Peer Review File

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Reviewer A

The paper titled "Surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy of rectal adenocarcinoma with penile metastasis: a case report" is interesting. All of the previously reported cases of penile metastasis from rectal cancer since 1870 have been reviewed. Yet, the metastatic prognosis remains poor regardless of the treatment options, except for lesions where metastasis is only limited to the penis. We found that the patient may derive more benefit from strategic therapies including surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) This study has only one case, which is too limited. It is recommended to increase the comparative analysis of the same or similar cases with other regions or countries.

Reply 1): We have modified our text as advised (see Page 3, line 83 to 86 and Page 6, line 200 to 202).

Changes in the test: (Page 3, line 82 to 84) and the table revealed the ages, interval of metastasis, treatment of rectal cancer, treatment of metastasis, other metastasis and prognosis of patients from different regions or countries.

(Page 6, line 199 to 201) The patient received chemotherapy for metastatic disease until disease progression and unacceptable toxicity and he died with progressive disease, and

2) What are the characteristics and evaluation criteria of immunotherapy? What are the effects of immunotherapy on tumor metastasis? It is recommended to add relevant content.

Reply 2): We have modified our text as advised (see Page 5, line 154 to 165).

Changes in the test: Tumor response was investigator assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1)(10) or immune Response Evaluation Criteria in Solid Tumors(iRECIST)(11). Cancers develop in complex tissue environments, depending upon for sustained growth, invasion and metastasis. Cancers develop in complex tissue environments, depending upon for sustained growth, invasion and metastasis. The establishment of an antitumor immune response and memory can eliminate tumor cells, prevent recurrence in the primary region and inhibit metastasis to distant sites(12). Cytokine therapy(13), cancer vaccines(14), immune checkpoint blockade (ICB)(15) and adoptive cell transfer (ACT) therapy(16) focus on boosting immune response and immune cell activities in the tumor microenvironment(TME), and the ICB function can inhibit local tumor progression and systemic metastasis.

3) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.
Reply 3): We have modified our text as advised(see Page 3, line 75 to 80).
Changes in the test: The knowledge gaps and limitations of prior study is low incidence of rectal adenocarcinoma with penile metastasis, inexperience of treatment and the lack of standard treatment guidelines. The clinical significance of this study is early detection, precise diagnosis, patient's positive psychology that we guided and comprehensive treatment including surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy.

4) What should be done if there are cutaneous manifestations of metastatic disease to the penis? It is recommended to add relevant content.
Reply 4): We have modified our text as advised (see Page 4, line 132 to 133)
Changes in the test: We disinfect with iodophor (the available iodine content,4.4g/L to 5.0 g/L) twice a day if there are cutaneous manifestations of metastatic disease to the penis.

5) With the discovery of new drug targets and the continuous emergence of new combination treatment options, what breakthroughs will be made in the treatment of rectal adenocarcinoma with penile metastasis in the future? It is recommended to add relevant content.
Reply 5): We have modified our text as advised(see Page 7, line 223 to 226)
Changes in the test: More clinical treatment research exploration, multi-center study and public network database to achieve global sharing of cancer data may bring greater clinical benefits to the patients. More comprehensive analysis of multiple markers may also provide the appropriate strategy in the future.

6) What are the predictors of efficacy of immunotherapy? It is recommended that relevant information be added to the discussion.
Reply 6): We have modified our text as advised(see Page 5, line 166 to Page 6, line 181)
Changes in the test: Immunotherapies have led to substantial changes in cancer treatment and been a popular topic in cancer research. Mismatch repair deficiency or microsatellite instability-high is significantly associated with long-term immunotherapy-related responses and better prognosis in colorectal and noncolorectal malignancies treated with immune checkpoint inhibitors(17). The efficacy of PD-1/PD-L1 inhibitors varies among different classic driver oncogene mutations(18), KRAS-mutant patients appeared to respond better to anti-PD-1/PD-L1 immunotherapy, while patients with EGFR mutant disease may obtain benefits from immunotherapies, but the detailed mechanistic explanation needs further research. As a specific RNA adaptor, THUMPD1(19) is significantly associated with immune cell infiltration, tumor mutational burden (TMB), microsatellite instability (MSI), immune checkpoints and neoantigen. Patients with higher THUMPD1 expression exhibited a better prognosis in kidney renal clear cell carcinoma (KIRC) and rectum adenocarcinoma (READ), while

worse prognosis in liver hepatocellular carcinoma (LIHC) patients, so THUMPD1 may be a novel predictor to evaluate cancer prognosis and immune therapy efficacy in diverse cancer types. More predictive require further exploration and research.

