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Review A

Excellent commentary. Need to update with recent presentation from ASCO 2023. Lian et al CDDP/Temozolomide vs antiPD1

Authors' response

Thank you for your suggestion. In accordance with your advice, we have added the data of the recent presentation by Lian et al. regarding CDDP/Temozolomide vs. anti-PD-1 antibody at ASCO 2023 in the second paragraph of "How should we interpret the results of adjuvant toripalimab versus HDI in MM?" (See Page 10-11, line 179-182).

Change: "In fact, a very recent prospective trial reported by Lian *et al.* (23) compared the therapeutic effect of temozolomide plus cisplatin with that of toripalimab in patients with resected MM in the adjuvant setting."

Reviewer B

Nakamura and Mori have submitted an editorial surrounding adjuvant systemic therapy for mucosal melanoma (MM). Some comments for their considerations:

Intro line 24 – it is unclear what is meant by "Even after 23 the advent of immune checkpoint inhibitors (ICIs), the unfavorable prognosis of mucosal melanoma compared to cutaneous melanoma (CM) remains unchanged." Do the authors mean that ICI do not have a therapeutic effect in MM? That seems obviously not correct. Alternatively, do they mean that the patients still have inferior outcomes to CM? If so then it would be helpful to more clearly state that.

Authors' response

Thank you for your comment. We have changed the expression of the sentence you pointed out (see Page 2, line 27-29).

Change: "Even with advances in development of immune checkpoint inhibitors (ICIs), the therapeutic effect of ICIs is not as effective in patients with MM, even if resected in early stages, as that in patients with cutaneous melanoma in advanced stages."

Intro line 30 – the authors reference the 2013 study suggesting adjuvant activity for IFN-a2b in MM. It would be important to point out that this study was performed in study subjects of Chinese descent. These results

were quite at odds with previous studies in western/Caucasian populations HDI has no evidence to support adjuvant activity in MM (or potentially at all). Notably, the results of this study did not have an impact on the management of MM outside of Asia.

Authors' response

Thank you for your comment. In accordance with the reviewer's suggestion, we have clearly indicated in which countries/regions the clinical trials cited from the reference 3 and 4 were conducted and have stated that the clinical trial conducted in China (reference 4) had little impact on the adjuvant therapy of mucosal melanoma in the Western countries in the second paragraph of "Introduction" (see Page 2-3, line 31-39).

Change: "Before the advent of ICIs, clinical trials demonstrated that high-dose interferon (IFN)-α2b (HDI) therapy may improve relapse-free survival (RFS) and OS of high-risk resected patients with cutaneous melanoma in Western countries (4). Additionally, a phase II randomized trial conducted in China demonstrated that adjuvant therapy with HDI improved both, RFS and OS in patients with resected MM compared to observation alone (median RFS: 9.4 vs. 5.4 months; median OS: 40.4 vs. 21.2 months, respectively) (5), but did not impact on the adjuvant treatment of MM in Western countries."

Throughout the manuscript, discussion surrounding the genetic and genomic differences of MM (or melanoma more broadly) between eastern and western populations should be discussed in. There are multiple studies that emphasize these differences (lower TMB, lower interferon associated gene expression, high angiogenesis scores etc…) with one contrasting short report as PMID: 32487680.

Authors' response

Thank you for your suggestion. In accordance with your suggestion, we have elaborated the genomic differences between the races (East Asians and Caucasians) and cited your recommended report (PMID: 32487680) in the Section of "How does the therapeutic effect of PD1ab monotherapy for advanced MM predict the outcome of adjuvant therapy for MM?" (See Page 7-8, line 122-134).

Change: "Several research studies have indicated that MM is molecularly different from cutaneous melanoma showing lower mutation rate of *BRAF* V600, lower tumor mutational burden (TMB), and higher rate of copy-number alterations (CNA), leading to lower response to ICIs (15, 16). It is also generally known that the incidence rate of MM in East Asians is higher than that in Caucasians (17, 18). Furthermore, a recent study reported by Liu *et al.* (19) shows lower TMB in the Chinese population. Additionally, rates of selected gene mutations, including those in the MAPK signaling pathway, variegate between the different races. NRAS Q61 and NF1 gene alterations, and preexisting T cell inflammation markers were observed to be less frequent in the Chinese population. Those data will lead to the hypothesis of the lower therapeutic effect of PD1ab in the advanced setting in East Asians than in Caucasian population (20). These observations suggest lower therapeutic effect of adjuvant PD1ab monotherapy in MM than in cutaneous melanoma."

