al, for publication as a case report article in BMC Cancer.

Below is a point by point response to the reviewer’s comments.

Response to the reviewers:

Referee: Kevin Kim

Major issues:

1-Please consider deleting the first paragraph of the Background section as this doesn’t seem to be essential to be included. Also, the second and the third paragraphs of Background may be a better fit for Conclusion/Discussion section.

We have shortened the first paragraph of the Background section to situate the clinical case that we are reporting as follows:

“We are conducting a Phase II/III clinical trial in which the effectiveness of the vaccine CSF470 is compared to IFN-alpha 2b treatment (Clinical Trials.gov ID: NCT01729663) in stage II-III cutaneous melanoma (CM) patients. The CSF470 vaccine consists of a mixture of four melanoma cell lines rendered apoptotic/necrotic by gamma-irradiation, and BCG and rhGM-CSF are used as adjuvants [1, 2].”

and moved the second and third paragraphs of the Background to the Discussion/Conclusion section as suggested by the reviewer. (Page 5)

2-In Case Presentation, Please mention whether the liver was evaluated with the ultrasound at the time of the serum LDH of 369 U/L and a normal CXR between November and December 2011. It will be important to clarify that the liver was clear at the time of the initiation of Progesterone.

In Case presentation (Page 4) the following paragraph was changed in order to clarify this point:

“At that time her serum LDH was 369 U/L and all other blood laboratory values were normal. Chest radiography was normal and abdominal ultrasound only showed a 4 cm diameter ovarian cyst, without adenopaties. Three months later, the patient came to a new follow-up visit and ultrasound showed multiple liver heterogeneous diffuse nodules (20-25mm) that were further confirmed by...
CAT scan, along with expansive lesions in both suprarenal glands and a spleen nodule (Figure 1E-F)."

For:

“At that time (11/20/2011) her serum LDH was 369 U/L and all other blood laboratory values were normal. Chest radiography was normal and abdominal ultrasound only showed a 4 cm diameter ovarian cyst, without adenopaties. No CAT scans were made at that time. In March 19 2012, the patient came to a new follow-up visit and ultrasound showed multiple liver heterogeneous diffuse nodules (20-25mm) that were further confirmed by CAT scan, along with expansive lesions in both suprarenal glands and a spleen nodule (Figure 1E-F).”

3- In Case Presentation, in which month the new liver mets were seen for the first time on an ultrasound? The authors stated “three months later”, but was it 3 months after the initiation of the progesterone treatment or 3 months after the completion of the 3 cycle of the progesterone treatment?

The new liver metastases were first seen in March 2012, three months after the completion of the three cycles of Progesterone treatment. The previous abdominal ultrasound was in November 2011 and showed normal liver and all other organs.

4- In the Conclusion/Discussion, the authors stated “After a one month Pg treatment for her ovary cysts a rapid disease progression ensued”. From the Case presentation, it appears that the disease progression occurred within 3 months, so it may not be accurate to state “after one month”.

We changed the corresponding sentence (Page 6) to:

“Three months after completing Pg treatment for her ovary cysts a rapid disease progression ensued”.  

5- There are many grammatical errors. The manuscript should be revised with a help of a scientific editor.

We have revised the manuscript and corrected grammatical errors to our best.

Minor issues:

1. In the Abstract, I am not sure what “(001-CSS)” means. If not essential info, please delete.

001-CSS was the patients´ code for the clinical study. We have deleted it.

2. In the abstract and the body of the manuscript, “Breslow index” and “Clark’s stage” should be replaced with “Breslow thickness” and “Clark’s level”, respectively.

We have corrected the text as suggested by the reviewer.
3. In the abstract, “CSF470” may be replaced with “melanoma cell lysate vaccine” because it will be easier for the readers to understand. It is appropriate that CSF470 was explained in the text body.

Since CSF470 vaccine is not a lysate of melanoma cells but a suspension of lethally irradiated apoptotic/necrotic melanoma cells, we have corrected the text as follows:

“a vaccine composed of irradiated melanoma cells”

4. In Case Presentation, I would suggest that which sentinel lymph node basin was examined. Was it bilateral axillary node or left or right axillary nodes?

