

Reviewer 1 (Sokchea Khou)

Basic reporting

Overall, the manuscript is improved. The authors addressed some of the points that have been raised except a few listed below.

From the body of data that has been added, it seems that nivolumab crossed react with murine PD-1 although authors did not provide binding assay to confirm the affinity of this antibody.

Author still haven't investigated the source of Plexin-B1 that interacts with Sema4D on the tumor cells, mediating PD-1 resistance effect.

⇒ Authors have still not addressed this point at all. I think it's critical to at least discuss what would be the possible source of Plexin-B.

1. Line 49-50: precise what type of treatment

Changes have been made. Please see the line 49-50.

2. Line 62: what type of cancer?

Melanoma, changes have been made. Please see lines 62.

⇒ Not found in the manuscript.

3. These two paragraphs should be combined together

<<Sema4D and Plexin-B1 overexpression in nivolumab resistance B16-F10 cells

To investigate the mRNA and protein expression of Sema4D and Plexin-B1 in B16-F10R and B16-F10 groups, Sema4D and Plexin-B1 were detected. Compared with B16-F10 group, mRNA and protein expression of Sema4D and Plexin-B1 were significantly overexpressed in B16-F10R group (Figure 1A-E).

PD-L1 overexpression in nivolumab resistance B16-F10 cells

mRNA and protein expression of PD-L1 in B16-F10R and B16-F10 cells were detected. Compared with B16-F10 group, mRNA and protein expression of PD-L1 were significantly overexpressed in B16-F10R group (Figure 1A, F-G).>>

Changes have been made here, Please see lines 223-232.

4. Line 251: Please explain what is the method and what is the source of database?

Thanks for your suggestions, we have fully revised corresponding fields. Please see lines 251.

⇒ Not addressed. Line 251 in the old version referred to figure 2A. Authors states the correlation between Sema4 and PD-L1 but do not describe what are the type of cells that express those markers.

5. Figure 3: What technique and markers used to detect apoptosis? what is the X axis (Alexa674) of the dot plot?

Cell apoptosis was detected by PI and Annexin V/Alexa Fluor 647 double staining, Alexa Fluor 647 was a fluorescent dye binding with Annexin V, reflecting early

apoptosis of cells. In addition, x-axis of Figure 3E was modified. Please see lines 173-179 and Figure 3.

Line27: respond

Line31: analysis

345-347: Need to be rephrased