Peer Review File

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Reviewer A

The authors described a case of small cell neuroendocrine prostate cancer (SCNC) with adenocarcinoma components mimicking prostatitis, which is rarely reported.

Initially, the authors stated that after 6 cycles of carboplatin and etoposide in combination with androgen deprivation therapy (ADT), the Eastern Cooperative Oncology Group (ECOG) performance status improved from 3 to 0, in addition to achievement for reduction in pain.

Furthermore, reflecting treatment efficacy, both PSMA and FDG PET/CT revealed a significant reduction of metastatic load in line with clinical improvement.

Overall, it is a well-written case report. However, several revisions to the points indicated below would make this report more valuable.

Major points:
#1. Although the title of this report mentions "mimicking prostatitis," only a small part of the case presentation is devoted to this point. Since it is included in the title, more discussion should be given to this point. Or, there is room to reconsider the title.

Reply #1: We would like to thank the reviewer on this important note concerning the title of the case report.

Changes in the text #1: We have changed the name of the manuscript to “Small cell neuroendocrine prostate cancer with adenocarcinoma components – case report and literature review” on page 1, lines 2-4.

#2. In the case presentation section, the authors noted that at the initial examination, the patient presented with "pain in the left lower quadrant of the abdomen with projection in the groin and frequent micturition". They also stated that the PSA value before the start of treatment was 72 µg/l, CEA and chromogranin A were negative, and LDH was elevated at 501 U/l. I believe that these clinical symptoms and changes in PSA and LDH with respect to the course of treatment should be described. For example, International

Prostate Symptom Score could be substituted for urinary symptoms, and for pain, the degree of improvement of symptoms could be evaluated by showing the change in analgesic dosage, and I request the addition of a figure that includes this information.

Reply #2: We would like to thank you the reviewer for a valuable addition to the manuscript.
After the third cycle of chemotherapy, the LDH levels went down to 260 U/l (normal range between 135 and 225 U/l) and PSA levels to 0.42 ng/ml. Surprisingly, Chromogranin A levels went up to 248 µg/l (normal range <102 µg/l), but after the last cycle of the chemotherapy went down to 61.1 µg/l. LDH levels after the sixth cycle of the chemotherapy went down to 256 U/l and PSA levels remained stable. As CEA and CA-19 were negative at diagnosis, these parameters have been further controlled after the sixth cycle of the chemotherapy and remained at normal range (CA-19 <9kU/l and CEA <1.8 µg/l).
The International Prostate Symptom Score (IPSS) at presentation showed a score of 16 which has improved to 5 after finishing the chemotherapy.
Considering the pain medication, before starting the chemotherapy, the patient was taking Tramadol 100 mg and additionally Metamizole 500 mg/ml up to 3 times daily. After the second chemotherapy cycle the patient was pain free and all pain medication was totally stopped.

Changes in the text #2: We have added following text "Before starting the chemotherapy, the patients pain medication included Tramadol 100 mg and additionally Metamizole 500 mg/ml up to 3 times daily. After the first cycle of chemotherapy, the pain medication dosage was bisected and after the second cycle the patient was pain free and all the pain medication was discontinued. To assess the urinary symptoms, The International Prostate Symptom Score (IPSS) was used. At presentation the patient had a score of 16 which has improved to 5 after finishing the chemotherapy. After the third cycle of chemotherapy, the LDH levels decreased to 260 U/l (normal range 135 - 225 U/l) and PSA levels to 0.42 ng/ml. Surprisingly Chromogranin A levels went up to 248 µg/l (normal range <102 µg/l), but after the last cycle of chemotherapy decreased to 61.1 µg/l. LDH levels after the sixth cycle of chemotherapy went down to 256 U/l and PSA levels remained stable. As CEA and CA-19 were negative at diagnosis, these parameters have been further controlled after the sixth cycle of the chemotherapy and remained at normal range (CA-19 <9kU/l and CEA <1.8 µg/l)." on page 7-8, lines 158-170.