Reviewer B

1. Please arrange the events and interventions in chronological order with dates.
Reply 1: We have added the Figure 6(see Page 5, line 136 and Page 12, line 350 to 352).
Changes in the test: We have added the Figure 6 as a timeline figure to arrange the events and interventions in chronological order with dates.
(Page 5,line 135) (Figures 4,5,6)
(Page 12, line 349 to 351) Figure 6 A timeline figure DT, Dose total; Gy, Gray; PET-CT, Positron emission tomography / computed tomography; CT, Computed tomography.

2. Justify the outcome of your strategic treatment approach and quality of life.
Reply 2: We have modified our text as advised (see Page 3, line 86 to 88).
Changes in the test: The prognosis of our patient is 54 months after being diagnosed with metastasis of penile cancer, and the patient benefited from the strategic treatment and improved the quality of life.

3. complete the list of reported metastatic penile cancer.
Reply 3: We have modified our text as advised (see Page 3, line 83 to 86).
Changes in the test: and the table revealed the ages, interval of metastasis, treatment of rectal cancer, treatment of metastasis, other metastasis and prognosis of patients from different regions or countries.

4. reconstruct the paragraph with grammatical corrections.
Reply 4: We have modified our text as advised (see Page 3, line 69 to Page 8, line 259)
Changes in the test: We reconstructed all the paragraph with grammatical corrections.

5. Provide the information mentioned in the comment box
Reply 5: We have modified our text as advised (see Page 2 to Page 16)
Changes in the test: We have provided all the information mentioned in the comment box.

Reviewer C

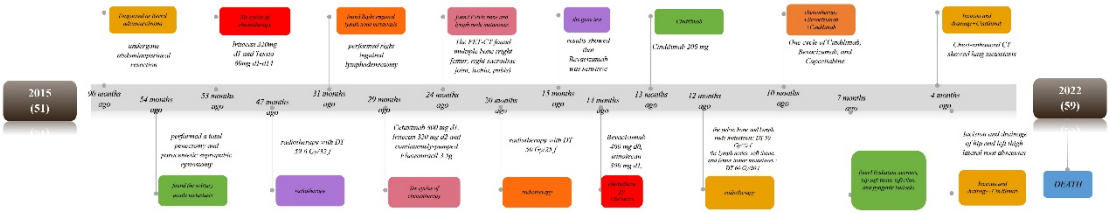
Comment 1: First, the abstract needs some revisions. The background is not unclear, please explain why the case deserved to be reported, the case per se or its immunotherapy. The rarity and unique clinical contribution should be described. The case presentation is not detailed enough, which needs to report the clinical characteristics of this patient, i.e., sex and main complaints, clinico-pathological

findings, treatment efficacy and adverse events, follow up, progression, and prognosis of this case. The conclusion needs more detailed comments for the effective management of similar cases.
Reply 1: We have modified our text as advised (see Page 1, line 34 to Page 2, line 47).
Changes in the text: (Page 1, line 34 to Page 2, line 47). The patient presented with penile pain and dysuria for 6 months when he was 54 years old, and Immunohistochemical staining showed that the origin was the rectum after total penectomy. The patient received surgery, chemotherapy, radiotherapy, targeted therapy and immunotherapy positively and still survived for a further 4 years and 6 months following penectomy despite the late metastasis of rectal cancer. There are two major changes and progress after penectomy, all of which have undergone surgical treatment during continuous treatment and follow-up, the patient completed right inguinal lymphadenectomy when his right regional nodes metastasis was found 23 months after penectomy. While the patient suffered from radiation injury after 47 months after penectomy, which led to radiation necrosis and hip soft tissue infection, and the patient tended to lay prone instead of lying on the back because of the hip pain. The patient ultimately died of multiple organ failure.

Comment 2: Second, the introduction of the main text needs a detailed review on the clinical management of penile metastases and its prognosis, with particular focus on the difficulties for the treatment. Please briefly describe the findings from previously published studies, not to mention the summary table alone.
Reply 2: We have modified our text as advised (see Page 3, line 79 to line 89).
Changes in the text: (Page 3, line 79 to line 89).We added some previously published studies “Beatriz NUNES reported a patient with a metastasis to the glans penis from a rectal adenocarcinoma underwent palliative treatment with radiotherapy and chemotherapy, remaining asymptomatic and disease-free at one year follow-up(3). Boubacar Efared disclosed a patient with penile metastasis from rectal adenocarcinoma who accepted his adjuvant chemotherapy (XELOX regimen, oxaliplatin plus capecitabine) (4) . A patient underwent palliative chemotherapy treatment and still alive 4 months after diagnosis of penile metastases(5). Taek-Gu Lee reported a patient with metastatic carcinoma from the rectal cancer and the patient was still alive after receiving palliative chemotherapy with modified FOLFOX-6 (mFOLFOX-6; oxaliplatin with 5-fluorouracil and folinic acid) plus bevacizumab (6)”.

