

Cancer cell-derived IL-1 β reverses chemo-immunotherapy resistance in non-small cell lung cancer

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed all of my comments and concerns, which I hope were considered useful. The revised version of the manuscript is even more exciting and rich in important information. I would like to congratulate the authors on this excellent study, which will surely become a reference for investigators in the field.

Reviewer #2

(Remarks to the Author)

Perrichet et al. have provided a novel mode of action of anti-tumor efficacy of a combination of either MEK or MDM2 inhibitors with chemo/immunotherapy in lung cancer in their publication. They propose a pathway whereby MEKi or MDM2i act synergistically with chemotherapy to induce cytosolic mtDNA, AIM2 activation which results in IL1b secretion by the tumor cells. IL1b can in lung cancer increase CXCL10 secretion leading to a higher number of CD8 T cells in the tumor. The revisions have added some additional relevant information, however, the reviewers feel that some of these data should also be added to the manuscript. In addition, major comments 2 and 9 have not been addressed fully and the conclusions drawn in the data presented as part of major comment 6 should be corrected. A point-by-point response can be found below:

- Major comment 4/5/7/10 and all minor comments: The authors have addressed these comments sufficiently.
- Major comment 1: The authors observe a significant weight loss and have added this in the figures but should also mention this in the text and discuss it in the discussion.
- Major comment 2:
 - o While the reviewer understands that a full comparison of the models is too extensive for this study, the reviewer feels the tumor growth graphs of the KPC model should be included and discussed in the manuscript since they provide valuable additional information, suggesting that the observed added benefit of IL1b is both dependent on the model and on the chemotherapeutics used. In addition, the authors should more clearly indicate in the manuscript why the FOLFIRINOX treatment regimen was used in the KPC model.
 - o While we appreciate the additional experiments in the KPC model, in the B16 model, the question remains if there is an added benefit of IL1b in combination with CDDP/Pem/anti-PD1. Currently, the authors only evaluated the added benefit of IL1b with anti-PD1. Without these extra experiments, the reviewer feels the added value for the manuscript of the current data in B16 are limited.
- Major comment 3: The additional graphs in supplements indeed clarify the effect of IL1b monotherapy, however the reviewer feels this should also be discussed at least briefly in the results and discussion.
- Major comment 6: The authors evaluated the impact of CXCL10 overexpression on tumor growth alone or in combination with chemo/immunotherapy. Since in their proposed model, CXCL10 is the downstream actor of the combination of IL1b/CDDP/Pem/Immuno, it would indeed be expected that CXCL10 alone already has a major benefit. Therefore, these data confirm their proposed hypothesis. In my opinion, the fact that there is no combinatory effect of CXCL10/CDDP/Pem/Immuno is then expected and fully in line with the model. Therefore, these data should also be added to the publication and the conclusions of the authors should be corrected. In view of these results, IL1b does not provide additional signals together with CXCL10 as the author suggests, but the effect of IL1b/CDDP/Pem/Immuno is equivalent to

the effect of CXCL10.

- Major comment 9: While we appreciate the effort of the authors performing IV injection of tumor cells to evaluate the impact of the combination treatment in an orthotopic lung tumor model, this model is a model of experimental lung metastasis and not a primary orthotopic lung tumor model. In addition, IL1b was still injected intratumorally in the subcutaneous tumor. Therefore, these results rather show that there is a systemic response occurring upon treatment with IL-1b/ CDDP/Pem /immuno. This could be either via the minimal circulating IL1b (which would be suggested by the reduction in body weight) or via a systemic anti-tumor immunity induced by the effects observed in the primary tumor. However, it does not show if this combination therapy would also be functional if IL1b is overexpressed locally in the lung tumor nodules and therefore the current data should not be presented as a primary orthotopic lung cancer model. This is a significant shortcoming of the study that remains and therefore should at least be discussed clearly in the discussion section, including a clear discussion on how the local lung environment would react to IL1b treatment/overexpression in the tumor cells and how this could potentially be translated towards a clinical therapy.

- General comment: The gating strategy described in Supplementary Figure 27 raises questions about the CD8 T-cell gating since there remains a high number of CD4 and CD8 negative T-cells. This could potentially indicate an inefficient staining of the CD8 marker. In addition, the CD8 T-cell flow cytometry data presented in supplementary figure 5 should rather be represented as bar graphs with means and standard deviation than pie charts. This would also allow to add statistical testing to the data.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

In the revised version, the authors have now appropriately addressed all my concerns. The manuscript has been greatly improved and will likely have a broad impact in the field.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

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REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

- Major comment 4/5/7/10 and all minor comments: The authors have addressed these comments sufficiently.
- Major comment 1: The authors observe a significant weight loss and have added this in the figures but should also mention this in the text and discuss it in the discussion.

