Author's response to reviews

Title: Impact of a tenofovir disoproxil fumarate plus ritonavir-boosted protease inhibitor-based regimen on renal function in HIV-infected individuals: a prospective, multicenter study

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Author's response to reviews: see over
Dear editor,

Thank you for considering our manuscript.

We appreciate the reviewer’s valuable comment on our study and our responses are below. I have addressed their concerns and resubmit an amended version in light of the comments. I hope my paper will be returned to the reviewers for further consideration.

I hope this e-mail finds you well and I look forward to hearing from you soon.

Sincerely

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Author’s responses to Reviewer:

MAJOR:

(i) Given that patients in TDF group were HIV-infected for a longer duration and had been exposed to other ART agents in the past, how might this have impacted your results? Authors should discuss and/or provide additional analysis adjusted for HIV duration if possible. Present discussion is unclear/insufficient.

Answer: We appreciate the reviewer’s valuable comment on our study. It is an ideal design comparing treatment naïve patients receiving TDF with treatment naïve individuals in China. However, only a few of patients can afford the cost of TDF if TDF was used as first-line therapy drug. Patients who failed first-line treatment could receive cART containing TDF. The reason was that TDF was only free as second-line therapy drug provided by the Chinese government to HIV-infected individuals who failed first-line treatment since mid-2009 in China. So patients in TDF group were HIV-infected for a longer duration than control group and had been exposed to other ART agents in the past. Although patients in TDF group have a longer duration HIV seropositive, they have received first-line cART (e.g., AZT + 3TC +NVP/EFV, D4T +
(iii) How do your results compare to the TDF-associated change in eGFR in western population?

Answer: Nancy Crum-Cianflone et al. [20] evaluated the impact of tenofovir and a boosted PI on renal dysfunction among 150 HIV-infected patients mainly based on Caucasian. They found that those initiating both tenofovir and a boosted PI had a great change in kidney function: 78 (52%) had a reduction in eGFR, the median change of eGFR was -12.1 ml/min/1.73m² [95% confidence interval (CI), -9.1 to -14.1 ml/min/1.73m²], and 30% had a loss of >10 mL/min/1.73m² during the 2-year follow-up period. In the study of Goicoechea et al. [20], they compared the estimated decline in renal function among western HIV-1 infected patients receiving a TDF+PI/r- (n =51) or non-TDF-containing (n=66) regimen. They demonstrated that patients receiving TDF+PI/r had a greater rate of decline in creatinine clearance (CrCl) than did the non-TDF-containing group (for MDRD, -14.7 vs. -4.78 mL/min/1.73m²/year). Consistent with observations in western population, in our study after 48 week the median changes of eGFR were respectively -8.8 ml/min/1.73m² in TDF+PI/r and 6.4 ml/min/1.73m² in control groups. Patients treated with TDF and PI/r was associated with greater declines in renal function over 48 weeks compared with non-TDF-containing regimens.

This part was added in discussion Paragraph 3.
(iii) Given that some PI agents could be nephrotoxic (such as indinavir, atazanavir and possibly lopinavir/r), at very least provide description of which PIs were being used in the TDF group.

Answer: The description of which PIs were being used in the TDF group was not clearly indicated in my former paper. Now I have adopted your suggestions to give this detail in Methods Paragraph 1 of the revised version. In TDF+ ritonavir-boosted protease inhibitor (PI/r) group, patients all received second-line cART consisting of TDF+ Lamivudine (3TC) + ritonavir-boosted lopinavir (LPV/r). So, in the TDF group, LPV/r was being used in the TDF group.

(iv) Statistics: Comparing differences, as well as difference in changes in creatinine and eGFR at 48 weeks using Wilcoxon rank-sum test seem appropriate. However, methods used for second part (Results paragraph 3) focused on trends in creatinine and eGFR need improvement. The conclusion that “.. creatinine rapidly increased in first 4 weeks and then stabilised..” and “.. eGFR declined in first 4 weeks and then stabilised..” are not sufficiently backed up by statistical analysis. Ideally, a random effect model (to account for repeat measurements and matching) having exposure group variable and time variable (categorised at say < or > 4 weeks) and possibly the interaction between time and exposure group, should be assessed for each of the endpoints (creatinine and eGFR).