#3. I think that etoposide/carboplatin therapy plus ADT for SCNC is a rather common treatment, but what is the point of originality in this report?

Reply #3: We would like to thank the reviewer on the comment considering the originality of our report. We present one of not many successful cases of treatment of SCNC where the patient was able to improve his overall quality of life drastically from having an ECOG 3 status on presentation to ECOG 0 and discontinue all the pain medication. Moreover, the presence of adenocarcinoma in addition to SCNC is rare and may be an important factor on treatment response. Moreover, the co-occurrence of a *BRCA2* genetic mutation in conjunction with the administration of poly (ADP-ribose) polymerase (PARP) inhibitors is noteworthy in instances of disease progression.

#4. The authors mentioned in the case presentation in the Abstract that the patient is currently being treated with ADT plus apaltamide, but no mention of apaltamide appears in the Case presentation section in the text. Where is your rationale for using Apalutamide?

Reply #4: We would like to thank the reviewer on the question considering the choice of the further therapy. A multidisciplinary team discussion was hold after the patient have finished the chemotherapy on further treatment. Our team has decided against abiraterone as we didn't want to prescribed cortisol due to possible side effects. The patient didn't want treatment with enzalutamide due to possible fatigue as a side effect as he wanted to regain an active lifestyle he had before his illness. The apalutamide has been chosen as it didn't have any interactions with patients' long-term medication.

Changes in the text #4: Following changes have been made to the manuscript: "A multidisciplinary team discussion was hold after the patient has finished the chemotherapy on further treatment. The apalutamide has been chosen as it didn't have interactions with patients' long-term medication, didn't requires the addition of cortisone with its side effects (abiraterone) and didn't have fatigue as a side effect (enzalutamide) as patient wanted to regain an active lifestyle he had before his illness. "on page 8, lines 179-183.

#5. The authors do a good job of describing the review of SCNC, but lack the perspective of how to apply it to this case. For example, based on these citations, what treatment should be considered at the time of recurrence in this case?

Reply #5: We would like to thank the reviewer on this question concerning the further perspective in case of recurrence. Due to BRCA2 mutation, in case of recurrence we would administer a PARP inhibitor.

Changes in the text #5: We have added the following changes to the manuscript "In line with these finding, in case of progression, the therapy with poly (ADP-ribose) polymerase (PARP) inhibitor e.,g. with olaparib would be initiated." on page 9, lines

192-194.

Minor points:

#1. What do you think was the cause of ECOG-PS 3 in this case?

Reply #1: We would like to thank the reviewer for this question. In our case the main reason for ECOG 3 was the pain that the patient experienced from the extensive metastatic load and chronic inflammation due to pro-inflammatory cytokines such as TNF- α, IL-1β, IL-6, IL-8, IL-11 that were linked to the proliferation and higher metastatic potential of cancer cells.

Changes in the text #1: We have added following subordinate clause to the following sentence: "Remarkably, after the first chemotherapy cycle the performance status of the patient dramatically improved from ECOG 3, caused by pain the patient experienced from an extensive metastatic load and chronic inflammation due to pro-inflammatory cytokines, to ECOG 0." on page 8, lines 155-157.

#2. It seems that the prognostic factors in the Introduction section have not been matched with this case. What do you think on this point?

Reply #2: We would like to thank the reviewer on this question. As we went through the case reports on SCNC, we have noticed that in many cases it has not been stated if SCNC occurred in pure form or mixed with adenocarcinoma of prostate. In our case, SCNC with a small percentage of the classic adenocarcinoma, is considered as a favorable prognostic factor as we have mentioned in the introduction. Although post hoc analysis, performed by Aggarwal et al. (doi: 10.1200/JCO.2017.77.6880), didn't show the difference in survival rates in patients with mixed tumors in comparison to pure SCNC, this analysis was performed only on treatment-related SCNC. We have mentioned, that the patients under the age of 60 have more favorable outcomes, our patient has just turned 60, so we can assume that age $\leq$60 can be considered a favorable prognostic factor.

