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Association of losartan with outcomes in metastatic pancreatic cancer patients treated with chemotherapy

Anup Kasi, Jessica Allen, Kathan Mehta, Prasad Dandawate, Subhrajit Saha, Stefan Bossmann, Shrikant Anant, Weijing Sun

Corresponding author
Anup Kasi
University of Kansas Medical Center Division of Medical Oncology, Kansas, USA

Handling editor:
Michal Heger
Department of Pharmaceutics, Utrecht University, the Netherlands
Department of Pharmaceutics, Jiaxing University Medical College, Zhejiang, China

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Ref.: Ms. No. JCTRes-D-20-00149
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Dear Ms. Allen,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Feb 24, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Excellent study, the title of the paper is informative, grabs readers' attention quickly. The aim of the paper is clear and relevant. Authors have inappropriately added some studies in their metanalysis, which caused erroneous results. References are recent and referenced correctly. The introduction and background of the topic is very detailed and informative. Researchers have raised and outlined an important question relevant to the topic, attempted to analyze the effect of losartan use at the time of diagnosis on overall survival (OS), progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR) in metastatic pancreatic cancer patients treated with chemotherapy. Data is presented in an appropriate way, figures are clearly presented, title and columns are clear and correctly placed. The discussion section is detailed from multiple angles and placed in the context of the topic, It doesn't look under or overly interpreted. In fact, they have discussed the results by comparing other studies from the past on the same topic such as hao et al., Murphy et al.. The conclusion is around the objective of the study and resonates with the results. Limitations seem reasonable for a retrospective chart review design. There are some minor errors/suggestions to consider reviewing.

1. Abstract: In order to confirm if the benefit of losartan + FOLFIRINOX seen in neoadjuvant setting for locally advanced cancer also applies to metastatic cancer, our findings need to be validated in a larger cohort (consider rewrite this sentence, this seems confusing and lengthy)
2. Thus, there is desperate need for efficient methods of early detection (consider a suitable appropriate word for " desperate) i-e critical
3. The effect of renin-angiotensin system(RAS)-modulating drugs on treatment outcomes in pancreatic cancer can be explained by the role RAS plays in the tumor microenvironment. (please provide reference to this statement)
4. The outcomes between patients using ARB losartan and a control group of patients not on losartan were compared using logrank trends tests and Kaplan-Meier survival curves. (please correct the font size)
5. high-dose losartan, defined as 100mg daily based on the maximum dose for hypertension in adults, at diagnosis versus control patients (please provide a reference to this that 100mg of dose is recognized as high dose in clinical settings).

Reviewer #2: Briefly association of losartan with outcomes in metastatic pancreatic cancer patients treated with chemotherapy is a retrospective review of the metastatic pancreatic ductal adenocarcinoma patients treated at KU from 2000-2019 on the overall survival (OS), progression free survival (PFS), objective response rate (ORR), and disease control rates (DCR) between the patients on losartan compared to patients not on losartan as a control. Statistical analysis showed there is no significant difference in the above outcomes except for slight trend towards better progression free survival in patients treated with FOLFIRINOX. Introduction was well written explaining the basis of Renin angiotensin system in the pathogenesis of the cancer.

Suggestions:
--Another table with the OS,PFS, ORR,DCR might help the readers in easily following the results.
--The subgroup of patients on losartan who got FOLFIRONOX are only around 24% compared to the control for the same chemotherapy group was 49%. This might have effected the p value not being statistically significant and just showed a trend. explaining this in the discussion section might help the readers.

Study has drawbacks of being a retrospective review but it was clearly mentioned in the discussion section.

Reviewer #4: Dear Authors, Please refer to the reviewers comment document for specific suggestions. Overall, the manuscript needs more discussion in the methodology, choice of statistical analysis, and elaborate discussion about the limitations, to inform the readers about the implications of this observation. The matter of the study is in the scope of the journal, and the research question is clearly described, with clear measurement of outcomes. However, given the small sample size, there needs to be specific discussion about the scope of subgroup analysis, especially in the Folfirinox subgroup. A discussion about controlling for variables like age, smoking, alcohol is also preferred.