From the toripalimab study, it is noted the clinically relevant 6 month greater RFS in the PDL1+ population is noted. Perhaps the authors could comment on this further noting that this analysis was likely underpowered given the overall sample size of the study. While PDL1+ is not a clinically impactful biomarker for CM, it seems likely that it would be an important marker to have adequately stratified and/or sub-group powered for analysis given the substantially lower interferon associated gene expression known in MM vs CM.

Authors' response

Thank you for your comment. We have elaborated potential higher median PFS in PD-L1 patients and probability of being underpowered given the smaller overall sample size of this trial in the Section of "How should we interpret the results of adjuvant toripalimab versus HDI in MM?" (See Page 10, line 163-168).

Change: "Additionally, the potential superiority of median RFS in the toripalimab arm in PD-L1 positive patients is that this analysis was likely underpowered given the smaller overall sample size of the study. While PD-L1 positivity is not clinically an impactful biomarker for cutaneous melanoma, it can be a potentially significant marker for an adequately stratified and/or sub-group powered for analysis in patients with MM."

The section "What do we know about ICI in melanoma" seems incomplete without greater focus on discussion of anti-PD1 in stage II disease (KN-716 and CM-76K). These data are especially relevant in MM where as the authors note, many patients present with high-risk localized disease without nodal involvement that eventually progresses to metastatic disease. Despite this need to add discussion for the relevance of stage II data, this section seems over long and the authors should look for ways to shorten this.

Authors' response

Thank you for this comment. Although you state "seems incomplete without greater focus on discussion of anti-PD1 in stage II disease (KN-716 and CM-76K)." in your comment, you also state "Despite this need to add discussion for the relevance of stage II data, this section seems over long and the authors should look for ways to shorten this."

We are being asked to shorten this section from you even though we are also required to describe stage II adjuvant therapy in detail. With all due respect, the proposed revision from you is contradictory.

We regret that we are unable to comprehend your intentions and that we are unable to respond to your suggested revision.

The authors provide an excellent and comprehensive review of the various retrospective studies evaluating anti-PD1 in MM. It would be helpful if possible to develop a table that could summarize all of this text given that it is rather dense and becomes difficult to follow.

Authors' response

Thank you for your comment. In accordance with your suggestion, we have added a table regarding summary of studies for anti-PD-1 antibody monotherapy for advanced mucosal melanoma, as Table 1, and in the first paragraph of the section "How does the therapeutic effect of PD1ab monotherapy for advanced MM predict the outcome of adjuvant therapy for MM?", we have elaborated the two representative retrospective studies with larger sample sizes.
(See Page 7, line 111-122).

Change: "In two larger representative real-world datasets, the JMAC study reported by Nakamura *et al*. (13) investigated 329 Japanese patients with advanced MM who were treated with ICIs, including 263 patients treated with PD1ab alone (nivolumab or pembrolizumab). The therapeutic effects of the PD1ab alone group showed downward trend (ORR: 26%; median PFS: 5.9 months; median OS: 20.4 months) than those in the phase III clinical trials such as CheckMate 067 and KEYNOTE-001 (13). Another larger international retrospective study by Dimitriou *et al.* (14) also investigated 545 patients with advanced MM treated with ICIs, including 348 patients treated with PD1ab alone. Similarly, the therapeutic effects of the PD1ab alone group demonstrated a downward trend (ORR: 29%; median PFS: 5 months; median OS: 19 months) than those in those in the forementioned trials (14)."

One point missing from the discussion of adjuvant studies is the complexity in risk stratification for MM where primary lesions carry substantial risk even without nodal involvement. Perhaps they should also consider reference to or discussion of Alliance for Clinical Trials in Oncology A091903 (NCT05111574) which is investigating nivolumab plus cabozantinib in this population.

Authors' response

Thank you for your comment. In accordance with the reviewer D recommendation, that is similar recommendation to yours, we have added the list of ongoing clinical trial as a table, which includes clinical trial investigating nivolumab plus cabozantinib. We have briefly elaborated this ongoing clinical trial and complexity in risk stratification regarding primary lesions in the Section of "Future perspectives: current ongoing clinical trials of ICIs for MM in neoadjuvant and/or adjuvant setting" (see Page 11-12, line 195-203).

Change: "The complexity in risk stratification for the location of MM may also carry the substantial risk for ineffectiveness of adjuvant therapies, even without regional nodal metastasis. Therefore, novel neoadjuvant and/or adjuvant combination therapies are warranted to obtain improved survival of patients with MM. Several clinical trials are presently ongoing to investigate the therapeutic effect and safety of various agents or treatment modalities in combination with PD1ab in patients with MM (Table 2). Those combination immunotherapies targeting different cancer-immunity cycles may be more promising strategy for resectable or resected MM."

