Author's response to reviews

Title: Efficacy of c-Met inhibitor for advanced prostate cancer

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Author's response to reviews: see over
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Dear Dr. Bristow:

Please find attached our revised manuscript entitled “Efficacy of c-Met inhibitor for advanced prostate cancer” (1146154576398237). We greatly appreciate the constructive comments made by you and the reviewers. As you requested, we have carefully revised the manuscript and addressed each of the comments in detail below:

Associate Editor’s comments:

1) The authors need to address previous papers in the field by Dai et al., and Munshi et al.

Actually, both the Dai et al. and Munshi et al. papers were published in June of 2010, after we submitted our manuscript to BMC on May 22, 2010. In addition, both papers investigated different c-met inhibitors than the ones that we used in this study. Therefore, a direct comparison between these experiments would be difficult. Nevertheless, we have cited their works and addressed their results in this revision.

2) There are a number of Methods missing and the authors should make the Methods, Results and Discussion more clear as per the reviewers

In response to the comments, we have carefully revised the manuscript and made the Methods, Results and Discussion sections more explicit. Particularly, we have added some detailed experimental procedures and addressed the novelty of the study to the field.

3) Please verify findings with competing out the effect of drug with HGF and/or (ii) carrying out K/down of the c-met gene.

This is a very important point. Actually, Maeda et al reported that knockdown of c-Met using specific c-Met siRNA inhibited the induction of c-Met expression by androgen depletion and repressed prostate cancer cell growth (Biochem Biophy Res Commun; 8;3474:1158-65, 2006), which has been cited in this manuscript (Reference 31). In addition, we also performed the similar experiments and observed an inhibitory effect of c-Met sRNA in LNCaP cells (Verras and Sun unpublished data). Moreover, Kim et al. (reference 26) demonstrated that decreasing c-met expression using ribozyme technology resulted in reduced prostate cancer cell proliferation and orthotopic tumor formation. These above data directly demonstrated that inhibition of c-Met expression represses the growth of prostate cancer cells, which formed the basis for the current study. We have modified the manuscript and made the above points clearer in the revision.

4) Please assess apoptosis or rebut why not assessed.

This is another very good point. At the beginning of the study, we examined the apoptotic effect of the inhibitors in the prostate tumors using TUNEL assays, but did not observe a significant difference between the treated and control mice. The observation is similar to the previous report in another tumor...
mouse model (Zou et al, reference 14). Thus, the above data directed us to focus on the anti-proliferative effect of the inhibitors in the study.

5) Show data for MET inhibition in non-castrated animals or rebut why this is not an appropriate control.

We appreciate the reviewer’s comment. Our previous study showed that AR suppressed c-Met transcription resulting in increased c-Met expression in prostate cancer cells, suggesting a novel mechanism underlying the pathogenesis of CRPC. Therefore, in this study, we chose the orthotopic mouse experiments to directly test whether co-inhibition of androgen and c-Met signaling pathways had more inhibitory effect on the growth of prostate cancer cells than castration alone. The main goal of these experiments was to test whether inhibition of c-Met signaling could be an adjunct to standard androgen deprivation therapy in reducing the development of castration resistance tumors. Thus, we carefully carried out the orthotopic xenograft mouse experiments to assess the effect of castration alone versus castration combined with PF-2341066 and showed an 85% reduction in proliferation in the presence of c-Met inhibitor. In addition, we evaluated the inhibitory effect of PF-2341066 using the non-castrated subcapsular renal model and showed a 45% reduction in proliferation based upon Ki67 index in the presence of c-Met inhibitor. Taken together, the current experimental results clearly demonstrated that PF-2341066 represses the growth and progression of prostate cancer tumor xenografts in mice.

Reviewers’ other comments:

1) Author JCG is an employee of Pfizer. This needs to be declared in the conflicts of interest section.

The employment has been declared.

2) Most of the effects found with the compounds were at concentrations of 2.5um. How does this level relate to the serum level achievable? Or to the IC50 of these compounds on c-met?

Previous studies have shown that the IC50 of PHA-665752 or PF-2341066 was 9-40 nmol/L (Christensen et al. reference 12) or 5-20 nmol/L (Zou et al, reference 14), respectively. Thus, we used the recommended concentrations of the compounds in this study accordingly.

3) The authors need to add to the western blot Figure 1D, the effects of inhibition of downstream pathways of c-met, for example, are PI3k and MEK required for the effects of this axis?

Again, the similar results have been reported in the previous studies. It has been shown that PHA-665752 and PF-2341066 affect the downstream proteins of c-Met, such as Akt and Erk in human tumor cells (Christensen et al. (reference 12) and Zou et al. (reference 14). Thus, we did not make the effort in this study to duplicate the above experiments.

4) Please correct all CRPR to CRPC

We apologize for the confusion and have corrected the error.

5) The second paragraph of the background is written poorly. In particular, it is unclear in the latter two sentences of this paragraph whether the authors are referring to the current or previous manuscript. Use of the past tense here would clarify things.

We have revised the related section accordingly.
6) A point of interest from the author’s previous studies is whether c-met inhibition could delay the time to androgen resistance. This issue is only addressed in a limited fashion in the current manuscript and the paper would achieve greater impact if this clinically relevant issue was addressed.

We agree with the reviewer that the potential impact of this study is significant in the field. In this current study, we used both in vitro and in vivo approaches to address the effect of the two newly developed c-met inhibitors on progression of prostate cancer. Data generated from this study have provided novel information for us to design more relevant experiments to further test a possible therapeutic strategy for advanced prostate cancer.

7) Discuss why c-met inhibition is dependent on hormone status.

Multiple studies have reported an inverse correlation between the expression of AR and c-Met that has been observed in prostate epithelium and prostate cancer cell lines. In our previous publication by Verras et al. (reference 11), we further demonstrated that c-Met expression was normally repressed by androgen action through the AR. Either reduction of androgens or repression of AR expression resulted in increased c-Met expression in prostate cancer cells and prostate tumor xenograft models.

We believe that we have appropriately addressed all of the points raised by you and the reviewers, and this revised manuscript has been significantly improved. We also highlighted the major changes in the manuscript as you requested. Again, we greatly appreciate the comments and suggestions from you and the reviewers. We are looking forward to hearing from you soon.

Sincerely,

William Tu and Zijie Sun