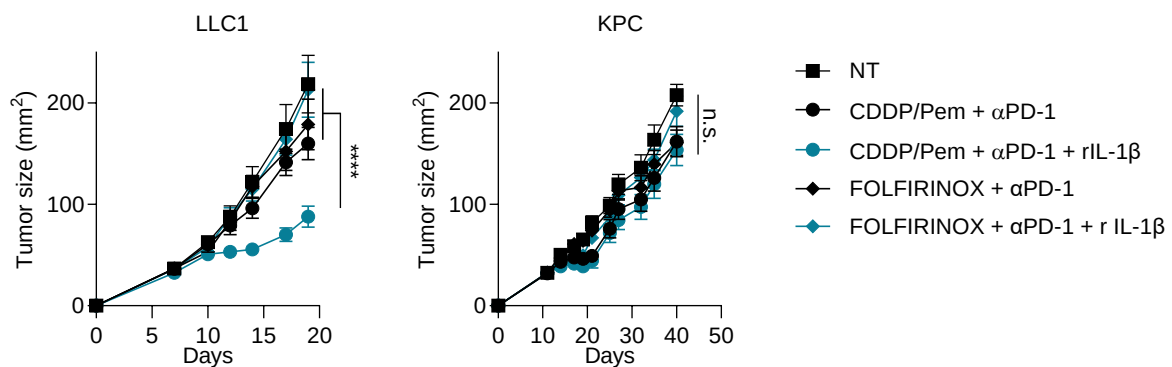
Answer: The weight loss was mentioned and discussed in the text as follows:

In the text: "Because the use of recombinant IL-1 β in mice leads to weight loss (Supplementary Figure 1B) and the treatment of cancer patients by intra-tumor injections of IL-1 β seems complex, we searched for ways to induce IL-1 β production by cancer cells."

In the discussion: "Because IL-1 β has some cytotoxic effects in mice (loss of weight) and that its injection to patients is difficult to consider, we aimed at finding inducers of TXNIP expression and consequently IL-1 β production."

- Major comment 2:
 - o While the reviewer understands that a full comparison of the models is too extensive for this study, the reviewer feels the tumor growth graphs of the KPC model should be included and discussed in the manuscript since they provide valuable additional information, suggesting that the observed added benefit of IL1b is both dependent on the model and on the chemotherapeutics used. In addition, the authors should more clearly indicate in the manuscript why the FOLFIRINOX treatment regimen was used in the KPC model.

Answer: The following figure comparing tumor growths in LLC1 and KPC with different treatments was added:



B, LLC1 or KPC cells were s.c. injected into C57BL/6 mice. Tumor-bearing C57BL/6 mice were treated with cisplatin (6mg/kg ip), pemetrexed (100mg/kg ip) (CDDP/Pem) or with FOLFIRINOX (see above) at

d9 (for LLC1) and d15 (for KPC) and with anti-PD-1 mAb (10mg/kg ip, 3 times a week from chemotherapy treatment, for 3 weeks). Recombinant IL-1 β (rIL-1 β - 50 μ g/kg) was daily injected in the tumor from d7 to d15 or from d13 to d21. Tumor size (n=10) was monitored.

The figure was described and discussed in the text:

“Moreover, the effects of IL-1 β seem restricted to lung cancer models treated with CDDP/Pem + anti-PD-1. No impact of IL-1 β on tumor growth was found in the KPC pancreatic cancer model, treated with CDDP/Pem + anti-PD-1 or FOLFIRINOX (5-FU, calcium folinate, irinotecan, oxaliplatin – one of the chemotherapy regimens usually used for pancreatic cancer patients) + anti-PD-1 or in the LLC1 model treated with FOLFIRINOX + anti-PD-1 (Supplementary Figure 2).”

“When we compared the effects of IL-1 β in association with cisplatin/pemetrexed + anti-PD-1 or FOLFIRINOX + anti-PD-1 in lung or pancreatic cancer models, a therapeutic effect was observed only when IL-1 β was associated with cisplatin/pemetrexed + anti-PD-1 in the lung cancer model. This suggests that the effects of IL-1 β are restricted to lung cancer models treated with cisplatin/pemetrexed and anti-PD-1. However, our investigation should be extended to other murine cancer models and further experiments are needed to understand why IL-1 β has therapeutic effects only in some conditions.”

o While we appreciate the additional experiments in the KPC model, in the B16 model, the question remains if there is an added benefit of IL1b in combination with CDDP/PEM/anti-PD1. Currently, the authors only evaluated the added benefit of IL1b with anti-PD1. Without these extra experiments, the reviewer feels the added value for the manuscript of the current data in B16 are limited.

