

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript. Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made corrections which we hope meet with approval. The main corrections in the paper and the response to the reviewer's comments are as following:

Reviewer 1 (Reviewer's code: 00003345):

1. In this manuscript entitled "Complete response to trastuzumab and chemotherapy in recurrent urothelial bladder carcinoma with HER2 gene amplification: A case report" the authors report that treatment of a patient with trastuzumab in combination with chemotherapy induced a clinically complete remission for 34 months. This was associated to the decrease of serum ferritin and related to the amplification of HER2 gene (analyzed by NGS). The authors conclude that NGS as a promising tool to accurate exploration of genetic alterations could identify gene targets that help better determining which patients could more benefit from trastuzumab therapy.

This study raises some questions and comments: - This study does not allow this type of conclusion. These results must be found on other patients.

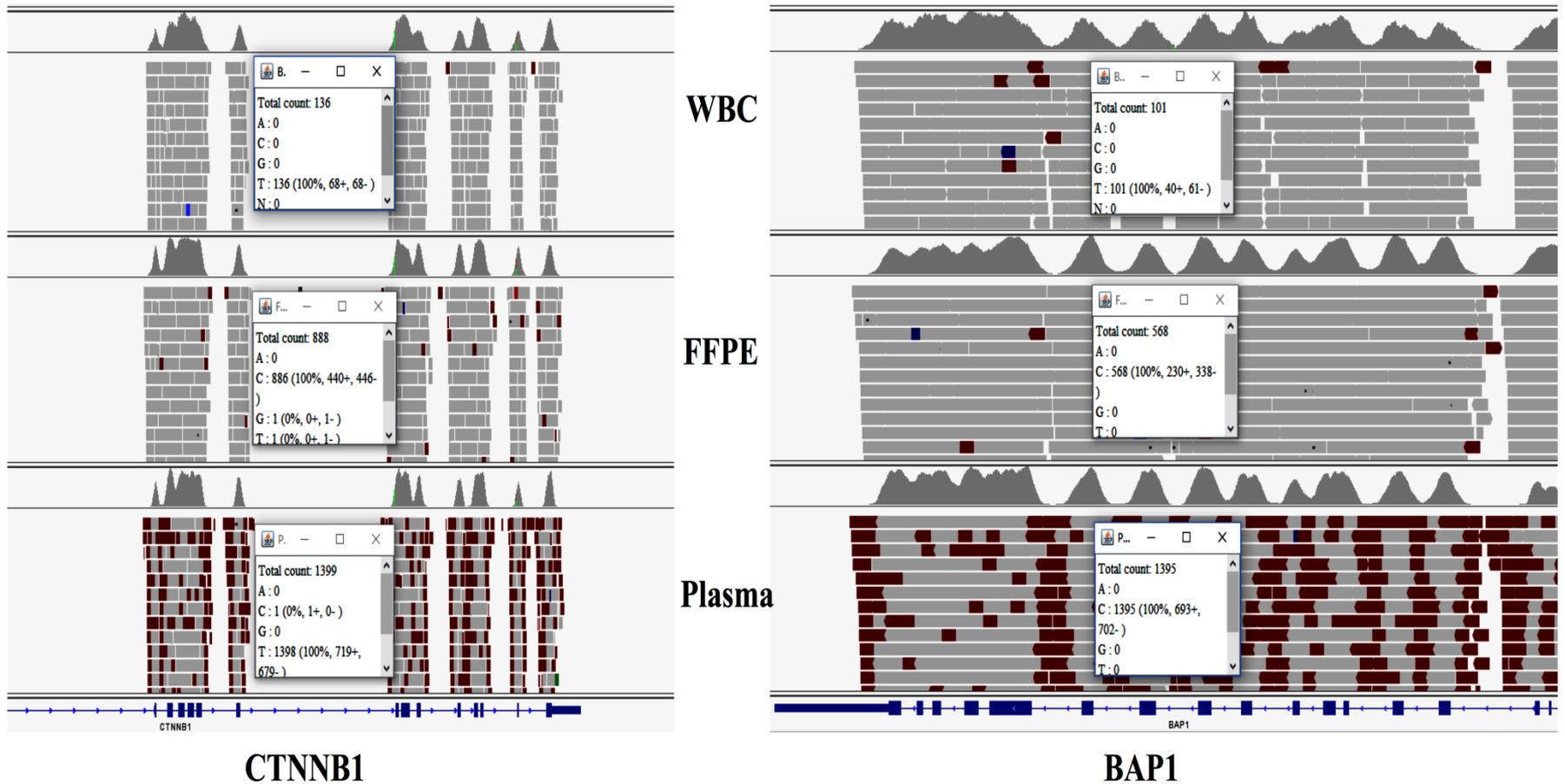
Response: We are very sorry for the lack of rigor in the conclusion. We have revised the conclusion and added "more studies and cases will be needed to confirm our findings." Details are as follows:

