

Format for ANSWERING REVIEWERS



May 15, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name:33517-revised.doc).

Title: Use of PD-L1 assay to predict the outcomes of non-small cell lung cancer patients treated with immune checkpoint inhibitors.

Author: Carmelo Tibaldi, Alice Lunghi and Editta Baldini.

Name of Journal: *World Journal of Oncology*

ESPS Manuscript NO: 33517

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer :

Reviewer n.1, n.2, n.3 :

(1) "*there are numerous mistakes of grammar and spelling in English.*" As requested by the Reviewer we we have subjected the manuscript to a native english reviewer for the relative corrections.

Reviewer n.1:

1) the title "*Predictive role of PD-L1 assay in NSCLC patients treated with immune checkpoint inhibitors.*" was changed in "Use of PD-L1 assay to predict the outcomes of non-small cell lung cancer patients treated with immune checkpoint inhibitors"

2) we deleted the sentence "*historically considered not immunogenic*"

3) *..."determined by immunoistochemistry (IHC) since this receptor seems to be"* we rephases in determined by immunoistochemistry (IHC) since this protein seems to be...

4) we changed the titles of tables as requested by the Reviewer.

Reviewer n.2:

(1) "*Check carefully all abbreviations, and their occurrence*". As requested by the Reviewer we we have carefully checked and corrected all abbreviations as soon as they occur.

(2) "*Introduction section. CTLA4 and PD1 are not expressed only on CD8+ T cells*" As requested by the Reviewer we have better explained the CTLA4/PDL1 role as follows: "Some key immune checkpoint proteins have been identified: cytotoxic T-lymphocytes antigen 4 (CTLA-4) and programmed death-1(PD-1). In the priming phase, which occurs in lymph-nodes, the CTLA-4 receptor, located on the surface of the lymphocyte T cells, binds the B7- receptor on the cellular membrane of the dendritic cell. In the effector phase, which occurs peripherally, the PD-1 located on the cellular membrane of lymphocyte T cells binds PD-L1 and PD-L2, which are expressed by tumor cells, stromal cells, or both. These observations have led to the development of a monoclonal antibody-directed against CTLA4 and PD1/PD-L1 proteins such as ipilimumab (anti-CTLA-4), nivolumab and pembrolizumab (anti PD1), atezolizumab, durvalumab, avelumab (anti-PD-L1). "

3) "*Section 2.1.1. 4th line: "by a 5% expression threshold"* is not needed since the scoring is described in the next sentence" as requested by the Reviewer we deleted this sentence.

4) "*Section 2.2.1. Does the results reported refer to the training or to the validation cohort of KEYNOTE-001 study?*" we have responded that the results are referred to the validation cohort.

5) The sentence "*in patients with tumors, PD-L1 positivity ...*" has to be rephrased. As requested we

rephrased as follows: "However, in patients with PD-L1 positivity $\geq 50\%$ the HRs for OS was 0.54 (p=0.0002) in the pembrolizumab 2 mg/Kg arm and 0.50 (p= <0.0001) in the Pembrolizumab 10 mg/kg treatment arm respectively; in addition, in this PD-L1 selected subgroup of patients also PFS was significantly longer than with chemotherapy (see Table 2)."

6) "Please rephrase "[95% CI: 6.7 was not reached] ". We rephrased as follows: " [95% CI: 6.7 to 'not reached']"

7) "Section 4.1. "; score = 2 positivity between $\geq 5\%$ and $<50\%$ " refers to both IC and tumor cells?" we rephrased:" score = 2 positivity between ≥ 5 and $<50\%$ on TC or PD-L1 expression on IC between ≥ 5 and $<10\%$;"

8) "Please spell out ITT" we spelled out: "intention to treat"

9) "Section 4.3. End, "Median PFS was 11.6 weeks...". If data refers to Verschraegen's study, the authors reported mPFS in the table as NS". The median PFS reported in the text is referred to the total population of the study whereas in the table we reported the mPFS according to PDL-1 expression.

10) "Section 4.2. "Overall ORR was 25% (11/43 PD-L1 positive; 1/8 PD-L1 negative)." Should be deleted as it does not add valuable information to the next sentence. As requested by Reviewer we deleted this sentence.

11) "Authors should combine **Table 1 and 2; 3 and 4; 5 and 6, 7 and 8.**" As requested by Reviewer we combined the tables.

12) "Authors could comment on whether PD-L1 positivity on tumor vs. immune cells has a different effect on outcome/response to treatment and on the potential functional correlates, as well as on whether other molecules involved in the PD1/PD-L1 signaling axis are being or could be explored as predictive markers of response to these immunotherapies" . In the conclusion section we have added the following sentences: "it is not clear whether PD-L1 positivity has a different effect on outcome/response to treatment, compared to PD-L1 positivity on immune cells. PDL-1 expression was evaluated in tumor cells in the majority of studies. The immunoresponse is a delicate balance between inhibitory checkpoints and activating signals such as LAG-3, OX40 etc. "

Reviewer n.3:

1) " I would have valued a table summarising properties of the various assays at technical level". In the tables we specified which antibody and platform were used in the immunohistochemistry assay .

- References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Oncology*

Sincerely yours,



Dr. Carmelo Tibaldi
Division of Oncology-Department of Oncology
S. Luca Hospital
Via Guglielmo Lippi Francesconi 1, 55100 LUCCA, Italy
e-mail: carmelo.tibaldi@uslnordovest.toscana.it