#3. The authors state that there is no standard treatment for SCNC and that chemotherapy with etoposide and "platin agents" (Page 11, line 259) is used based on the protocol for SCLC, but it is appropriate to state "platinum-based agents" rather than "platin agents".

Reply #3: We would like to thank the reviewer on providing the correction on the correct name of platinum-based agents.

Changes in the text #3: The changes in the manuscript from "platin agents" to "platinum-based agents" have been completed on page 14, line 298.

Reviewer B

Figure 1: Please add immunostaining confirming small cell prostate cancer diagnosis. Figure 1D shows only H&E morphology.

Reply #1: We thank the reviewer for an important comment on pathology findings. We further added a figure 1C, that shows few cells with immunohistochemical membranous CD56 positivity. Although the amount of the positive cells is low, according to WHO criteria for SCNC may completely lack synaptophysin and chromogranin A labelling but still be diagnosed on the basis of characteristic nuclear morphology alone. Moreover, the essential criteria for the diagnosis are characteristic high-grade histology, including nuclear and architectural features such as small or absent nucleoli, nuclear molding, high nuclear-to-cytoplasmatic ratio, cell necrosis. Positive immunostaining for synaptophysin or chromogranin A are considered desirable but not essential features for the diagnosis. We have also included a Figure 1F, which is the magnified version of Figure 1E showing tumor area with neuroendocrine morphology. The nuclei show finely granular chromatin, high nuclear-to-cytoplasmic ratio, inconspicuous nucleoli and nuclear molding. The tumor grows in solid sheets. The arrows point at multiple mitotic figures, the arrowheads point at tumor necrosis with apoptotic bodies.

Changes in the text #1: Figure 1C was added showing immunostaining on page 6 with following descriptions: "(C) Few cells showing immunohistochemical membranous CD56 positivity. Nevertheless, they show the characteristic morphology of neuroendocrine cancer cells. (CD56, 40x)" lines 113-115, page 5. Also Figure 1F was added showing the morphological features of SCNC with following description "Magnified version of Figure 1E showing tumor area with neuroendocrine morphology. The nuclei show finely granular chromatin, high nuclear-to-cytoplasmic ratio, inconspicuous nucleoli and nuclear molding. The tumor grows in solid sheets. The arrows point at multiple mitotic figures, the arrowheads point at tumor necrosis with apoptotic bodies." lines 117-121, page 5. We would like to notice that we have changed the order of the Figures 1C and 1D due to the two new ones (Figures 1E and 1F), that were added to the revised manuscript.

TABLE 1 Characteristic of reported cases on de novo SCNC

Please consider doing comprehensive review to capture de novo small cell prostate

cancer cases. Eg: PMID 37144976 case not included

81-year-old man who was found to have a pelvic mass at abdominal and pelvic CT during workup for left-sided flank pain and lower urinary tract symptoms. Prostate-specific antigen (PSA) level measured 0.62 ng/mL (reference range, ≤4.0 ng/mL). The patient underwent prostate biopsy for tissue diagnosis, which showed small cell carcinoma. The cells stained positive for synaptophysin and negative for chromogranin, PSA, prostate-specific acid phosphatase, and GATA3. He was initially administered carboplatin and etoposide and was later switched to nivolumab immunotherapy. Because of a poor response to chemotherapy and immunotherapy, he was transitioned to hospice care and died 9 months after initial presentation.

Reply #2: We would like to thank the reviewer for his/her valuable addition to the reported cases on de novo SCNC. We have added the case report, mentioned above, to our overview.

Changes in the text #2: We have added the case report from Husken et al. (doi 10.1148/rycan.230013) to the Table 1, page 10-11, line 225-226.