Answer: We appreciate the reviewer’s stringent comment. We know a random effect model is very fit to deal with the problem. However, we are not proficient in random effect model. We consulted some statisticians who worked in our hospital. In their opinion, the Wilcoxon signed rank test could be used to assess trends in serum creatinine and eGFR. So in this revision, in order to assess trends in serum creatinine and eGFR, the Wilcoxon signed rank test was used.

The median values of serum creatinine or eGFR for baseline and the 4-week point were compared using Wilcoxon signed rank test. The median values of serum creatinine or eGFR for the 4-week point and the 48-week point were also compared using Wilcoxon signed rank test. We found that compared with baseline, the median
values of serum creatinine for the 4-week point were significantly higher ($P=0.002$). However, the median values of serum creatinine between the 4-week point and the 48-week point were no significant differences ($P=0.719$). So it showed that the values of serum creatinine increased significantly initially (0-4 weeks), and then (5-48 weeks) maintained at the stable level. We noticed that compared with baseline, the median values of eGFR for the 4-week point were significantly lower ($P=0.001$). However, the median values of eGFR between the 4-week point and the 48-week point were no significant differences in TDF+PI/r group ($P=0.725$). So it showed that the median values of eGFR decreased significantly initially (0-4 weeks), and within 5-48 weeks, median eGFR remained relatively stable in participants treated with TDF + LPV/r + 3TC.

(v) It seems counter intuitive that the impact of TDF was largely in first 4 weeks and then stabilised. Results from larger cohorts (see EUROSIDA study) suggest cumulative exposure to TDF could result in increasing renal toxicity. Similarly, negligible impact of TDF in those with abnormal baseline eGFR (n=23) is also surprising. It is possible that the study was simply not powered to pick up these differences given that expected impact of TDF on GFR is generally small. Authors should attempt to make a statement on power of their study or discuss in limitations.

Answer: For our analyses of kidney function over time among patients using TDF+LPV/r+3TC, we noticed that the impact of TDF+ PI/r was largely in first 4 weeks and then stabilized during 48 weeks. Consistent with observation in the Development of Antiretroviral Therapy (DART) trial in a large cohort of HIV-infected African adults from Uganda and Zimbabwe, changes in eGFR are also observed predominantly during early exposure to containing-TDF cART regimens, with stabilization of kidney function after ~4 weeks [29]. On the contrary, in the EuroSIDA (AIDS across Europe) study, data from larger European cohorts have recently demonstrated an association between TDF cumulative exposure and increasing renal toxicity [30]. Andrew N. Phillips et al. performed a prospective study and white
participants took up more than 85% in their study. 89.8% enrolled participants were cART experienced patients and 99.7% patients have already received containing-TDF cART regimen at or before baseline. Many of enrolled participants had preexisting risk factors for CKD. In TDF group, 21.7% patients used nephrotoxic drugs, such as pentamidine, cidofovir, acyclovir, foscarnet or amphotericin B at or before baseline. However, in our study, patients were Chinese and more than 90% was Han race. In addition, all patients in TDF+PI/r group never received TDF and/or PIs-containing cART regimens and never used nephrotoxic drugs at or before baseline. Thus, the most plausible explanation for this discrepancy is the longer follow-up of the EuroSIDA cohort, the genetic nature, the different treatment history of patients and the inclusion of unselected patients, many of whom had pre-existing risk factors for CKD, which have been demonstrated to further increase the risk of TDF-related CKD [31].

