Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "**Circulating tumor DNA genomic profiling reveals the complicated olaparib-resistance mechanism in prostate cancer salvage therapy: Case report** " (Manuscript NO: 60981). Those comments are all valuable and very helpful for revising and improving our paper, as well as, the important guiding significance to our research. We have studied the comments carefully and have made the appropriate corrections. We hope the revision could meet with your approval.

Yours sincerely,

Hong Zhou

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Responds to the reviewer's comments:

Reviewer #1:

1. **Page 3, line 18-19: "Serum total prostate-specific antigen (TPSA) level reduced and symptoms remitted for 4 mo." What is 'mo'?**

   Response: Thank for your kind question. 'mo' means months and in order to avoid to have confusions, we have replace 'mo' with 'months' in the whole article.

2. **The authors are advised to revise the 'Core tip'**

   Thanks. The 'core tip' have been revised

3. **Please add more strong keywords**

   Thanks for your suggestion. Based on our principle and objective, we have added some strong keywords: mCRPC; olaparib; ctDNA; PALB2; resistance mechanism; reverse missense mutations.

4. **Page 5, line 2-3: "Prostate cancer is the sixth most commonly occurring malignant tumor in mainland China." Please add more epidemiology of prostate cancer.**

   We have added some epidemiology of prostate cancer based an update on the global cancer burden that the International Agency for Research on

Cancer produced using the GLOBOCAN 2020 estimates of cancer incidence and mortality.

5. **Page 5, line 9-10: "Poly (ADP-ribose) polymerase (PARP) inhibitors have displayed promising clinical results…" Would you please enlist the names of PARP inhibitors?**

   Thanks, we have added the names of PAPR inhibitor, such as olaparib, rucaparib, niraparib or talazoparib which had been granted for the tumor treatment by the Food and Drug Administration(FDA).

6. **Page 5, The whole introduction section is general. Authors are advised to revise the introduction section carefully and add more data to make an association between each paragraph to support the problem statement. It is recommended to add literature in the introduction section to create a research gap.**

   Thanks very much. We have rewritten the whole introduction, focused on the research gap of ctDNA for genomic sequencing as a surrogate of tumor tissue in advanced prostate cancer and the lack of clinical efficacy date about the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib on the prostate cancer carrying the other homologous recombination repair such as PALB2 besides the two BRCA1 and BRCA2 genes.

7. **Page 5: What is the novelty of the present study?**

   Thanks a lot. After we rewritten the introduction, the novelty of the present study is obvious. By genomic sequencing results on ctDNA in the absence of the tumor issue, we researched the rapid response and resistance mechanism of the PAPR inhibitor olaprib. Our case emphasizes the importance of blood-based liquid biopsies and genomic profiling by means of ctDNA. Besides, we also proved that the advanced prostate cancer with HRR gene PALB2 mutation could be sensitive to the PARP inhibitor olaprib and the reverse mutation of PALB2 gene and others affecting known cancer-related signaling pathways may be the resistant mechanism to olaprib.

8. **Page 5, line 30-31: "A 61-year-old man presented to our hospital with complaint of continuous lumbosacral pain that had lasted for 3 mo" Please indicate the name of the hospital.**

   Thanks. We have indicated the name of the hospital: Chongqing University Cancer Hospital

9. **Page 6, line 27: "ADT in combination with docetaxel was applied as the initial treatment." How much drug was used? And for how many days?**

   Thanks. We have added the information of the usage on ADT and docetaxel. As below:

   ADT therapy (triptorelin, an LHRH agonist) in combination with docetaxel **(three weekly doses of 75 mg/m2**), but without prednisolone, was applied as the initial treatment fitting with the patient's mHSPC diagnosis and high tumor burden. **Two cycles** of this therapeutic intervention led to significant relief in the patient's self-reported pain as well as a substantial drop in TPSA level to 0.45 ng/mL

10. **The discussion section is not up to the mark; authors only discussed general literature without any comparison of results. No limitations are enlisted, and no overall conclusion is added. Overall, the discussion section needs extensive revision.**

    We really appreciate your constructive suggestion on the discussion section. We have rewritten and revised the discussion part by discussing the patient's clinical outcome compared with the literature results, enlisted three limitations about our case, and at the end, we also added overall conclusion.

11. **Authors are advised to proofread the whole manuscript to overcome grammatical mistakes**

    Thanks. We have sent the revised manuscript to a professional English language editing company (Filipodia Publishing, LL) and a native English-speaking expert had polished the manuscript further.

12. **The figures need proper interpretation and appropriate captions, and proper**

labelling

Thanks. We have added proper interpretation, captions and labelling about the three figures and the table.

13. **Please revise the references according to the journal instructions**

Thanks. We revised the references according the journal instructions ,such as add PMID and DOI number, besides, we also added some high impact journals and replaced some abstracts with some articles on published or on line which are related with our case.

