Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-21-989

Reviewer A

Comment 1: Page 91-95 (please mention the evolution of neurological symptoms) over 1 week or 4 weeks. Looks like subacute presentation but time frame of disease evaluation is important if that information is available. Feel the spinal tap was done at least one month after presentation given we saw a very normal csf findings.

Reply 1: we have modified our text as advised (page 3, line 92)

Changes in the text: "with a three weeks progressive course"

Comment 2: line 182 and 183 are not mutual. PCD generally is not a treatable condition irrespective of tumor or antibody. So we cannot equate non-responsive PCD to poorly responsive tumor. Recommend removing this argument.

Reply 2: we have removed this argument as recommended.

Comment 3: Authors could also explain a second neurological worsening after third line chemo in December 2020 could be related to possible more onconeural antigen release as there is radiological response (given CT Jan 2021 showed no evidence of tumor).

Reply 3: we have modified our text as advised (page 3, line 129)

Changes in the text: "a possible underlying mechanism could be an increased onconeural antigen release following disease response to treatment".

Comment 4: Authors could mention if liquid biopsies should be considered for NSCLC & SCLC when known NSCLC patients develop PCD -- given tumors could be very small or microscopic.

Reply 4: we have included the suggested text (page 6, line 175).

Changes in the text: "Taking into account that the onset of a paraneoplastic syndrome may entail disease progression even if it is not detectable by standard radiological techniques, the use of liquid biopsy should be considered for patients with known NSCLC in complete response when a paraneoplastic syndrome is suspected".

Reviewer B

Comment 5: The authors summarize the neurological symptoms and signs as PCD, which is

predominantly cerebellar. However, they also describe that the patient has drowsiness, somnolence, cognitive sequelae and frequent memory lapses. How drowsy/somnolent was the patient? What were the cognitive sequelae? Was cognitive testing (formal or bedside) performed? This raises the question whether the patient was suffering from additional brainstem and/or limbic encephalitis.

Reply 5: we have modified our text to include some data about the somnolence and cognitive sequelae (page 3, line 92; page 3, line 113). It is true that some of the symptoms were not typical manifestations of PCD, so there is a possibility that other CNS areas were affected by the encephalitis.

Changes in the text: "GCS 14/15", "MiniMental State Examination of 21 and anterograde amnesia".

Comment 6: The patient was treated with 0.4 mg/kg/day of IVIG for 5 days. This should probably be 0.4 g/kg/day. This should be corrected in text and in figure 3.

Reply 6: we have modified our text as advised (page 3, line 111)

Comment 7: The authors found high levels of anti-Zic4 by immunoblot and by indirect immunofluorescence (IFI). Authors should report the provider of the immunoblot assay. How was the IFI performed and can the authors describe the result of the IFI? I.e. was there staining of nuclei, mainly in the granular layer of the cerebellum? A picture of IFI would in my opinion be as (or more) informative as the immunoblot.

Reply 7: the information about the assay provider has been included (page 3, line 107). We have included the IFI report and an additional image (page 9).

Changes in the text: "Euroimmun" "Figure 3. Indirect immunofluorescence showing staining of the nuclei mainly in the granular layer of the cerebellum (EG). Purkinje cells (CP) and neurons of the deep cerebellar nuclei (EM) reveal a lower intensity staining."

Comment 8: In the discussion the authors state that anti-Zic4 antibodies tend to occur along with other antibodies such as anti-Hu, anti-CRMP5 or anti-Ri. I agree with anti-Hu and anti-CRMP5 but really doubt anti-Ri. Can the authors provide a reference indicating frequent co-occurrence of anti-Zic4 with anti-Ri?

Reply 8: after a thorough research, the association between anti-Zic and anti-Ri is unclear. This part of the text has been modified as recommended (page 4, line 159).

Comment 9: The authors rightfully state that anti-Zic4 is usually associated with SCLC and not with NSCLC. However, their statement (1st line discussion) that this case report is 'the first time a paraneoplastic syndrome associated with isolated anti-Zic4-antibodies is communicated in NSCLC' is not completely true. Bataller et al.{Bataller, 2004 #1605} report anti-Zic4 in one

patient with adenocarcinoma of the lung. Because this patient also had anti-Hu, these authors speculate that the patient probably had a mixed tumor.

Reply 9: we intended to highlight that this is the first time that isolated anti-Zic4 have been detected in NSCLC without coexistence with more common onconeural antibodies (such as anti-Hu).

Comment 10: Likewise, Pozas et al speculate that the tumor in the current case report could possibly have transformed to SCLC, although they deem this unlikely. The link between anti-Zic4 and the EGFR mutated NSCLC could easily be strengthened by looking for Zic4 expression in the tumor. {Bataller, 2004 #1605} .

Reply 10: we contacted with our immunologist and, unfortunately, the appropriate techniques for the detection of Zic4 expression in tumor samples are not available in our institution at the moment.

Comment 11: The discussion stating that 'in most cases of PCD, upfront immunosuppressor treatment led to a mild clinical course and rapid symptomatic improvement' is in my opinion misleading. The authors refer to reference 6 in support of this statement. {Loehrer, 2020 #2492} In this reference, 15 patients with isolated Zic4 antibodies are summarized. In 10 of 15 no data on treatment response is available. In 5 patients with treatment data, 2 had no response, 2 improved somewhat with persisting symptoms and only 1 achieved complete clinical remission. None of the 3 patients who responded had the typical association with SCLC. In 1/3 there was a painful neuropathy which has a very questionable relation to anti-Zic 4, as Zic4 is only expressed in the granular layer of the cerebellum. On top of that, PCD in general has a poor outcome, in particular when associated with antibodies directed against intracellular antigens (as is Zic4).

Reply 11: we have modified our text as recommended (page 4, line 164)

Changes in the text: "Upfront immunosuppressive treatment was the elective therapeutic approach. Nonetheless, PCD has a poor outcome, in particular when associated with antibodies directed against intracellular antigens as is Zic4. In this review, out of 5 patients with treatment data, two had no response, two improved somewhat with persisting symptoms and only one achieved complete clinical remission".

Reviewer C

Comment 12: The authors report a rare EGFR-mutated NSCLC case with paraneoplastic syndrome associated with anti-Zic4 antibody. The manuscript is well written, and the results are clearly presented.
The reviewer has one minor comment. Paraneoplastic syndrome is more likely to be seen at the time of diagnosis with malignant disease, and the symptoms may improve with treatment for malignant disease (lung cancer). In this case, the emergence of the paraneoplastic syndrome is

the timing when lung cancer is well under control with EGFR-TKIs. Progression to brain metastasis and meningitis are often encountered in patients with EGFR mutated NSCLC. In particular, meningitis may not be identified by MRI imaging or CSF examination, as in this case report. Therefore, the reviewer is concerned that the symptoms in this case may result from progression to meningitis.
For example, were there any changes in tumour markers such as CEA? If there were no deterioration of tumour markers, this case presentation would be more convincing.

Reply 12: tumour markers are not routinely monitored in lung cancer in our institution, so unfortunately they were not available in our patient.