If you agree with the following suggestions, please consider revising the methods, statistical analysis and the discussion sections before resubmitting.

Thank you.
Sincerely,

Reviewer 4

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

Authors' response

Dear Editorial Board of JCTRes,

RE: RESPONSES TO REVIEWER COMMENTS

We would like to sincerely thank you for reviewing our manuscript, and for providing important comments. We have taken all of the referees' comments into consideration and

have revised the manuscript accordingly. Below, please find our point-by-point responses to the referees' comments.

Reviewer 1:

1. Abstract: In order to confirm if the benefit of losartan + FOLFIRINOX seen in neoadjuvant setting for locally advanced cancer also applies to metastatic cancer, our findings need to be validated in a larger cohort (consider rewrite this sentence, this seems confusing and lengthy)

   Response: Edited the sentence to provide further clarification (line 33-35); "Our findings should be validated in a larger cohort to confirm if the benefit of losartan and FOLFIRINOX seen in a neoadjuvant setting for locally advanced cancer also applies to metastatic cancer."

2. Thus, there is desperate need for efficient methods of early detection (consider a suitable appropriate word for " desperate) i-e critical

   Response: Removed the word 'desperate' from the sentence (line 61); "Thus, there is need for efficient methods of early detection, prognostic markers to guide treatment decisions, and treatments with greater efficacy in increasing overall survival."

3. The effect of renin-angiotensin system(RAS)-modulating drugs on treatment outcomes in pancreatic cancer can be explained by the role RAS plays in the tumor microenvironment. (please provide reference to this statement)

   Response: Added citation (line 79); "8. Pinter M, Jain RK. Targeting the renin-angiotensin system to improve cancer treatment: Implications for immunotherapy. *Sci Transl Med*. 2017;9(410):eaan5616. doi:10.1126/scitranslmed.aan5616 "

4. The outcomes between patients using ARB losartan and a control group of patients not on losartan were compared using logrank trends tests and Kaplan-Meier survival curves. (please correct the font size

   Response: Fixed font, changed to times new roman 12 pt (lines 109-110)

5. "high-dose losartan, defined as 100mg daily based on the maximum dose for hypertension in adults, at diagnosis versus control patients" (please provide a reference to this that 100mg of dose is recognized as high dose in clinical settings)

   Response: Included citation (line 114) and updated citation list, corrected citation numbers; " 11. Sica DA, Gehr TW, Ghosh S. Clinical pharmacokinetics of losartan. Clin Pharmacokinet. 2005;44(8):797-814. doi: 10.2165/00003088-200544080-00003. PMID: 16029066."

Reviewer 2:

1. Another table with the OS,PFS, ORR,DCR might help the readers in easily following the results.
   Response: Added a table displaying the results of PFS, OS, DCR, and ORR including p values (line 169).

Table 2

Group	Median OS (days)	*p*	Median PFS (days)	*p*	DCR *p*	ORR *p*
Losartan	274	0.466	83	0.919	0.497	0.621
Control	279		111			
Losartan +Gemcitabine + Abraxane	312	0.916	69	0.314		
Gemcitabine + Abraxane without Losartan	221		136			
Losartan + FOLFIRINOX	347	0.916	350	0.0604		
FOLFIRINOX without Losartan	333		101			
Losartan 100mg	261	0.727	84	0.790		
Control	279		11			

2. The subgroup of patients on losartan who got FOLFIRONOX are only around 24% compared to the control for the same chemotherapy group was 49%. This might have effected the p value not being statistically significant and just showed a trend. explaining this in the discussion section might help the readers.

   Response:

   - Clarified the numerical difference in subgroup analysis of FOLFIRINOX and how this may have impacted our p value (lines 179-185); “Both subgroup analyses based on chemotherapy regimen, FOLFIRINOX and gemcitabine plus abraxane, found no statistically significant difference in OS and PFS between experimental groups and control groups. However, there was notable numerical difference in PFS in the

FOLFIRINOX group, with a median PFS of 350 days in the losartan group compared with a median PFS of 101 days in the control group. There was no significant difference seen in OS and PFS in patients on the maximum dose of losartan and control patients."

