Peer Review File

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Reviewer A

The authors investigated the efficacy of keishibukuryogan (TJ-25) for hot flashes in prostate cancer patients undergoing androgen deprivation therapy. This is an interesting study to address the effect of TJ-25 and cytokines on hot flashes.

#1 Important information is missing from the abstract. The number of patients is not stated in the abstract. How many? The study design was not shown in the abstract. Is it a single-arm prospective study?

Reply 1: Thank you for your important comments. The current study is a sub-analysis of serum hormonal and cytokine levels extracted from the single-arm prospective study. In addition, number of patients was described in the abstract and results.
Changes in the text: I have added the sentence in abstract as following; 'The current study is a sub-analysis of serum hormonal and cytokine levels extracted from the single-arm prospective study.' In addition, number of patients (25 patients) was described in the abstract and results.

#2 The sample size estimate (based on the previous prospective study [9]) should be shown in the methods.

Reply 2: Thank you for your important comments. I have shown the sample size estimate in the methods.
Changes in the text: I have added the sentence in the methods as following; 'This was a sub-analysis of a prospective observational pilot study [9]. In the original study, the sample size had been calculated based on the number of patients undergoing hormone therapy per year at research facilities and incidence rate of hot flashes.'

#3 The author should state the primary endpoint. What do the authors want to know most? Is it the relationship between VAS and hot flash frequency (Fig.1)?

Reply 3: Thank you for your kindly comments. The present study is a sub-analysis of our prior study, and therefore primary endpoint was not set. In the present study, we attempted to elucidate the mechanisms of TJ-25 efficacy by focusing on hormonal and cytokine levels. In order to understand them easily for the readers, the results had been revised.
Changes in the text: The results which we wanted to investigate in the present study were described with subtitle; 'The relationship between the intensity and frequency of hot flashes and hormone/cytokine levels' and 'Responder analysis based on hormone/cytokine levels'.

#4 The effect of TJ-25 was similar (or non-inferior?) to the previous study [9]? The present study showed "hot flush intensity (VAS) (mm) 51.8 ± 21.3, hot flush frequency 4.2 ± 2.2", but it is not easy to understand whether the present data were feasible or not.

Reply 4: Thank you for your comments. The current study is a sub-analysis of serum hormonal and cytokine levels extracted from the prospective single-arm prospective study. Therefore, data regarding VAS and frequency of hot flashes are the same with the previous study [9]. In accordance with your suggestions, we had revised the results.
Changes in the text: In result, we added the sentence as following; 'As shown in our previous study [9], mean values of VAS and frequency of hot flashes were 51.8 ± 21.3 and 4.2 ± 2.2 at baseline, and 37.6 ± 15.8 and 3.7 ± 2.0 at the 12-week visit, and TJ-25 treatment for 12 weeks had significantly improved strength and frequency of hot flashes.'

Minor; Line 87 Error in "Frequency. S"

Reply: I have revised this error.
Changes in the text: I have deleted "S".

Reviewer B

This study evaluated the relationship of keishibukuryogan (TJ-25) for hot flashes in prostate cancer patients undergoing androgen deprivation therapy, with hormonal and cytokine values.
Comments:
1. In the abstract, the background section suggests that the aim of the study is 'The current study looked at the efficacy of keishibukuryogan (TJ-25) for hot flashes in prostate cancer patients undergoing androgen deprivation therapy. That is not true, as there is no overall efficacy data of TJ-25 on hot flashes presented in the paper, only correlation with hormonal and cytokine values. Please clarify the aim or present the overall efficacy data.

Reply 1: Thank you for important comments. Certainly, aim of the current study is not to elucidate overall efficacy of TJ-25. Therefore, I have revised 'aim' of introduction according to your suggestion.
Changes in the text: I have revised 'background' in introduction as following; **'**The current study attempted to elucidate the mechanisms of keishibukuryogan (TJ-25) efficacy by focusing on hormonal and cytokine levels. The is a sub-analysis of serum hormonal and cytokine levels extracted from the prospective single-arm prospective study.'

2. It is not clear what are the exact parameters that determine separation into the 2 groups, and there does not appear to have any data for the 2 groups in table 1.

Can the authors clarify what are the 2 groups?