Reviewer #2:

1. **About the effective olaparib-abiraterone tx for a patient without damage repair gene alterations, the authors should provide more minute explanation of its mechanism.**

We really appreciate your constructive suggestion on the explanation of the mechanism and we also added some relevant information. As below:

' In addition, there is a dual model of synergy between PARP inhibitor and ADT[23, 24]. PARP is involved in AR-dependent transcription and PARP inhibitor impairs this process, at the same time, the AR regulates transcription of DNA repair genes and androgen depletion impairs HRR, which might produce a so-called "BRCA-ness" phenotype that renders susceptibility to PARP inhibitor. Amplification of the AR gene, as observed via ctDNA profiling of our patient, would lead to continuous activation of downstream signaling pathway(s), overcoming the extrinsic androgen inhibition[25, 26] and precluding triggering of the "BRCA-ness" phenotype. The synergy between PARP inhibitor and ADT would not be able to be established in such a patient, which would explain why our patient's clinical response was worse.

2. **Description concerning genetic counseling is totally lacking. The authors**

should explain this aspect of issue within genetic examination.

Thanks. We had added some description concerning genetic counseling. As below:

PALB2 is a cancer susceptibility gene that may increase the risk of breast cancer (absolute risk: 41%-61%). The National Comprehensive Cancer Network (commonly known as NCCN) Genetic/Familial High-Risk assessment: Breast, Ovarian and Pancreatic (Version 1.2022) recommends that the PALB2 germline mutation carrier should start annual mammograms, with consideration of tomosynthesis and breast MRI with contrast, at age of 30 years and that the healthcare team open discussions into the option of risk-reducing mastectomy. PALB2 gene mutations are also associated with susceptibility to cancers of the ovary (absolute risk: 3%-5%), pancreas (absolute risk: 5%-10%), and breast in males. The NCCN: Prostate cancer (Version 1.2022) also recommends germline multigene testing that includes (at least) BRCA1/2 and PALB2 in its testing panel. Our patient carried a PALB2 germline pathogenic variation, so that his offspring would carry a 50% likelihood of harboring the same variation. As such, we would suggest that first- and second-degree relatives visit a genetic counselor to further evaluate whether they carry the proband's same PALB2 mutation; if so, the relative should receive genetic counseling to gain a sufficient understanding of the correlative cancer risk, further screening options and risk-reduction strategies. This will allow the positive carrier to better protect against the onset of related cancers or at least promote their ability to suspect and seek timely assessment to detect a cancer much earlier. Although we made such suggestions to our patient's relatives, none have accepted our suggestion as of the writing of this case report; nonetheless, this is part of our routine strategy of care and we will continue such efforts in the future.

***Science editor*:**

1. **With all due respect but this report requires some additional work, as ideas and purpose of presentation are rather unclear. From the title it is not clear what is the main purpose of this case report. Is it the genetic sequencing, the response to therapy?. Authors should be more specific about this supposedly patients in the real-world situation. How was this patient different? Why should this patient be taken as a proof of whatever authors want to highlight with this case. Why is this case special and different from the others. It is rather confusing what authors are trying to demonstrate from the beginning. Please re-review the whole document, order your ideas and try being more specific about the importance of this case**

We really appreciate your constructive suggestion. We had re-reviewed the whole document and rewritten or revised the core tip, key words, introduction, discussion and conclusion. The main purpose of this case report is to emphasized the feasibility of genomic sequencing on ctDNA as a sample choice of tumor genomic sequence in the absence of the tumor issue and on the sequencing results of ctDNA, we can research the rapid response and resistance mechanism of the PAPR inhibitor olaprib. Besides, we also proved that the advanced prostate cancer with HRR gene PALB2 mutation could be sensitive to the PARP inhibitor olaprib and the reverse mutation of PALB2 gene and others affecting known cancer-related signaling pathways may be the resistant mechanism to olaprib. This patient could be taken as a proof to be described because that the case showed the feasibility of ctDNA sequencing to guide treatment, indicate prognosis and analyze resistance and its underlying mechanisms, with ctDNA serving as a surrogate for limited or unavailable tumor tissue. Overall, though, the case provides a real-world example of how timely multigene testing can be of great importance for selecting the most precise therapeutic approach for cancer patients, especially for those with mCRPC.

Company editor-in-chief:

1. **Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.**

   Thanks. We had provided the original figure documents or decomposable figures by a single PowerPoint file and uploaded to the file destination of "Image File or Table File".

2. **Please upload the approved grant application form(s) or funding agency copy of any approval document(s).**

   Thanks. We had uploaded the approved grant application form(s) or funding agency copy of any approval document(s).

Remark: Explanation on the adjustment of the article related fund revision:

Dear Editors, due to the completion of the National Natural Science Foundation of China, No.81702452 during the article submission period, aand the consideration of the subsequent manuscript fee support, in the revised article, we had replaced it with the another fund: the Chongqing technological innovation and application development - Major theme projects, No. cstc2019jscx-fxydx0008.