Peer Review File

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Reviewer A

Comment 1: The discussion of the inhibition of cancer cell stemness is an emerging area in lung cancer. I have no further recommendations for revision.

Reply 1: Thank you for your comment.

Reviewer B

Comment 1: The editorial does not criticize the main study. It simply summarizes their findings but does not add a critical perspective or question the results. For example. If an anti-PD1 antibody blocks the growth of PD1 positive tumor cells, why it does not affect the growth of T lymphocytes? In fact is the opposite, what is the co-expression of PD1 and PDL1 in tumor cells? What antibody did they use? (there is no mention about it in the paper) There are several unanswered questions in the study and an editorial is supposed to raise them.

Reply 1: Based on your comment, we noted the variance in downstream signaling of PD-1 between T cells and tumor cells. We have modified our text as advised (see Page 2, lines 24-27). We also described the co-expression of PD-1 and PD-L1 in tumor cells. We have modified our text as advised (see Page 4, lines 8-10).

Changes in the text: Page 2, lines 24-27. Page 4, lines 8-10.

Comment 2: Line 28. Non-small cell lung cancer (NSCLC) patients. Please change for “patients with non-small cell lung cancer (NSCLC)”

Reply 2: As you pointed out, we changed to “patients with non-small cell lung cancer (NSCLC)”. We have modified our text as advised (see Page 2, line 9).

Changes in the text: Page 2, line 9.

Comment 3: Line 49: “prognosis prediction marker”. Please use “predictive biomarker”

Reply 3: As you pointed out, we changed to “predictive biomarker”. We have modified our text as advised (see Page 2, line 32).

Changes in the text: Page 2, line 32.

Comment 4: Line 53. Therefore, treatment with cytotoxic agents alone for recurrent NSCLC tumors

highly expressing PD-1 after the administration of cytotoxic agents is likely to fail.

This assessment is not justified with the current data, it is just a hypothesis (in fact, there is evidence that docetaxel works as second line after cisplatin failure. Docetaxel has not been tested in this experiment.

Reply 4: As you pointed out, we deleted this assessment.

Changes in the text: We deleted the sentence “Therefore, treatment with cytotoxic agents alone for recurrent NSCLC tumors highly expressing PD-1 after the administration of cytotoxic agents is likely to fail.”.

Comment 5: Line 66: please change “demonstrated” by “suggest”

Reply 5: As you pointed out, we changed to “suggest”. We have modified our text as advised (see Page 3, line 16).

Changes in the text: Page 3, line 16.

Reviewer C

Comment 1: This is a study mainly based on RNA and cell lines. Since the gold standard of biomarker expression (such as PD-L1) is IHC, future studies with protein profiling on FFPE human samples are needed, either with IHC, mIF or other protein profiling platforms such as DSP or phenocycler, which can also provide insights about the tumor microenvironment and other proteins related to the PD-1-PD-L1 axis.

Reply 1: Based on your comment, we pointed out the necessity of protein profiling on FFPE human samples. We also described other protein profiling platforms such as DSP. We have modified our text as advised (see Page 3, lines 10-14).

Changes in the text: Page 3, lines 10-14.

Comment 2: One of the limitations on the methodology of Rotolo et al is that the PD-1 differential expression found using IF in monolayers vs pneumospheres is not well described, only that the images were visualized using ImageJ software, but there's nothing about the method of quantification of the cells (There's no need to add anything about this, just wanted to bring to your attention).

Reply 2: Thank you for your comment. We pay attention to the points.

Comment 3: The statement "The findings obtained indicated that liver cancer, renal cancer, urothelial cancer, testis cancer, and melanoma have subgroups of tumor cells with higher PD-1 expression levels than lung cancer" is confusing. Indeed, in the human protein atlas there are a few examples with very low expression of PD-1 in tumoral cells, but I don't understand the point of

comparing it with other tumors. I would state the finding (that in lung is not that seen) as a limitation and justification to study in a large cohort of human samples.

Reply 3: As you pointed out, we deleted the statement. We stated the necessity of analyzing a large cohort of human samples. We have modified our text as advised (see Page 4, lines 7-8).

Changes in the text: Page 4, lines 7-8.

Comment 4: Do you have any comments about hyperproliferative disease after immunotherapy? do you think there's a link between these tumors that resist therapy and the tumor PD-1 expression?

Reply 4: As you pointed out we described hyperproliferative disease after immunotherapy. We also stated the relationship between these tumors that resist therapy and the tumor PD-1 expression. We have modified our text as advised (see Page 4, lines 10-12).

Changes in the text: Page 4, lines 10-12

Reviewer D

Comment 1: The authors raise no criticisms or concerns about the results/conclusions presented in the Rotolo et al study. It could be interesting if the commentary format was used to also highlight eventual shortcomings identified by the authors of the Rotolo et al. study.

Reply 1: Based on your advice, we noted the variance in downstream signaling of PD-1 between T cells and tumor cells. We have modified our text as advised (see Page 2, lines 24-27). We also described the co-expression of PD-1 and PD-L1 in tumor cells. We have modified our text as advised (see Page 4, lines 8-10).

Changes in the text: Page 2, lines 24-27. Page 4, lines 8-10.

Comment 2: PD-L1 is only introduced very briefly in lines 46-48. The statement that PD-L1 is a biomarker for PD-1 immunotherapy efficiency can be disputed. At least some new medical guidelines recommend immunotherapy irrespective of PD-L1 expression level. Albeit very speculative, it could also be interesting to have some thoughts from the authors on eventual PD-1/PD-L1 interactions at cancer cell surfaces (and thereby not involving immune cells), if such interaction could mediate meaningful intracellular cancer cell signaling, how/if PD-L1 antibodies could act in concert with the PD-1 antibodies, and why there could be a selective advantage of the cancer cells to express PD-1?

Changes in the text:

Reply 2: As you pointed out, we discussed the limitation of PD-L1 expression as a biomarker for PD-1 immunotherapy efficiency. We also described PD-1/PD-L1 interactions at cancer cell surfaces. We have modified our text as advised (see Page 2, lines 34-35, and Page 4, lines 8-10).

Changes in the text: Page 2, lines 34-35. Page 4, lines 8-10

Comment 3: Lines 31-35 are difficult to read and interpret. Please rewrite for clarity.

Reply 3: Based on your advice, we rewrite the sentence. We have modified our text as advised (see Page 2, lines 12-13).

Changes in the text: Page 2, lines 12-13.

Comment 4: Line 42. Please help the reader with a more in-depth background description of ´pneumospheres`. This cannot be expected to be common knowledge.

Reply 4: As you pointed out, we explained pneumospheres. We have modified our text as advised (see Page 2, lines 20-22).

Changes in the text: Page 2, lines 20-22.

Comment 5: Lines 26-28. Is there a reference for driver mutation target therapies (e.g. use of TKI's) as well?

Reply 5: We could not find a reference for driver mutation target therapies. We have found a reference for the phase 1b study of sintilimab Plus anlotinib as first-line therapy in patients with advanced NSCLC (J Thorac Oncol. 2021 Apr;16(4):643-652). Eligible patients were treatment-naive and had unresectable stage IIIB/C or IV NSCLC without EGFR/ALK/ROS1 mutations.