This case hinted that the recurrent UBC patient with HER2 gene amplification may benefit from targeted trastuzumab. Further studies and cases are needed to further investigate the status of HER2 gene and better determine trastuzumab in the management of UBC, particularly after failure of routine therapy.

It is very light like amplification of HER2 gene. Not sure that this could have an impact on HER2 activation. Normally, this type of amplification is much larger.

Response: We do treasure the suggestions from the reviewer. We re-consulted the operators and analysts involved in NGS testing for the NGS results. They did the FFPE NGS again and came to the same conclusion.

The number of bases of the HER2 gene in the tissue (FFPE) is significantly higher than the number of bases of the reference gene ALK gene (HER2 2092 vs ALK 670; Figure 1), indicating HER2 gene amplification. In addition, we compared two other reference genes (CTNNB1 and BAP1) with HER2 gene and also found amplification in HER2 gene (base number: HER2 2092 vs CTNNB1 888 vs BAP1 568).



- Table 1 and Figure 1 must be presented in a clearer form.

Response: Thanks for reviewer's suggestion. We have added a note into Figure 1 to make the NGS result more understandable. Meanwhile, we deleted Table 1 which duplicates the results in Figure 1.

- I do not see Figure legend for Fig 1 and 2?

Response: Thanks for reviewer's suggestion. In fact, we have written the figure legends previously, as follows:

Figure 1: ERBB2 (HER2) amplification in FFPE by the Integrative Genomics Viewer

Figure 2: The change of serum ferritin level in this patient

Reviewer 2 (Reviewer's code: 01560504):

I enjoyed reading the manuscript. A few details about the surgical management would give a complete clinical profile of the patient - 1. Why regional lymph nodes were not addressed during partial cystectomy (first surgery)? what was the margin status?

Response: Thanks for the suggestions from the reviewer. Since radical surgery that removing the bladder will have a great impact on the quality of life of patients, we performed local electroresection of the bladder tumor and postoperative chemotherapy for the patient through his own choice. Pathology confirmed that the surgical margin was negative.

2. Why ureterocutaneostomy and not ileal conduit during second surgery?

Response: Ileal conduit is indeed one of the mainstream ostomy methods for radical surgery of bladder cancer. We reviewed the patient's condition again and communicated with the surgeon, and found that the patient was in poor physical condition at the time and was not suitable for the relatively complicated ileal conduit with a longer operation time. Therefore, a simpler ureterocutaneostomy was selected, which was conducive to the rapid recovery of patients after surgery.

3. What was the third surgery undertaken?

Response: We are very sorry for the lack of the detailed information of the third operation. In the third operation, we only removed the left inguinal lymph node that was pathologically confirmed to have tumor infiltration.

Reviewer 3 (Reviewer's code: 02505493):

The manuscript can be accepted for publication.

Response: We deeply appreciate the reviewer's evaluation and consideration of the paper for publication.

Reviewer 4 (Reviewer's code: 02446191):

The authors have described a case of recurrent urinary bladder cancer where HER2 gene status was used as a tool for deciding treatment modality. Study highlights that patient with HER2 gene amplification tested by targeted next-generation sequencing (NGS), benefits from targeted trastuzumab. This is a case study, nevertheless many such cases are required to investigate for HER2 status before taking treatment decisions.

Response: We are very sorry for the lack of rigor in the conclusion. We have revised the conclusion and added "more studies and cases will be needed to confirm our findings." Details are as follows:

This case hinted that the recurrent UBC patient with HER2 gene amplification may benefit from targeted trastuzumab. Further studies and cases are needed to further investigate the status of HER2 gene and better determine trastuzumab in the management of UBC, particularly after failure of routine therapy.