Reviewer C

de novo SCNC is a rare disease in prostate cancer and standard treatment is yet to be established. The authors reported a case of de novo SCNC, which has been successfully treated with carboplatin and etoposide. I have the following comments and suggestions that may help to further improve the manuscript.
We would like to thank the reviewer on a positive feedback and on suggestions to improve the manuscript.

Minor critics:

• There are grammatical errors. Please revise the manuscript using an application like Grammarly.
Response #1: We would like to thank the reviewer on his/her suggestions concerning the grammatical error. A professional review has been performed correcting the grammar. All the spelling changes have been done in green color.

Changes in the text #1: Following changes have been made in the manuscript: "Elevated levels of C-reactive protein (CRP) of 7.89 ng/ml (standard range between 0

and 0.5 ng/ml) and dolent prostate during digital rectal examination were matching the diagnosis." on page 5, lines 95-96.

• The patient was initially diagnosed with prostatitis. Did the patient have pyuria?

Response #2: We would like to thank the reviewer on this question regarding the patients' symptoms at presentation.

No pyuria was observed, however a digital rectal examination revealed tender and dolent prostate. In addition, serum inflammation parameters (CRP; IL6) were elevated.

• Clinical TNM stage information should be provided.

Response #3: We would like to thank the reviewer on this comment regarding TNM staging. It has been added to the manuscript.

Changes in the text #3: We added TNM staging "Based on the clinical staging of prostate cancer using tumor, node and metastasis (TNM) system, the patient had a clinical stage T3c N1 M1." on page 6, lines 131-133.

• According to Ref 3 (Aggarwal, Rahul, et al. "Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: a multi-institutional prospective study." Journal of Clinical Oncology 2018), the presence of deleterious mutations and/or copy number loss in DNA repair pathway genes including BRCA2 was almost entirely mutually exclusive with SCNC. Interestingly, as opposed to Aggarwal et al.'s findings, this SCNC showed BRCA2 copy number loss. This may be attributed to the difference between treatment-emergent SCNC and de novo SCNC. The authors should touch on this fact and discuss it in the discussion section.

Response #4: We would like to thank the reviewer on proposing some additional information on BRCA alteration on patients with SCNC. In the work mentioned above by Aggarwal et. al. (doi: 10.1200/JCO.2017.77.6880), 12 patients with treatment-related SCNC (t-SCNC) (4 with mixed t-SCNC and 8 with pure t-SCNC) were included in somatic targeted genomic sequencing. Variants predicted to lead to loss of function in TP53 and/or RB1 were found in 10 of 12 patients with t-SCNC. None of the 12 patients had alterations in BRCA1 or BRCA2 genes. In our case, as the reviewer also kindly noted, the patient had copy number loss in BRCA2 gene, which can be related to differences its treatment and response in patient with de novo SCNC in comparison to t-SCNC.

Changes in the text #4: Following changes were made in the manuscript on page 12, lines 256-263 "Considering the difference between de novo SCNC and t-SCNC, the work of Aggarwal et al., that was mentioned previously, looked closely into genomic

characteristics of t-SCNC. Twelve patients (4 with mixed t-SCNC and 8 with pure t-SCNC) were included in somatic targeted genomic sequencing. Variants predicted to lead to loss of function in *TP53* and/or *RB1* were found in 10 of 12 patients with t-SCNC. None of the 12 patients had alterations in *BRCA1* or *BRCA2* genes (3). In our case, the patient had copy number loss in *BRCA2* gene, which can be related to differences in treatment and response in patient with de novo SCNC in comparison to t-SCNC."

• Because this report discusses a rare case of de novo SCNC, providing diagnostic pathological information is important. Can the authors provide the magnified version of Figure 1D showing the characteristic features of SCNC such as rosette formation, necrosis, crush artifact, darkly stained homogeneous chromatin, lack of nucleoli, nuclear molding, mitotic and apoptotic figures?

Response #5: We would like to thank the reviewer on this comment on pathological features of SCNC.