The impact of TDF+PI/r-based cART regimen on renal function was analyzed in patients stratified by baseline eGFR. Baseline eGFR was stratified into 2 categories (eGFR \(\geq 90\) and \(90 > \text{eGFR} \geq 50\) ml/min/1.73m\(^2\)). Before results were known, in our opinion, we also thought that a significant decline in renal function should be observed in patients with baseline \(90 > \text{eGFR} \geq 50\) ml/min/1.73m\(^2\). However, the viewpoint was not supported by our data. It was also surprised us. We checked the data. The data was correct. Maybe patients with baseline \(90 > \text{eGFR} \geq 50\) ml/min/1.73m\(^2\) are not sensitive to side effects of drugs such as nephrotoxicity, patients with baseline \(\text{eGFR} \geq 90\) ml/min/1.73m\(^2\) were more sensitive to renal dysfuntion, but this requires confirmation in further studies. It is also possible that baseline \(\text{eGFR} \geq 90\) and \(90 > \text{eGFR} \geq 50\) ml/min/1.73m\(^2\) was simply not powered to pick up these differences given that expected impact of TDF on GFR is generally small. Larger and further studies may be needed.

Also in Discussion, Paragraph-4 “In TDF+PI/r group...” is unclear.
Answer: The Paragraph-4 “In TDF+PI/r group...” was replaced by “The impact of
(vi) Since this was a prospective study designed for renal endpoints, other risk factors (smoking, diabetes, hypertension) were surprisingly absent. They should be presented if available, else discuss this important limitation.

Answer: We quite agree with the reviewer’s opinion. Indeed we lacked data for renal risk factors, such as smoking, diabetes and hypertension in our former paper. We added the data in the revised version as the reviewer noted.

Minor:
- It seems the abstract is missing.
Answer: The abstract was already added in the revised version.

- Clearly state if both the groups were initiating (i.e. not already receiving) respective ART regimen at baseline.
Answer: The control group was not initiating ART regimen at baseline, but the TDF+PI/r group was already receiving the first line ART regimen at baseline and then was switched to the second line ART regimen (TDF + 3TC+ LPV/r) . We added this explanation in Methods Paragraph 1 of the revised version.

- Confirm is ethics approval was obtained from the mentioned review board.
Answer: The ethics approval was obtained from the Institutional Review Board of Peking Union Medical College Hospital (PUMCH). It was written in Methods Paragraph 1.

- Mention full form of all abbreviations at first use.
Answer: The full form of all abbreviations was not given when they were at first use in my former paper. Now I have carefully corrected them.

- Mention if matching was 1 to 1 or based on frequencies of the matched variables?
Answer: Seventy five HIV-1 infected cART-naïve patients exactly matched with 75 HIV-1 infected patients in TDF group for gender, age, serum creatinine and eGFR selected from a multicenter cohort study population constituted the control group.

-Methods Paragraph 1, Inclusion criteria point 2: Mention frequencies of given cART regimens.
Answer: Thanks for your nice suggestion. Thirty five patients treated with Zidovudine (AZT) + 3TC + Nevirapine (NVP) / Efavirenz (EFV), twenty patients treated with Stavudine (D4T) + 3TC + NVP / EFV, fifteen patients treated with AZT + Didanosine (DDI) + NVP / EFV and five patients treated with D4T + DDI + NVP / EFV.

-Discussion: Discussion on mechanism of TDF toxicity could be reduced/minimised as it was not the main focus of the study.
Answer: We quite agree with the reviewer’s opinion. The mechanism of TDF toxicity was already minimized.

-Replace all P values given as “0.000” to “<0.001”.
Answer: I have replaced them.

-Fig-1 and Fig-2 presentations are different (p values in fig-1 and actual eGFR values fig-2). Please be consistent.
Answer: Actual Scr values were also shown in Fig-1. Now they are consistent.

-Figure-3: change “90>eGFR>=group” to “90>eGFR>=50” in the table.
Answer: As the reviewer noted, in the table of Figure-3 “90>eGFR>=group” is an error, I have changed it to “90>eGFR>=50 group”.

-There were other several minor grammatical errors which I have mentioned as track changes in the word document as they were too many to be populated here.
Answer: Thank you for carefully considering our manuscript. The written English was
improved by my native-English speaking colleague.

Discretionary comment:
It would be of interest to give the sensitivity analysis after calculating eGFR using Thai eGFR formula (recently validated and published, see Praditpornsilpa et al AIDS 2012 26:1781-1788). This eGFR calculation may be more closer to Chinese population than that derived on white and black populations.