Comment 3: Third, in the case presentation, a timeline figure is needed to describe the diagnosis, treatments, progression, follow up, and prognosis of this case. Fourth, in the discussion, please explain why the current treatment strategies have relative good prognosis and how to improve the clinical management of similar cases.
Reply 3: Third, we added a timeline figure: Figure 6, (see Page 5, line 143). Fourth, have modified our text as advised (see Page 5, line 159 to line 163; Page 6, line 205 to line 210).
Changes in the text: Third， we have added a timeline figure: Figure 6, the last figure(Page 5, line 143). Fourth, page 5: Sintilimab is a recombinant humanized lgG4

antiPD-1 antibody, with affinity to human programmed cell death protein 1 (PD-1). Chuan Lv(13) reported that a patient of recurrent and metastatic penile squamous cell carcinoma obtained progression-free survival with continuous sintilimab. Page 6: The choice of treatment of our patient have a palliative intention，but these therapeutic options may lead to superior treatment outcomes especially when considering the patient's positive attitude. We speculate that comprehensive treatment including surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy and helping patients have a positive and optimistic attitude may contribute to improve the clinical management of similar cases.



Reviewer D

1. Figure 2

a) Please explain HE in the legend.

Reply 4: we have modified our text as advised and explained HE (see Page 14, line 402 to 403)

Changes in the text: HE (X100, HE:Hematoxylin-eosin staining).HE staining can reveal the adenocarcinoma cells.

b) Please double check the legend, HE was not mentioned in the legend, please state the situation (positive or negative or others) of HE staining.

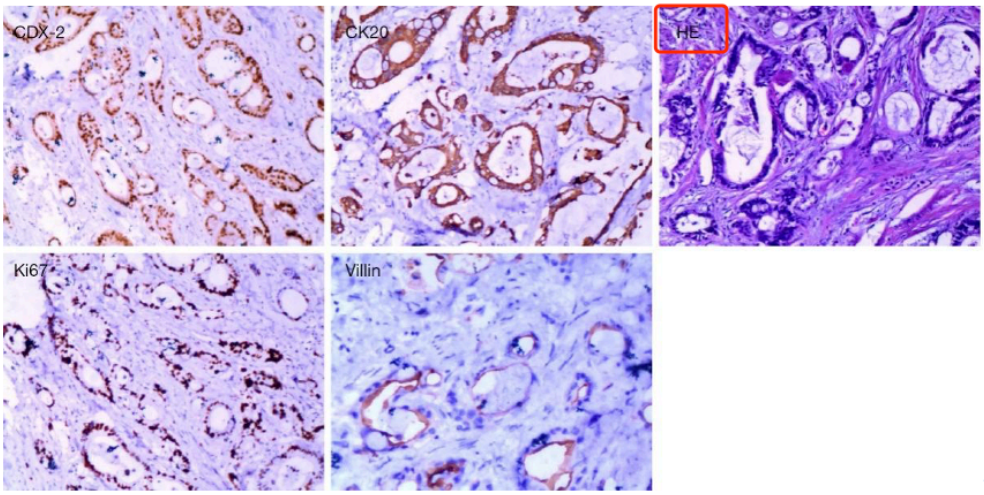


Figure 2 Immunohistochemistry, positive for CDX-2(X100), Villin(200) and CK20(X100), Ki-67index 50%(X100).

Reply 4: we have modified our text as advised, we have added X in the Villin(200) and explain HE (see Page 14, line 401 to 403). HE staining can reveal the adenocarcinoma cells, and we do not state the situation whether it's positive or negative or others.
Changes in the text: Villin(X200), HE (Hematoxylin-eosin staining, X100).HE staining can reveal the adenocarcinoma cells.

2. Figure 3

Please explain SPECT and CT in the legend.
Reply 5: we have modified our text as advised (see Page 14, line 407 to 408).
Changes in the text: SPECT/CT, Single-Photo Emission Computed Tomography/Computer Tomography.

3. Table 1

a) Please check if the author's name matches with the citation.

Gbenou (16)	2011	79	24
Seok Seo (17)	2011	72	7
Khubchandni (45)	1986	71	4

Reply 6 a): we have modified our text as advised (seePage4 and Page 16).
Gbenou(16) is right, the full name is Maximilien C Goris Gbenou.
Seok Seo(17) is right, the full name is Han Seok Seo.
Khubchandni(45) should be changed to Khubchandani(45)
Changes in the text: Khubchandni(45) was changed to Khubchandani(38)

b) Please add this study to the reference list and add the citation in the table.
*Please note that the references should be cited numerically and consecutively in the order of appearance**.**

Eberth	1870	40

Reply 6 b): we have modified our text as advised. (see Page 5 and Page 17).
Changes in the text: We have added this study to the reference list and added the citation in the table Eberth was changed to Eberth(3).

c) Please explain CRT in the table footnote.
Reply 6 c): we have modified our text as advised. (see Page 5, line 99 and Page 17, line 420).
Changes in the text: CRT, chemoradiotherapy.