Changes in the text #5: We have added a magnified version of a specimen showing the features with SCNC on page 6 with following description "(F) magnified version of Figure 1E, showing tumor area with neuroendocrine morphology. The nuclei show finely granular chromatin, high nuclear-to-cytoplasmic ratio, inconspicuous nucleoli and nuclear molding. The tumor grows in solid sheets. The arrows point at multiple mitotic figures, the arrowheads point at tumor necrosis with apoptotic bodies. (H&E, 40x)", lines 117-121, page 5. We would like to notice that we have changed the order of the Figures 1C and 1D due to the two new Figures 1E and 1F, that were added.

• Also, the authors stated the tumor was partially positive for CD56. Can authors show the CD56 IHC result in Figure 1?

Response #6: We would like to thank the reviewer on highlighting the importance of including the Figure showing the CD56 positive cells. We have added a figure with immunostaining that confirms the small cell prostate cancer diagnosis. We have added a figure, that shows few cells with immunohistochemical membranous CD56 positivity. Although the amount of the positive cells is low, according to WHO criteria for SCNC may completely lack synaptophysin and chromogranin A labelling but still be diagnosed on the basis of characteristic nuclear morphology alone. Moreover, the essential criteria for the diagnosis are characteristic high-grade histology, including nuclear and architectural features such as small or absent nucleoli, nuclear molding, high nuclear-to-cytoplasmatic ratio, cell necrosis. Positive immunostaining for

synaptophysin or chromogranin A are considered desirable but not essential features for the diagnosis. We have also included a Figure F, which is the magnified version of Figure 1E showing tumor area with neuroendocrine morphology. The nuclei show finely granular chromatin, high nuclear-to-cytoplasmic ratio, inconspicuous nucleoli and nuclear molding. The tumor grows in solid sheets. The arrows point at multiple mitotic figures, the arrowheads point at tumor necrosis with apoptotic bodies.

Changes in the text #6: We have added Figure 1C showing immunostaining on page 6 with following descriptions "(C) Few cells showing immunohistochemical membranous CD56 positivity. Nevertheless, they show the characteristic morphology of neuroendocrine cancer cells. (CD56, 40x)" lines 113-115, page 5. Additionally, as we have mentioned in the response to reviewers' previous comment, we have added a magnified version of a specimen, showing the features with SCNC on page 6 with following description "(F) Magnified version of Figure 1E showing tumor area with neuroendocrine morphology. The nuclei show finely granular chromatin, high nuclear-to-cytoplasmic ratio, inconspicuous nucleoli and nuclear molding. The tumor grows in solid sheets. The arrows point at multiple mitotic figures, the arrowheads point at tumor necrosis with apoptotic bodies (H&E, 40x)." on page 5, lines 117-121.

• Was there any reason why carboplatin was chosen instead of cisplatin such as deteriorated renal function?

Response #7: We would like to thank the reviewer for the question regarding the therapy choice. Carboplatin has been chosen as an agent regarding patient's deteriorated renal function at presentation with creatinine levels of 1,19 mg/dl and glomerular filtration rate (GFR) of 49 ml/min/1.73m^3 (GFR >60 ml/min/1.73m^3 is required for eligibility for cisplatin). Moreover, due to ECOG 3 status at presentation, carboplatin was chosen to reduce the toxicity of the treatment.

Changes in the text #7: We have added the following changes to the manuscript: "Carboplatin has been chosen as an agent due to deteriorated renal function at presentation with creatinine levels of 1.9 mg/dl and glomerular filtration rate of 49 ml/min/1.73m^3. Moreover, due to ECOG 3 status at presentation, carboplatin was chosen to reduce the toxicity of the treatment." on page 7, lines 148-151.

• As for the somatic genetic testing, was the sample admixture of SCNC and adenocarcinoma or SCNC dominant sample? If it is an admixture, can the authors provide the approximate ratio of adeno to SCNC of the analyzed sample? I ask this because I wonder if the adenocarcinoma part inherently harbors BRCA1 loss and RB1

nonsense mutation or if they are specific to SCNC.