Answer: We agree with reviewer that the B16 model was not suitable. Thus, we decided to remove it from the paper.

• Major comment 3: The additional graphs in supplements indeed clarify the effect of IL1b monotherapy, however the reviewer feels this should also be discussed at least briefly in the results and discussion.

Answer: The effects of IL-1 β monotherapy were described in the results and briefly discussed:

“To understand the effects of IL-1 β in these models, we used either recombinant IL-1 β intratumor injections or overexpressing cancer cells. While recombinant IL-1 β has no effect on tumor growth, its overexpression leads to opposite effects with an increase in LLC1 tumor growth and a delay in KP1.9 tumor growth (Supplementary Figure 1A).”

“These previous studies are in agreement with our results, i.e. 1) IL-1 β continuous overexpression can accelerate tumor growth in the LLC1 model (but not in the KP1.9 model, and this discrepancy might be

investigated) and 2) IL-1 β should not be inhibited, but rather induced or added in the context of chemo-immunotherapy treatment of NSCLC to trigger therapeutic effects.”

- Major comment 6: The authors evaluated the impact of CXCL10 overexpression on tumor growth alone or in combination with chemo/immunotherapy. Since in their proposed model, CXCL10 is the downstream actor of the combination of IL1b/CDDP/Pem/Immuno, it would indeed be expected that CXCL10 alone already has a major benefit. Therefore, these data confirm their proposed hypothesis. In my opinion, the fact that there is no combinatory effect of CXCL10/CDDP/Pem/Immuno is then expected and fully in line with the model. Therefore, these data should also be added to the publication and the conclusions of the authors should be corrected. In view of these results, IL1b does not provide additional signals together with CXCL10 as the author suggests, but the effect of IL1b/CDDP/Pem/Immuno is equivalent to the effect of CXCL10.

Answer: We agree with the reviewer that such data corroborate that CXCL10 is the downstream effector of IL-1 β /CDDP/Pem/anti-PD-1. We checked all the manuscript and correct/remove all the conclusions that sustain the idea that IL-1 β may provide an additional signal together with CXCL10. As proposed by the editor the data were not added in the manuscript. However, a sentence about the role of CXCL10 was added in the discussion: “The importance of CXCL10 was also sustained by the fact that its overexpression in cancer cells can delay tumor growth (unpublished data).”

- Major comment 9: While we appreciate the effort of the authors performing IV injection of tumor cells to evaluate the impact of the combination treatment in an orthotopic lung tumor model, this model is a model of experimental lung metastasis and not a primary orthotopic lung tumor model. In addition, IL1b was still injected intratumorally in the subcutaneous tumor. Therefore, these results rather show that there is a systemic response occurring upon treatment with IL-1b/ CDDP/Pem /immuno. This could be either via the minimal circulating IL1b (which would be suggested by the reduction in body weight) or via a systemic anti-tumor immunity induced by the effects observed in the primary tumor. However, it does not show if this combination therapy would also be functional if IL1b is overexpressed locally in the lung tumor nodules and therefore the current data should not be presented as a primary orthotopic lung cancer model. This is a significant shortcoming of the study that remains and therefore should at least be discussed clearly in the discussion section, including a clear discussion on how the local lung environment would react to IL1b treatment/overexpression in the tumor cells and how this could potentially be translated towards a clinical therapy.

Answer: We thank the reviewer for your insightful comment regarding the use of the intravenous (iv) injection model and the interpretation of our data. We agree that the model employed in our study, in which tumor cells are iv injected to establish lung nodules, is indeed a model of experimental lung metastasis rather than a true primary orthotopic lung tumor model. We also acknowledge that the intratumoral administration of IL-1 β was performed in subcutaneous tumors, not directly in the lung lesions. We recognize that these experimental choices limit the conclusions that can be drawn regarding the efficacy of local IL-1 β treatment in a true orthotopic (primary) lung tumor setting. As the reviewer correctly point out, our current data primarily demonstrate that the combination therapy can induce a systemic anti-tumor response, which may be mediated either by minimal circulating IL-1 β (as suggested by the observed reduction in body weight) or by systemic immunity initiated at the site of the subcutaneous tumor. However, our results do not directly address whether the combination therapy would be effective if IL-1 β was overexpressed or administered locally within lung tumor nodules. Maybe, we considered this project more at the clinical translation level than the biological level. Because injections of IL-1 β appears complicated (toxicity, ways of injection) in Human we decided to find tools to increase IL-1 β (MEKi, MDM2i) instead of exploring its role in all the mice. This is a point that we will investigate in our future experiments and we thank the reviewer to focus the attention on it. We will particularly develop real orthotopic lung cancer models using surgery. We now started to evaluate the impact of different ways of injecting IL-1 β . Our preliminary data show that ip injections of IL-1 β is able to control sc tumor growth arguing for a systemic effect of IL-1 β . However, this kind of experiment needs to be repeated together with other ways of injections in orthotopic model (intra-lung injection) to confirm the role of IL-1 β .