- Also noted the numerical difference in the Results section under 'Characteristics of Patients' (lines 133-138); "Conversely, FREQ procedure performed to test association between chemotherapy regimens (FOLFIRINOX, gemcitabine + abraxane, gemcitabine, capecitabine, other regimen, and no regimen) and losartan use found significant differences in groups [p=0.0137]. This explains the numerical difference between patients on losartan treated with FOLFIRINOX (14, or 24.6% of the total losartan sample) and control patients treated with FOLFIRINOX (28, or 49.1% of the total control sample)."

Reviewer 3:

1. Consider mentioning the study as an observational study where data were analyzed looking at a cohort of metastatic PDA patients retrospectively.

   Response: Added clarification of the study's retrospective, observational nature in abstract (line 10), added that the study was observational in the introduction (line 95) and methods (line 102)
2. Please mention what diagnostic code was used in the electronic database, and if there was any exclusion or any conflict while selecting the cohort.

   Response: We used our institution's medical informatics system, HERON, which we cited after mentioning it. We noted that we searched for patients based on losartan use and metastatic pancreatic cancer diagnosis. We then conducted chart review of patients we found via HERON to ensure that they were indeed using losartan when diagnosed and that losartan use continued throughout treatment for pancreatic cancer. ; "Patient groups based on losartan use at time of metastatic pancreatic cancer diagnosis were identified via KUCC's medical informatics system, HERON.[9,10] Chart review was then conducted to ensure losartan was used at time of diagnosis and continued throughout treatment. Patient data was stored on a secure REDCAPs database. All data was deidentified before analysis." (lines 106-109)

3. The subset of two subgroup analysis (lines 40-43) seems to be between the losartan and no losartan groups. Please clarify the groups in the sub group analysis.

   Response: Added clarification that chemotherapy subgroup analyses were between losartan vs non-losartan groups (lines 115-117); "A subgroup analysis assessing OS and PFS was conducted between patients on high-dose losartan, defined as 100mg daily based on the maximum dose for hypertension in adults, at diagnosis versus control patients.[11] Another subgroup analysis of OS and PFS was conducted between patients on losartan that were primarily treated with the chemotherapy regimen FOLFIRINOX versus control patients not on losartan treated with FOLFIRINOX and patients on losartan treated with gemcitabine and abraxane versus control patients treated with gemcitabine and abraxane."

4. There is no mention of alcohol use in both the groups. Please mention why this data was not reported or not collected. Overall, the manuscript lacks any discussion about the effects of the variables like alcohol, smoking, age, and gender. Though smoking and age data were reported, it would be helpful to mention these factors as possible confounding factors in the observation.

Response:

- In the 'Characteristics of Patients' section of the Results we also added information on statistical analysis of distribution between losartan and control groups based on race, age, chemo regimen, and smoking status (lines 130-141); "FREQ procedure was done to test for association between covariates such as gender and losartan use [p=0.851], race and losartan use [p=.323], and smoking and losartan use [p=0.492]. No significant association was found between any of these variables and losartan use, indicating even distribution between groups. Conversely, FREQ procedure performed to test association between chemotherapy regimens (FOLFIRINOX, gemcitabine + abraxane, gemcitabine, capecitabine, other regimen, and no regimen) and losartan use found significant differences in groups [p=0.0137]. This explains the numerical difference between patients on losartan treated with FOLFIRINOX (14, or 24.6% of the total losartan sample) and control patients treated with FOLFIRINOX (28, or 49.1% of the total control sample).

TTEST procedure found a significant difference in age of the losartan group and the control group [p=0.0034]. Control group patients had a lower median age (61 years) when compared to patients on losartan (68 years)."

- The discrepancy in age between groups is also listed as a limitation (line 201) and is numerically pointed out in the results section (lines 137-139; see above).

- We did not collect data on alcohol use, and this was listed as a limitation in the discussion (line 202; "Other limitations include differences in demographic characteristics, such as age, between experimental and control groups, lack of collection of other demographic characteristics such as alcohol use, and possible homogeneity due to sample selection from only one treatment center which limits generalizability of results."

