Peer Review File

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Review Comments:

Comment 1: A major limitation of this study is the fact that relies on dipstick as the method of choice to assess proteinuria. It would have been more appropriate to use proteinuria/creatinine ratio to assess proteinuria which most likely is available for at least a subset of the patients studied and should be included in their analysis.

Reply 1: Thanks for your valuable comments. We agree that proteinuria/creatinine ratio is a better way to assess proteinuria than dipstick. As this is a retrospective study and urine albumin creatinine ratio was not routinely examined in our hospital, proteinuria /creatinine ratio was unavailable for any patients in this study. In further studies, we will routinely test proteinuria/creatinine ratio to evaluate proteinuria. However, dipstick is also widely used in the literature including some high quality clinical trials[1-3]. We add using dipstick to evaluate proteinuria as a limitation in the end of "Discussion".

Changes in the text: We add this as a limitation at page 14 lines 310-313. It reads as: "*Forth, urine albumin creatinine ratio, which is considered as a more reliable parameter to assess proteinuria, is unavailable in the current study due to its retrospective design. In further studies, we will routinely test urine albumin creatinine ratio to evaluate proteinuria.*"

Comment 2: In addition given the known effects of TKIs on blood pressure, this should also be included in their analysis as well as the type of drugs used to control hypertension as they may also have an effect on proteinuria.

Reply 2: Thank you for pointing out this important issue. We are very sorry for not including hypertension and hypertension medications in the analysis. In the revised manuscript, we added the information about hypertension and hypertension medications.

Of the 141 patients, thirty-six (26%) patients had hypertension and 20 patients were on long-term hypertension medications. In uni- and multivariate analysis, hypertension and hypertension medication were not independent factors for predicting an increase of the level of proteinuria (**Table 2**). While hypertension was defined as a significant predictor (OR:2.81, 95%CI: 1.25-6.28) for predicting a 15% drop in eGFR (**Table 3**).

Changes in the text: We added some data in our tables. The description was in page 8 lines 166-167 *"Thirty-six (26%) patients had hypertension and 20 patients were on long-term hypertension medications"* and page 9 lines 204-205 *"In univariate analysis, older age (OR:3.56, 95% CI:1.63-7.90), hypertension (OR:2.81, 95% CI:1.25-6.28), longer duration of TKI (OR:2.68, 95% CI:1.32-5.45), abnormal baseline CYSC (OR:1.94, 95% CI:0.98-3.81) were determined as predictors for significant renal function decline."*

Comment 3: There is no mention either whether patients at any time received corticosteroids for suspected AIN secondary to PD-1 inhibitors and whether any of the subjects had a renal biopsy.

Reply 3: We are very sorry for missing that information. Of the 36 patients treated with PD-1 inhibitors, 4 patients received corticosteroids due to suspected AIN based on clinical symptom. However, none of 4 patients had renal biopsies.

Changes in the text: We made some changes in page 11 lines 235-237. Now we state as: *"Besides, Of the 36 patients treated with PD-1 inhibitors, 4 patients forced to stop taking immunotherapy and received corticosteroids due to suspected acute interstitial nephritis (AIN)."*

Comment 4: Since partial nephrectomy is now more commonly used for the surgical treatment of renal masses the authors should also determine whether the type of nephrectomy played any role on the further development of proteinuria and reduced renal function.

Reply 4: Thanks for your comments. Twenty(14%) patients received partial

nephrectomy and 91 (65%) patients underwent radical nephrectomy before systemic therapy treatment (**Table 1**). However, the type of nephrectomy didn't play significant role on the further development of proteinuria and reduced renal function in our cohort (**Table 2 & 3**).

Changes in the text: We added some data in our tables.

Comment 5: Finally it's not clear either whether any of the changes in renal function and/or proteinuria led to discontinuation of treatment on whether they were linked to differences in survival.

Reply 5: We thank the reviewer for this important suggestion. A total of sixteen (11%) patients discontinued treatment because of severe renal insufficiency. We used Log-rank analysis to analyse the association between renal impairment and survival of patients after TKI treatment (**Figure 5**). The proteinuria increase (HR:2.99, 95%CI: 1.96-5.46) and eGFR decline (HR:2.50, 95%CI: 1.36-4.58) were all significant with patients' survival.

Changes in the text: We added figure 5 and some description in the revised paper. In the abstract (line 73-75), we added *"Log-rank analysis identified proteinuria deterioration and eGFR decline were both significantly associated with patent's survival*". In page 5, line 110-112, we added *"Patients' survival was defined as the time from the initiation of TKI treatment to the date of death as a result of any cause or was censored at the date of last follow-up.". I*n page 7, line 159-160, we added *"Log-rank analysis was used to analyse the association between renal impairment and survival of patients after TKI treatment".* We state in line 230-240 as: "After a 19.43 (22.32-8.37) months follow-up in 141 patients treated with TKI, we found 103 (73%) patients discontinued treatment or changed the type of TKI. Forty-nine (42%) patients had adverse reactions including renal impairment, hand-foot syndrome,

nausea, vomiting, diarrhea, thrombopenia and hypoleukocytosis. Sixteen (11%) patients stopped treatment due to severe renal impairment" and in line 231-235 as "Forty-three (30%) patients died because of drug-resistance and tumor progression. Log-rank analysis showed the proteinuria increase (HR:2.99, 95%CI: 1.96-5.46) and eGFR decline (HR:2.50, 95%CI: 1.36-4.58) were both significantly associated with patients' survival (**Figure 5**)"

1. Sorich, M. J., Rowland, A., Kichenadasse, G. et al.: Risk factors of proteinuria in renal cell carcinoma patients treated with VEGF inhibitors: a secondary analysis of pooled clinical trial data. Br J Cancer, **114:** 1313, 2016
2. Motzer, R. J., Hutson, T. E., Glen, H. et al.: Lenvatinib, everolimus, and the combination in patients with metastatic renal cell carcinoma: a randomised, phase 2, open-label, multicentre trial. Lancet Oncol, **16:** 1473, 2015
3. Ravaud, A., Barrios, C. H., Alekseev, B. et al.: RECORD-2: phase II randomized study of everolimus and bevacizumab versus interferon α-2a and bevacizumab as first-line therapy in patients with metastatic renal cell carcinoma. Ann Oncol, **26:** 1378, 2015