Peer Review File

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Reviewer A Comments:

Comment 1:

The development of antibody-drug conjugates as cancer therapeutics have been growing rapidly and many lessons were learned from the success and failure of the older generations of ADC. Sacituzumab govitecan is one of the game changing drugs in breast cancer and its clinical development definitely needs more insight into its biomarkers to identify patients who would benefit from the drug or who would not.

The authors started by summarizing the clinical trial results of sacituzumab govitecan in breast cancer and reviewed the cutting-edge studies which aimed to identify the predictive/resistant biomarkers. Also, they added the analysis of relative Trop-2 mRNA expression compared to normal tissue across various cancer types and challenged the notion of Trop-2 as a highly expressed ideal antigen target for ADC.

The authors made a point of saying Trop-2 expression may not be the crucial factor for sacituzumab govitecan to be effective and other factors such as bystander killing effect may also contribute the drug efficacy. I totally agree that there are many conflicting results regarding Trop-2, especially it is not mandatory to confirm Trop-2 expression as the clinical trial eligibility.

Reply 1: We thank the Reviewer for his/her positive comments.

Comment 2:

My suggestion is that the paragraphs or sentences that argue against Trop-2 expression as predictive biomarker should be grouped together. (i.e., P4 Line 72) For the readability, please consider start with results that indicate Trop-2 as predictive marker (please refer survival data or ORR data depending on Trop-2 expression in clinical trial if available) and then explain the results that do not. Also, please make sure that the lack of relative abundance of antigen expression compared to the normal tissue does not necessarily mean that it is not predictive because in some cases, little expression of antigen may still work (i.e., DS-8201 activity in HER2-low tumors) depending on affinity between antibody and antigen, cell membrane permeability or linker stability.

Reply 2: As suggested by the reviewer and to improve readability, the following paragraph was moved to page 4 line 67 "A genomic (WES) and transcriptomic (RNA-seq) analysis of pre- and post-SG tumor samples from three patients with metastatic TNBC treated with SG was reported, the post-treatment samples corresponding to multisite progressions harvested at rapid autopsy: TROP2 expression was a pre-treatment determinant of response, whereas acquired mutations involving the direct targets of antibody (TROP2) and of drug payload (TOP1) were identified in one patient in the post-progression samples."

Regarding ADC activity in context of relative abundance of antigen, the following sentence was added page 7 line 135: "Nevertheless, the lack of relative abundance of antigen expression compared with normal tissue does not preclude ADC activity. In

some cases, low antigen expression may still function (i.e., DS-8201 activity in low HER2 tumors) depending on antibody-antigen affinity, cell membrane permeability, or linker stability". This statement was complete with the following additional reference: Modi et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low–Expressing Advanced Breast Cancer: Results From a Phase Ib Study. *J. Clin. Oncol*. 2020, 38, 1887–1896.

Changes in the text:

- modification of the position of a paragraph page 4 line 67 "A genomic (WES) and transcriptomic (RNA-seq) analysis of pre- and post-SG tumor samples from three patients with metastatic TNBC treated with SG was reported, the post-treatment samples corresponding to multisite progressions harvested at rapid autopsy: TROP2 expression was a pre-treatment determinant of response, whereas acquired mutations involving the direct targets of antibody (TROP2) and of drug payload (TOP1) were identified in one patient in the post-progression samples."
- Addition of a sentence page 7 line 135: "Nevertheless, the lack of relative abundance of antigen expression compared with normal tissue does not necessarily mean that it is not predictive because, in some cases, low antigen expression may still function (i.e., DS-8201 activity in low HER2 tumors) depending on antibody-antigen affinity, cell membrane permeability, or linker stability"
- Addition of a reference: Modi et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low–Expressing Advanced Breast Cancer: Results From a Phase Ib Study. *J. Clin. Oncol*. 2020, 38, 1887–1896.

Comment 3:

Overall, I think this is an informative review of sacituzumab govitecan and the biomarkers studies.

Reply 3: we thank the Reviewer for his/her positive comments.

Comment 4:

1) P3 Line 38 "Sacituzumab-govitecan" was stated as "sacituzumab govitecan" earlier.

Reply 4: We have homogenized the name of the drug throughout the manuscript.

Changes in the text: Title page (page 1): Sacituzumab-govitecan was modified to sacituzumab govitecan

Comment 5:

2) P4 Line 66 Please consider rephrasing "To date, it is the first and only FDA-approved ADC in TNBC".

Reply 5: the sentence was rephrased as follows: "To date, no other ADC has been approved by the FDA in TNBC".

Changes in the text: P4 Line 66, rephrasing of the phrase "To date, it is the first and only FDA-approved ADC in TNBC" to "To date, no other ADC has been approved by

the FDA in TNBC".

Comment 6:
3) P5 Line 82: If other mechanisms of resistance are suggested, please indicate the examples and cite the past reports.
Reply 6: examples of other mechanisms of resistance and a new reference have been included.
Changes in the text: P5 Line 82, addition of the following sentence: "(intratumor heterogeneity, elevated drug transporters such as MDR1 and MRP1, altered antibody trafficking, ADC processing, intracellular drug release, alteration of the payload target…)" and of one reference: Loganzo et al. Mechanisms of Resistance to Antibody-Drug Conjugates. *Mol. Cancer Ther.* **2016**, *15*, 2825–2834.

Comment 7:
4) P5 Line 83: Please make sure sentence ends with period, not with comma. "…with other ADCs, certainly exist for SG. The …"
Reply 7: Modification has been done.
Changes in the text: P5 Line 83 the comma was replaced by period.

Reviewer B Comments:
Please check the grammar of the text and make corrections, such as "Line 59: The incidences of grade 3 or higher toxicities was higher in the SG 60 arm than the chemotherapy arm".
Reply: Modification has been done.
Changes in the text: Line 59, "The incidences" was replaced by "The incidence".