Response #8: We would like to thank the reviewer on this question concerning the *BRCA2* and *RB1* mutations. Out of 15 biopsies samples, only 1 biopsy had < 0,1 cm of acinar adenocarcinoma. Proportion of tumor tissue in the NGS analysis was specified with 40% in the pathology report.

RB1 loss is seen regularly in NEPC and also castration resistant PCa. This holds true for a SCNC which evolves from a precursor adenocarcinoma and also de novo SCNC. Loss of *BRCA2* is also frequently seen in de novo SCNC as well as in prostate adenocarcinoma. So, it is not possible to say if the *RB1* and *BRCA2* mutations are specific to SCNC of adenocarcinoma exclusively. (Publications of Fraser et al (doi:10.1038/s41467-021-26-489-0) and Yamada et al (doi:10.1007/s11912-020-01003-9)

• Line 117: 72 ug/l. For all the other PSA values, unit ng/ml is used. Please make it consistent.

Response #9: We would like to thank the reviewer for pointing out the mistake made on the PSA value.

Changes in the text #9: We have corrected the PSA value to ng/ml on the page 6, line 124.

• Please make gene names italic.

Response #10: We would like to thank the reviewer for the proposal on font changes for the gene names.

Changes in the text #10: We have changed the gene names font to italic on pages 3, 9, 12, 13, 14 lines 56, 57, 193, 195, 196, 198, 242, 256, 258, 261, 268-270, 273, 274, 276, 279-286 and 298.

• The figures need to be in the right place in Figure 2. The figure legend says “FDG PET-CT whole-body scans showing the comparison of the metabolically active metastases before (A, B)”, but Figure 2B FDG PET looks like after treatment.

Reply #11: We would like to thank the reviewer for his/hers notes. We have changed the order of the Figures in the manuscript, Figure 3 shows both FDG PET-CT and PSMA PET-CT scans with concomitant CT scans.

Changes in the text #11: We have changed the layout of the Figure 2 on page 7, that now comprises both FDG PET-CT scans as well as PSMA PET-CT scans with the

following descriptions on page 6, lines 135-140: "**FIGURE 2:** FDG PET-CT (A) and PSMA PET-CT (B) whole-body scans showing the comparison of the metabolically active metastases before (on the left) and after 6 cycles of chemotherapy with an addition of GnRH antagonist (on the right).
FDG PET-CT (C,D) and PSMA PET-CT (E,F) scans with concomitant CT scans: at the diagnosis (C, E) and re-staging after 6 cycles of chemotherapy with etoposide/carboplatin with an addition of GnRH antagonist (D, F)."

• Likewise, the figure legend of Figure 3 says, "PSMA PET-CT scans with concomitant CT scans: at the diagnosis (A,C)", but Figure 3C looks like PSMA PET-CT after treatment.

Isn't it "PSMA PET-CT scans with concomitant CT scans: at the diagnosis (A,B) and

re-staging after 6 cycles of chemotherapy with etoposide/cisplatin with an addition of GnRH antagonist (C, D)"?

Reply #12: We would like to thank the reviewer noticing the mismatch between the scans and the descriptions.

Changes in the text #12: We have changed the layout of the Figure 2 on page 7, that now comprises both FDG PET-CT scans as well as PSMA PET-CT scans with the following descriptions on page 6, lines 135-140: "**FIGURE 2:** FDG PET-CT (A) and PSMA PET-CT (B) whole-body scans showing the comparison of the metabolically active metastases before (on the left) and after 6 cycles of chemotherapy with an addition of GnRH antagonist (on the right).
FDG PET-CT (C,D) and PSMA PET-CT (E,F) scans with concomitant CT scans: at the diagnosis (C, E) and re-staging after 6 cycles of chemotherapy with etoposide/carboplatin with an addition of GnRH antagonist (D, F)."