The sentinel lymph node biopsy was bilateral. We have included the detail in the text. (Abstract and Page 3)

5. In Case Presentation, please describe how frequent the patient received a cycle of 4 daily injections of CSF470 vaccine. Was it repeated every 4 weeks? Or 3 months?

We have modified the corresponding paragraphs as follows in order to clarify the treatment schedule: (Page 4)

“After giving written consent she entered a randomized Phase II/III clinical study comparing the CSF470 vaccine plus BCG plus GM-CSF versus IFN-alpha, and she was assigned to the vaccine arm. CSF470 vaccine consists in a mixture of four gamma-irradiated melanoma cell lines injected i.d. plus 10^6 cfu BCG plus 400µg rhGM-CSF (divided in four daily injections, 100 µg GM-CSF each).”

“She received a total of 13 doses of CSF470 vaccine (the first 4 doses, every three weeks, then every two months until completion of the first year and finally every three months in the second year)…..”

6. In Case Presentation, after the first description of the serum LDH level, please include the normal range in the parenthesis, so that it will be easier for the readers.

We have included the normal LDH level range in our Institution as suggested (Page 4).

7. In Case Presentation, please describe the specific grade 2 toxicity at the injection site. Pain? Rash? Erythema? Edema?

We have described the specific grade 2 toxicity at the injection site:

“…..presenting only grade 2 toxicity (erythema, edema and pain) at the vaccination site.” (Page 4).

8. In Case Presentation, Please describe the duration of the cycle of progesterone. Every 4 weeks? Every 2 weeks?

In Case Presentation (Page 4) we have clarified the paragraph as follows:
“Between November and December 2011 she was treated by her gynecologist with three cycles of Pg, 200 mg/day for ten days, every two weeks (MAFEL, Raymos Laboratories, Argentina), to treat her ovarian cyst”.

Referee: Mark B Faries

Since single case reports are difficult to use as evidence of a causal relationship, some supportive correlative data would bolster this presentation. Were there any correlative findings in this patient that suggested immune suppression related to the progesterone treatment? For example, were there changes in antibody titers, delayed-type hypersensitivity testing to vaccine cells or PPD that could support the suggestion that the progesterone led to loss of immune protection and release of tumor growth?

Since this patient was participating in a clinical study, immunological follow up was thorough only during the course of the study. Treg lymphocytes (CD3+/CD25+/FoxP3+) analyzed in patients’s PBMCs were low during the clinical study: 4.8% (Pre-vaccination), 5.2% (26 weeks post-treatment), 6% (50 weeks post-treatment) and 4.8% (110 weeks, at the end of treatment in July 2011). We did not analyze Treg levels after finishing the study. Patient´s serum samples taken at the same time points were tested (1/10 dilution) against the melanoma cells that compose the vaccine by flow cytometry. We observed that serum reactivity to vaccine cells increased from 15 % (pre-treatment) to around 60% (all three post-treatment samples). Again, we did not test serum reactivity after finishing the protocol. Finally, since the patient is HLA-A0201 negative, we could not address specific cellular immune responses (i.e anti-MART-1 or gp100 tetramer staining). DTH testing to the vaccine cells at the last visit of the study (August 2011) was strong, resulting in a 2.6 cm papula 48 h after the injection. DTH testing was not performed after finishing the clinical study.

1) Time relationship between intervention and reaction. With regard to this, the patient’s LDH was 309 in August 2011. What is the normal range in that institution? In our hospital that value would be considered elevated, suggesting the recurrence started before the progesterone injections.

In our Institution (Instituto Médico Especializado Alexander Fleming, Buenos Aires, Argentina) the normal LDH value range is 230 - 460 U/L. Therefore, a 309 value is not considered elevated. Patients´s LDH values fluctuated along the two years of vaccine treatment between 241 to 347 U/L. For that reason the last value of 309 at the end of the clinical study was not suggestive of melanoma recurrence. In fact, all CAT scans performed during CSF470 vaccine treatment were normal, including the one performed at the end of the protocol.

Looking forward to read your reply.

With best regards,

María Marcela Barrio, PhD