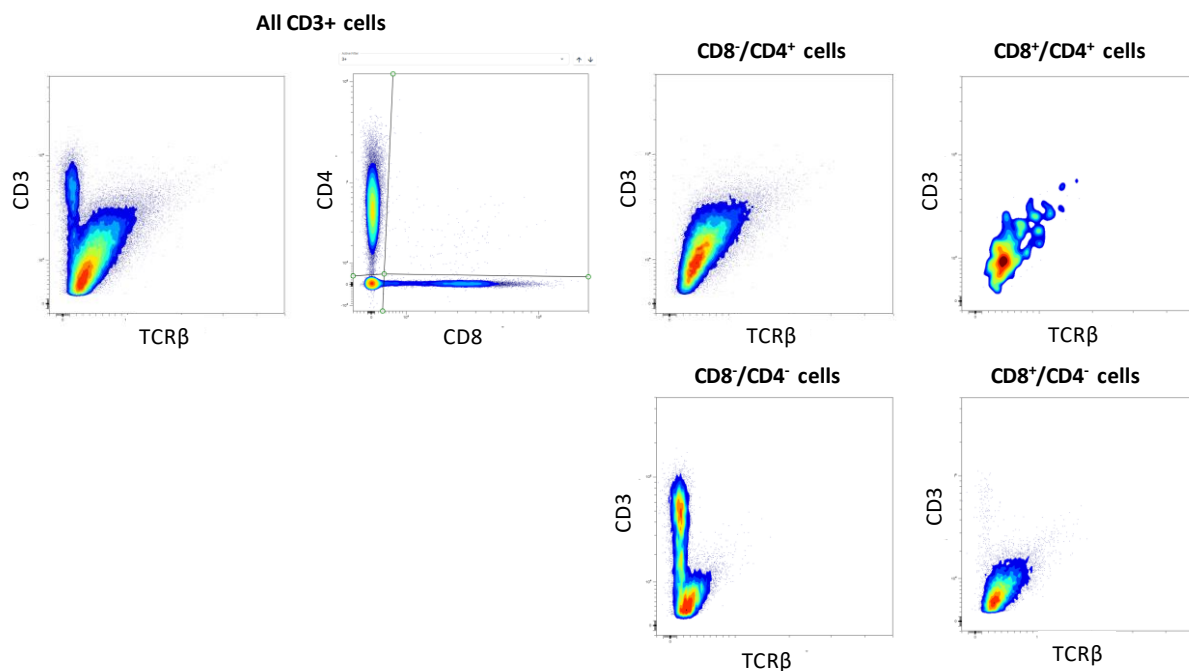
To address this significant limitation, we have revised the Discussion section of the manuscript to clearly state the constraints of our current model and to avoid presenting our data as evidence from a primary orthotopic lung cancer model. Specifically, we now discuss the following points:

“We demonstrate that local or systemic IL-1 β could reverse resistance to CDDP/Pem plus anti-PD-1 in subcutaneous lung cancer models. In addition, IL-1 β therapy can also reverse resistance of lung tumor foci. However, this last model only mimics lung metastasis and our experiments do not completely investigate in situ lung cancer. Thus, the local tumor microenvironment of orthotopic lung cancer model is different from the microenvironment of sc tumors and we cannot eliminate that it may limit the efficacy of our combination therapy.”.

- General comment: The gating strategy described in Supplementary Figure 27 raises

questions about the CD8 T-cell gating since there remains a high number of CD4 and CD8 negative T-cells. This could potentially indicate an inefficient staining of the CD8 marker. In addition, the CD8 T-cell flow cytometry data presented in supplementary figure 5 should rather be represented as bar graphs with means and standard deviation than pie charts. This would also allow to add statistical testing to the data.

Answer: We have also noticed the high proportion of CD4 and CD8 negative cells. We get deeper in the analysis of CD3⁺ cells in LLC1 tumors. While CD4⁺, CD8⁺ T cells express TCR β the CD4⁻/CD8⁻ cells are partly TCR β negative. This means that around half of these cells may be $\gamma\delta$ T cells usually described to be CD4⁻/CD8⁻. Concerning the other part (TCR β ⁺ cells), we also observed this population in other murine models (MC38, CT26) but in a very less frequency, suggesting that it should not be a problem of CD8 staining. Further experiments are required to understand the role of these cells.



As requested by the reviewer, the representation of flow cytometry results was modified as follows:

