TLCR TRANSLATIONAL LUNG CANCER RESEARCH
AN OPEN ACCESS JOURNAL FOCUSING ON CLOSING THE GAP BETWEEN "BENCH AND BEDSIDE"
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Peer Review File

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Reviewer Comments

In this case report the authors describe a case of a lady with NSCLC who develosp aphasia and right arm weakness a few months after initiating pembrolizumab with laboratory and electrodiagnostic work-up suggestive of anti-Ma2 paraneoplastic neuroligc syndrome. This is an interesting case report for the field of neurology and immunotherapy, particularly as it is the third case report of ICI-associated anti-Ma2 where anti-Ma2 antibodies were demonstrated to be present prior to the initiation of immunotherapy (and before the patient was symptomatic). These reports and others in other PNSs continue to raise the question as to whether pre-existing onconeuronal antibodies are a risk factor for developing ICI-associated PNS. Given the many other ICI-associated anti-Ma2 case reports and reviews and other case reports and series on ICI-associated PNSs, the pre-existence of anti-Ma2 antibodies is the most important focus of this particular case, though not entirely novel. The text, particularly the discussion, needs to be more precise and concise.

Comment 1: In the intro the authors write "this neurological toxicity has been linked to an increased incidence of paraneoplastic syndromes caused by onconeural antibodies (Abs)6." Research suggests in most, if not all, cases onconeuronal antibodies that target intracellular proteins in classic paranoeplastic syndromes do not directly cause pathology (this is in contrast to autoimmune encephalitis associated antibodies like anti-NMDAR that target cell surface proteins). Authors should change this sentence to "[associated with] onconeural antibodies".

Reply 1: we have modified our text as advised (see Page 2, line 69).

Changes in the text:

"this neurological toxicity has been linked to an increased incidence of paraneoplastic syndromes associated with onconeural antibodies (Abs)"

Comment 2: In the discussion the authors text also impies that the onconeural antibodies are directly pathogenic. We know though that patients with cancer can have these onconeural antibodies present without developing paraneoplatic neurologic symptoms and in some, if not all, cases these seems to be because the pathology is driven by T-cells, not by the antibodies. This needs to be clarified in multiple areas of the discussion. Given that anti-Ma2 antibodies can be incidental and not associated with pathology it is important to comment in the discussion that it is possible her lesion and symptoms were an independent autoimmune/inflammatory event not related to anti-Ma2 PNS.

Reply 2: we have clarified in multiple areas of the manuscript that onconeural Abs are not

directly pathogenic and their detection might be incidental (see Page 2, lines 231-234; Page 5, lines 308-309).

Changes in the text:

"Although onconeural Abs have not been demonstrated to be directly pathogenic, their expression is often associated to a neurological impairment due to an autoimmune or inflammatory response"

"Although anti-Ma2 Abs were sometimes found incidentally in asymptomatic patients, this correlation may suggest a particularly strong association between ICIs and anti-Ma2 encephalopathy"

Comment 3: The discussion is far too long for a case report and as a result lacks a focus. The discussion should not read like a rapid overview of a review paper on the topic of ICI-assocaited ICI-irAEs. The discussion should focus on discussing salient points relevant to this case report and its more novel features. There are many reviews out there on ICI-associated paraneoplastic neurologic synddromes. Here are some recommendations on text to remove, though some focusing of the discussion is also needed.

a. Remove "The same way, the persistence of anti-Ma2 Abs after clinical improvement due to steroid therapy and intra-venous immunoglobulins, but at much lower titers and only detectable by western-blot technique, suggests that the decrease of immune activitywith immune-suppressant drugs correlates with an attenuation of the clinical effects ofthe paraneoplastic reaction." Titers do not necessarily correlate with disease activity since the antibodies are not thought to be pathogenic (but do correlate with neoantigen expression as the authors state). The authors point out that the antibody titers were even lower when the patient developed symptoms than her initial titers which would go against this statement.

b. Remove the following text as it does not add to the discussion and is not directly related to the important points of this case: "A retrospective study of 50 patients with paraneoplastic neurologic disorders, examinedat the Memorial Sloan Kettering Cancer Centre, showed that small cell lung cancer(SCLC) accounted for 40% of the cases of PNS, whereas NSCLC was much less frequent (10%)28. In our case, no neuroendocrine component was found in the histological analysis of the tissue samples. A high TMB might be behind the predisposition to develop onconeural Abs independently from the histological characteristics of the tumor, although further research is needed to confirm this hypothesis. Among the previous reported cases of anti-Ma2 encephalitis, at least three of the patients developed immune-related adverse events other than PNS after the initiation of immunotherapy (new onset diabetes mellitus18, diarrhea19 and immune-related cutaneous rash20), although this information was not always specified."

c. Remove the following as it is incorrectly applied to this case report: "A real-world transverse study, enrolling 751 patients with advanced cancer treated with anti-PD1 agents, showed that among patients with pre-existing both active and inactive

autoimmune diseases (AIDs), incidence of irAEs was significantly higher when compared with patients without AIDs (65.9% vs 39.9%)29. Therefore, our patient history of previous immune-related hepatitis is consistent with the scientific evidence supporting a higher incidence of ICIs toxicity in patients with previous autoimmune disorders." This referenced study is incorrectly applied to this case report. The patient in this case report developed autoimmune hepatitis after starting pembrolizumab, which does not qualify as a pre-exisiting autoimmune disease. This would simply reflect that the patient developed multiple irAEs.

d. Trim down the section talking about treatment. Those guidelines, as referenced, already exist. It is still reasonable to comment on how the current guidelines are based on all irAEs, most of which are not Paraneoplastic, and thus there may be unique immunopathologic underpingings to these diseases that may require different therapy. Figure 1 and 6 could also be removed, but do provide some additional information but not necessarily one that changes the impact of this case report.

Reply 3: we have modified our text as advised and removed the previously mentioned paragraphs. The section talking about treatment has been shortened as advised (see Page 5, lines 354-385)

Comment 4. Not all figures seem necessary for a case report (and six figures is a lot of a case report). Figure 2 and 5 are the only one this reviewer feels are truly necessary. Figure 3 and Figure 4 provide no additional information beyond the text and are not necessary.

Reply 4: figures 1, 3 and 6 have been removed as advised. We have chosen to provisionally maintain figure 3 since we think it is interesting and illustrative of anti-Ma2 Abs detection, though we might remove it if reviewers still consider it is advisable.

Comment 5. The authors write "In the opinion of the authors, the performance of well-designed prospective studies would be of particular interest to evaluate the correlation between pre-existing anti-Ma2 Abs and the development of clinical encephalitis after the initiation of immunotherapy, as well as the monitorization of anti-Ma2 Abs titers both after the discontinuation of ICIs and the administration of steroids or intravenous immunoglobulins." Given anti-Ma2 and even all the Paraneoplastic neurologic disorders combined are very rare, how would the authors propose such "well-designed prospective studies" could be designed without exorbitant cost?

Reply 5: the text has been modified to include a reference about the low incidence of paraneoplastic syndromes as a clear limitation for the design of prospective studies. We have

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changed "anti-Ma2 Abs" by "onconeural Abs" since we think this concept can be extrapolated to other similar antibodies, what might facilitate the recruitment of an adequate number of patients (see Page 5, lines 389-396).

Changes in the text:

"In the opinion of the authors, the performance of prospective studies would be of particular interest to evaluate the correlation between pre-existing onconeural Abs and the development of clinical encephalitis after the initiation of immunotherapy, as well as the monitorization of Abs titers both after the discontinuation of ICIs and the administration of steroids or intravenous immunoglobulins. Although the low incidence of paraneoplastic neurologic disorders is a clear limitation, this line of research might be useful to clarify if the determination of onconeural Abs prior to the initiation of a treatment with ICIs is helpful to predict a higher risk of immune-related encephalitis, what might facilitate an earlier detection and better therapeutic management.

Comment 6. The authors write in the discussion "This may entail that precisely those patients who benefit from immunotherapy could be at a higher risk of developing immune-related toxicities, although this hypothesis has not been validated by prospective studies so far." This sentence implies there is little known about this association, but then later in the discussion the authors discuss a meta-analysis of several ICI studies that do suggest associations with irAEs and response to immune checkpoint inhibitors. This paragraphs should be combined.

Reply 6: these paragraphs have been combined according to the previous recommendations (see Page 5, lines 354-385).

Comment 7. The authors write in the discussion "This finding proves that Abs are present not only long time before the clinical debut of encephalitis, but also before the initiation of ICIs." This is an overstatement as these few case reports do not prove this point. Consider changing to "These findings demonstrate that onconeuronal antibodies are present before initiation of ICI-therapy in some, if not all, patients who develop ICI-associated paraneoplastic neurologic syndromes".

Reply 7: we have modified our text as advised (see Page 5, lines 330-334).

Comment 8. Does the DWI signal shown in Figure 2E and 2F have an ADC correlate?

Reply 8: there was a correlation between DWI signal and apparent diffusion coefficient (ADC). Both ADC and eADC have been mapped and included as part of *figure 1* (1G, 1H*)* (previously *figure 2*), where images from initial MRI study performed at diagnosis are shown.

Comment 9. Please provide actual CSF cell counts.

Reply 9: CSF cell counts have been included in the text (see Page 2, lines 128-129).

Comment 10. Was the acute onset global aphasia severe? Was she unable to speak at all? Please qualify the degree of her aphasia and her weakness (0/5, 1/5, 2/5 etc.) before and after therapies were initiated.

Reply 10: the text has been modified as advised, including the degree of paresia and weakness at hospital admission and the improvement after steroid therapy and immunoglobulins were administered (see Page 2, lines 110-112 and 163-166).

Comment 11. Was the patient initially treated with anti-seizure medication? Did these improve her seizures or her symptoms?

Reply 11: it has been specified in the case presentation that the patient initially received a combination of several anti-seizure drugs with a poor clinical response (see Page 2, line 115).

Comment 12. There are some minor grammatical errors in the text that should be corrected

Reply 12: minor grammatical errors have been corrected.