The authors seem to conclude that despite an insightful discussion of the limitations on current evidence surrounding anti-PD1 in adjuvant MM and that the toripalimab adjuvant study was negative per the pre-specified statistical plan that indeed anti-PD1 should be a standard of care with a future of anti-PD1 as the control arm for randomized studies. This seems hard to follow and rather these conclusions should be tempered to say that further evidence is needed to fully accept that anti-PD1 should set a standard.

Authors' response

Thank you for your thoughtful comment. In accordance with your comment, we have added the sentence "however, further evidence is required to fully accept that anti-PD1 antibody should be set as a standard of care." in the second paragraph of the Section of "How should we interpret the results of adjuvant toripalimab versus HDI in MM?" (See Page 11, line 175-180).

Change: "The validation of adjuvant PD1ab monotherapy for MM in a prospective trial, though still tentative, is a significant contribution to the field of this rare clinical subtype of melanoma. This study may lead to future clinical trials in which the PD1ab monotherapy arm can be used as the control arm for comparing with other novel adjuvant therapies for MM; however, further evidence is required to fully accept that PD1ab should be set as a standard-of-care."

Reviewer C

Line 21: may be better to use "post-operative" instead of adjuvant

Authors' response

Thank you for your comment. We have revised from "adjuvant" to "post-operative" according to your comment (see Page 2, line 30-31).

Change: "Therefore, effective and safe postoperative adjuvant therapies are urgently required for patients with resected MMs."

General comment on introduction—would be helpful to actually explain why prognosis of mucosal melanoma is so much worse than cutaneous—low mutation burden, delay in diagnosis etc…

Authors' response

Thank you for your comment. We have added the explanation of poorer prognosis and lower clinical efficacy or ICIs in patients with mucosal melanoma than cutaneous melanoma in the Section of "Introduction" (see Page 2, line 23-27)

Change: “The poorer prognosis of MM than cutaneous melanoma is derived from multiple factors, such as aggressive nature of MM, delayed medical examination, and difficulties in complete excision due to the tumor invasion or metastasis to other adjacent anatomical structures (3).”

and in the Section of “How does the therapeutic effect of anti-PD1ab monotherapy for advanced MM predict the outcome of adjuvant therapy for MM?” (See Page 7, line 123-126).

Change: “Several research studies have indicated that MM is molecularly different from cutaneous melanoma showing lower mutation rate of BRAF V600, lower tumor mutational burden (TMB), and higher rate of copy-number alterations (CNA), leading to lower response to ICIs (15, 16).”

Line 41: use of “as adjuvant setting” is not grammatically correct—try in the adjuvant setting instead

Authors’ response

Thank you for your comment. We have revised from “as adjuvant setting” to “in adjuvant setting” according to your comment (see Page 3, line 43-45).

Change: “Lian *et al.* performed a phase II randomized trial for investigation of the therapeutic effect of toripalimab, an anti-PD-1 antibody (PD1ab) versus HDI-α2b for MM in adjuvant setting.”

It’s really hard to state that the 2 retrospective studies (JMAC and Dimitriou et al, page 6-7) are truly conclusive on whether single agent or dual agent should be used in mucosal melanoma. My own personal experience is that single agent anti pd1 is very limited in activity in the metastatic setting and I favor dual agent. I think there are significant biases in these retrospective analyses that limit data interpretation.

Authors’ response

Our major discussion point in citing these two large retrospective studies is not whether anti-PD-1 or anti-PD-1 + anti-CTLA-4 antibodies are more effective, as you pointed out. As the title of this paragraph, the main theme in this paragraph is that we can predict the efficacy of anti-PD-1 adjuvant therapy of mucosal melanoma based on the data of the efficacy of anti-PD-1 antibodies in advanced-stage mucosal melanoma, compared with cutaneous melanoma. Therefore, we do not intend to discuss in depth in this paragraph whether monotherapy or combination therapy is more effective in advanced mucosal melanoma. Therefore, we have removed the description of nivo+ipi data from the first paragraph of “How does the therapeutic effect of PD1ab monotherapy for advanced MM predict the outcome of adjuvant therapy for MM?” (See Page 6-7, line 94-122).

The main issue I have with this manuscript as a whole is that the recent ASCO presentation by Jun Guo’s group of adjuvant chemo vs adjuvant pd1 showed superiority of chemo. Additionally, the prior study by this group demonstrated clear superiority of adjuvant chemo over HDI. These studies were completely neglected

in this commentary. I think the conclusion that pd1 may be superior to HDI is appropriate, but there seems to be data that adjuvant chemo is superior to anti PD1 so not sure how impactful this study really is.

Authors' response

Thank you for your suggestion. In accordance with your suggestion, we have added the data of the recent presentation by Lian et al. regarding CDDP/Temozolomide vs. anti-PD-1 antibody at ASCO 2023 in the second paragraph of "How should we interpret the results of adjuvant toripalimab versus HDI in MM?" (See Page 10-11, line 180-187).

Change: "In fact, a very recent prospective trial reported by Lian *et al.* (23) compared the therapeutic effect of temozolomide plus cisplatin with that of toripalimab in patients with resected MM in adjuvant setting. The temozolomide plus cisplatin arm showed significantly improved survivals compared with the toripalimab arm (median RFS: 28.2 vs. 12.0 months, $p = 0.04$), DMFS (median DMFS: 42.0 vs. 19.0 months, $p = 0.02$), and OS (median OS: 93.4 vs. 39.3 months, $p = 0.03$). This data suggests that adjuvant temozolomide plus cisplatin may be a better option for resected MM than adjuvant PD1ab even in the era of ICIs."

Reviewer D

I have just few minor comments for the authors:

- As the mucosal melanoma is a rare disease among Caucasian population and more frequent among Asians, I'd suggest to the authors to add some comments about the results of the study limited to Asian population, and the chance, if any, to extend the results or use the same drug among other ethnic groups

Authors' response

Thank you for your comment. In accordance with your suggestion, I have added sentence of the study limitation you pointed out in the second paragraph of "How does the therapeutic effect of PD1ab monotherapy for advanced MM predict the outcome of adjuvant therapy for MM?" (See Page 7-8, line 123-135).

Change: "Several research studies have indicated that MM is molecularly different from cutaneous melanoma showing lower mutation rate of *BRAF* V600, lower tumor mutational burden (TMB), and higher rate of copy-number alterations (CNA), leading to lower response to ICIs (15, 16). It is also generally known that the incidence rate of MM in East Asians is higher than that in Caucasians (17, 18). Furthermore, a recent research reported by Liu *et al.* (19) shows lower TMB in the Chinese population. Additionally, rates of selected gene mutations, including those in the MAPK signaling pathway, variegate between the different races. NRAS Q61 and NF1 gene alterations, and preexisting T cell inflammation markers were observed to

be less frequent in the Chinese population. Those data will lead to the hypothesis of the lower therapeutic effect of PD1ab in the advanced setting in East Asians than in Caucasian population (20). These observations suggest lower therapeutic effect of adjuvant PD1ab monotherapy in MM than in cutaneous melanoma."

- I recommend to add a paragraph or table with the ongoing trials for mucosal melanoma in the adjuvant setting, both as immunotherapy and targeted therapy or chemotherapy

Authors' response

Thank you for your comment. In accordance with your suggestion, I have added the list of ongoing clinical trial regarding (neoadjuvant) and/or adjuvant therapy for mucosal melanoma (Table 2) from ClinicalTrials gov (https://clinicaltrials.gov) and elaborated those ongoing clinical trials as future perspectives in the updated last Section of "Future perspectives: current ongoing clinical trials of ICIs for MM in neoadjuvant and/or adjuvant setting"(see Page 12, line 199-203).

Change: "Several clinical trials are presently ongoing to investigate the therapeutic effect and safety of various agents or treatment modalities in combination with PD1ab in patients with MM (Table 2). Those combination immunotherapies targeting different cancer-immunity cycles may be more promising strategy for resectable or resected MM."

- Do the authors think that radiotherapy on the primary tumor, combined with immunotherapy, may be an option for clinical trials and reduce the rate of relapse disease?

Author's response

Thank you for your comments. We already reported a retrospective study for ICIs in combination with radiotherapy for advanced mucosal melanoma (Umeda Y…Nakamura Y. Eur J Cancer. 2021 157:361-372). In this study, irradiation field's response rate is higher than ICI alone. In this respect, we speculate that radiotherapy in combination with ICIs may have potential benefits even in the advanced setting. We did not address this topic because such ongoing clinical trial is included in Table 2 (NCT4318717, Pembrolizumab+hypofractionated Radiation Therapy).

Reviewer E

Excellent commentary on an important clinical trial for mucosal melanomas.
No suggestions for revisions!
Congrats to the authors!