Reply 2: As responder analysis, according to the baseline median values of every hormone and cytokine, all subjects were split into two groups. The changes from baseline to 12-week visit in strength and frequency of hot flashes in both groups were compared by unpaired t-test. These descriptions had been cited by 'statistical analysis' in methods. As being understandable easily, these descriptions had been added in result (responder analysis), and median baseline value in each hormone and cytokine level were described in new Table 2.
Changes in the text: I have added the comments in results (responder analysis) as following; 'All subjects were divided into two groups according to the baseline median values (Table 2) of every hormone and cytokine, and the responder analysis was performed.' In addition, median baseline value in each hormone and cytokine level were described in new Table 2.

3. Table 1 should include the clinical characteristics of prostate cancer patients, eg. Stage, Gleason grade and PSA levels.

Reply 3: Thank you for your suggestion. I have added the clinical characteristics of prostate cancer patients (stage, Gleason score, and PSA) in table 1.
Changes in the text: As mentioned above, I have added the clinical characteristics of prostate cancer patients (stage, Gleason score, and PSA) in table 1.

4. In table 1, please clarify the use of antiandrogen therapy and duration – are these used as short term to prevent flare phenomenon during the initiation of ADT or used as combined androgen blockade (CAB), and whether these are 2nd generation novel hormonal agents eg. Abiraterone/ enzalutamide? Was there a difference seen on the addition of CAB or 2nd generation novel hormonal agents with hot flushes at baseline and difference in efficacy of TJ-25 in the single agent ADT vs double agent combination therapy?

Reply 4: Thank you for your kindly comments. In our population, no patients used 2nd generation hormonal agents, and then anti-androgenic therapy was bicaltamide (CAB therapy) in all cases. No difference in strength and frequency hot flashes between hormonal therapy was not observed in original therapy (reference 9). In the present report, this point was not mentioned because aim of the present study was to elucidate the mechanisms of TJ-25 efficacy by focusing on hormonal and cytokine levels.
Changes in the text: In revised table1, I have added the comments as following; 'All cases used bicaltamide.'

5. It is also important to state the median duration of ADT before TJ-25 was started – were all patients newly initiated with ADT or are stable on ADT for some time, as this may make a difference in the strength or frequency of hot flushes experienced?

Reply 5: Thank you for your important comments. Unfortunately, duration of ADT was not examined in the original study (reference 9), and then a correlation between hot flashes and duration of ADT was not discussed. Therefore, it can't be also mentioned in this study because of a sub-analysis. However, duration of ADT may have some effects on cytokine levels, which is an interesting concern. We have described this point in limitation.
Changes in the text: I have added the comments in limitations as following; 'Furthermore, duration of ADT was not examined in the original study [9], and then a correlation between hot flashes and duration of ADT was not discussed. Duration of ADT may have some effects on cytokine levels.'

6. Table 1 – please clarify if radiation therapy for treatment of the prostate as radical therapy vs treatment of metastases? Can the use of RT affect cytokine levels?

Reply 6: In all cases, radiation therapy was performed as radical treatment, but not as palliative treatment of metastasis. In addition, radiation did not affect hormonal and cytokine levels.
Changes in the text: In table 1, I have changed 'radiation' to 'radical radiation therapy'.

7. In table 1, please clarify if the hormonal and cytokine values are for baseline or at 12 weeks? Why are both data sets not presented in the table?

Reply 7: Thank you for your kindly comments. I have described the hormonal and cytokine levels at baseline and 12-week visit in new table 2 and results.
Changes in the text: I have described the hormonal and cytokine levels at baseline and 12-week visit in new table 2. In addition, I have added the sentence in results as following; 'Hormone and cytokine levels at baseline and 12-week visit are shown in Table 2. No significant changes in all of hormone and cytokine levels were observed by TJ-25 treatment.'

8. The word 'diary' is misspelled as 'daily' in several parts of the paper.

Reply 8: All misspelled words have been revised.
Changes in the text: The revised words are indicated by red color and borderline in the revised manuscript.

Reviewer C
Congrats on the job. Very relevant study for the daily practice of everyone who treats patients with prostate cancer. The complaint of hot flashes is very relevant and reduces the quality of life of patients. We look forward to future studies.

Reply: Thank you for your kindly comments! Hot flashes in the patients with ADT

reduces QOL of patients. I would like to conduct further studies involving a sizable number of participants and controls to elucidate the mechanism of TJ-25.