5. Please mention why sub group analysis was done with a relatively small n between Folfirinox-losartan group with the control group (14 vs 28). This limitation needs more exploration in the discussion section, where Murphy et al. study was cited, showing some benefit in the losartan Folfirinox group in the locally advanced PDA (Page 8, lines 45-55)

Response: Elaborated on how the numerical difference in the FOLFIRINOX subanalysis groups may have impacted our results in the newly added 'limitations' section (lines 208-213); "A specific limitation in the subgroup analysis between patients on losartan treated with FOLFIRINOX and control patients treated with FOLFIRINOX is the numerical difference between subjects in each group. Only 24.6% of the losartan group was treated with FOLFIRINOX while 49.1% of the control group was treated with FOLFIRINOX. This numerical difference (13 losartan + FOLFIRINOX patients versus 28 control +

FOLFIRINOX patients) could have affected the significance in difference in OS and PFS between these two groups."

- Also noted the numerical difference between the losartan FOLFIRINOX group and the control FOLFIRINOX group in the Results section under 'Characteristics of Patients' (lines 133-138); "Conversely, FREQ procedure performed to test association between chemotherapy regimens (FOLFIRINOX, gemcitabine + abraxane, gemcitabine, capecitabine, other regimen, and no regimen) and losartan use found significant differences in groups [p=0.0137]. This explains the numerical difference between patients on losartan treated with FOLFIRINOX (14, or 24.6% of the total losartan sample) and control patients treated with FOLFIRINOX (28, or 49.1% of the total control sample)."

6. Please consider discussing Nakai et al. study design (citation 10) and their sample size while discussing the significant effect on the OS and PFS in the mixed cohort of locally advanced and metastatic PDA. It would be useful to lay out the comparison with the current design and sample size to inform the readers about the implications of this observation.

Response:

- Clarified sample size of both of Nakai, et. al.'s studies (line 192).; "[6] Our findings differ from those of Nakai, et.al., which found that the use of an ACEi or ARB was associated with significantly increased OS and PFS in a mixed cohort of locally advanced and metastatic PDA patients in both a retrospective analysis (n= 155) and a phase I trial (n=14)."
- It is already noted that one study of theirs was a retrospective analysis similar to ours and another was a phase 1 trial (lines 189-190); see above.

- Specified that Nakai, et. al. only studied patients treated with gemcitabine (lines 192-193); "This difference could be explained by the heterogeneity of the patient population analyzed by Nakai, et. al., as their study included a mix of locally advanced and metastatic PDA[10] Additionally, Nakai et. al. only studied patients treated with gemcitabine.[10]"

~~4.~~ 7. Before suggesting future direction, it would be better to elaborate the limitation section either within the discussion subheading or in a separate subheading called "Limitations". Please elaborate what were the specific limitations of the retrospective study design. Elaborate on sample size, power calculation (though not mandatory in observational design, but mentioning it gives a clarity in the readers' minds), and subgroup analysis between groups with very small sample sizes. Likewise, the scope of generalizability needs to be discussed, as the study is done on one academic center.

~~5.~~

Response: Added a 'limitations' section prior to the 'future directions' section of the discussion (line 199). In this section, we expanded on why small sample size is a limitation (reduced study power) and why data from KUCC exclusively reduces generalizability of results.

- "*Limitations*

Limitations of this study include retrospective chart review design, which only allows identification of correlation between losartan use and increased PFS though it didn't meet statistical significance. Another limitation was the small size of analysis

(n=114), which reduces the study's power. Other limitations include differences in demographic characteristics, such as age, between experimental and control groups, lack of collection of other demographic characteristics such as alcohol use, and possible homogeneity due to sample selection from only one treatment center which limits generalizability of results.

A specific limitation in the subgroup analysis between patients on losartan treated with FOLFIRINOX and control patients treated with FOLFIRINOX is the numerical difference between subjects in each group. 24.6% of the losartan group was treated with FOLFIRINOX while 49.1% of the control group was treated with FOLFIRINOX. This numerical difference (13 losartan + FOLFIRINOX patients versus 28 control + FOLFIRINOX patients) could have affected the significance in difference in OS and PFS between these two groups."

2nd Editorial decision
28-Feb-2021

Ref.: Ms. No. JCTRes-D-20-00149R1
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Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: