Article information: http://dx.doi.org/10.21037/atm-20-4441

Point-by-point response to comments

Response to Reviewer #A

Comment 1: Re-construct the manuscript to focus on Breast, Lung and Pancreatic Cancer alone. Avoid using the collective term of 'cancers' as this appears misleading.

Reply 1: *Thank you very much for your suggestion. We strongly agree with the reviewer that we should re-construct the manuscript to focus on Breast, Lung and Pancreatic Cancer alone and avoid using the collective term of 'cancers'. In response to the reviewer's suggestion. We re-analyzed the efficacy of metformin in combination with standard treatment in breast, lung and pancreatic cancer separately. The revised results were shown as follows* ***(see page 9-11, line 179-210 in the manuscript)****:*

Changes in the text:

Efficacy of metformin in breast cancer

There are three RCTs including 226 participants investigating the efficacy of adding metformin to standard treatment in breast cancer. These RCTs reported the ORR, OS and PFS data. The pooled ORR was 30.3% (33/109) in the metformin combined with treatment group and 16.1% (18/112) in the placebo combined with standard treatment group, showing that the addition of metformin to standard treatment was beneficial to the ORR (RR 1.92, 95% CI 1.19–3.10, P = 0.008) with no significant heterogeneity (I^2 = 26.7%, P = 0.255) ***(See Figure. 1A below)****. However, the results of meta-analysis showed that OS and PFS were not significantly improved in patients who received metformin plus standard treatment compared with those who received placebo plus standard treatment without significant heterogeneity (OS: HR 1.02, 95% CI 0.71–1.46, P = 0.916, I^2 =23.8%, P_{het} = 0.269; PFS: HR 1.14, 95% CI 0.86–1.50, P = 0.366, I^2 = 0%, P_{het} = 0.945)* ***(See Figure. 1B-C below)****.*

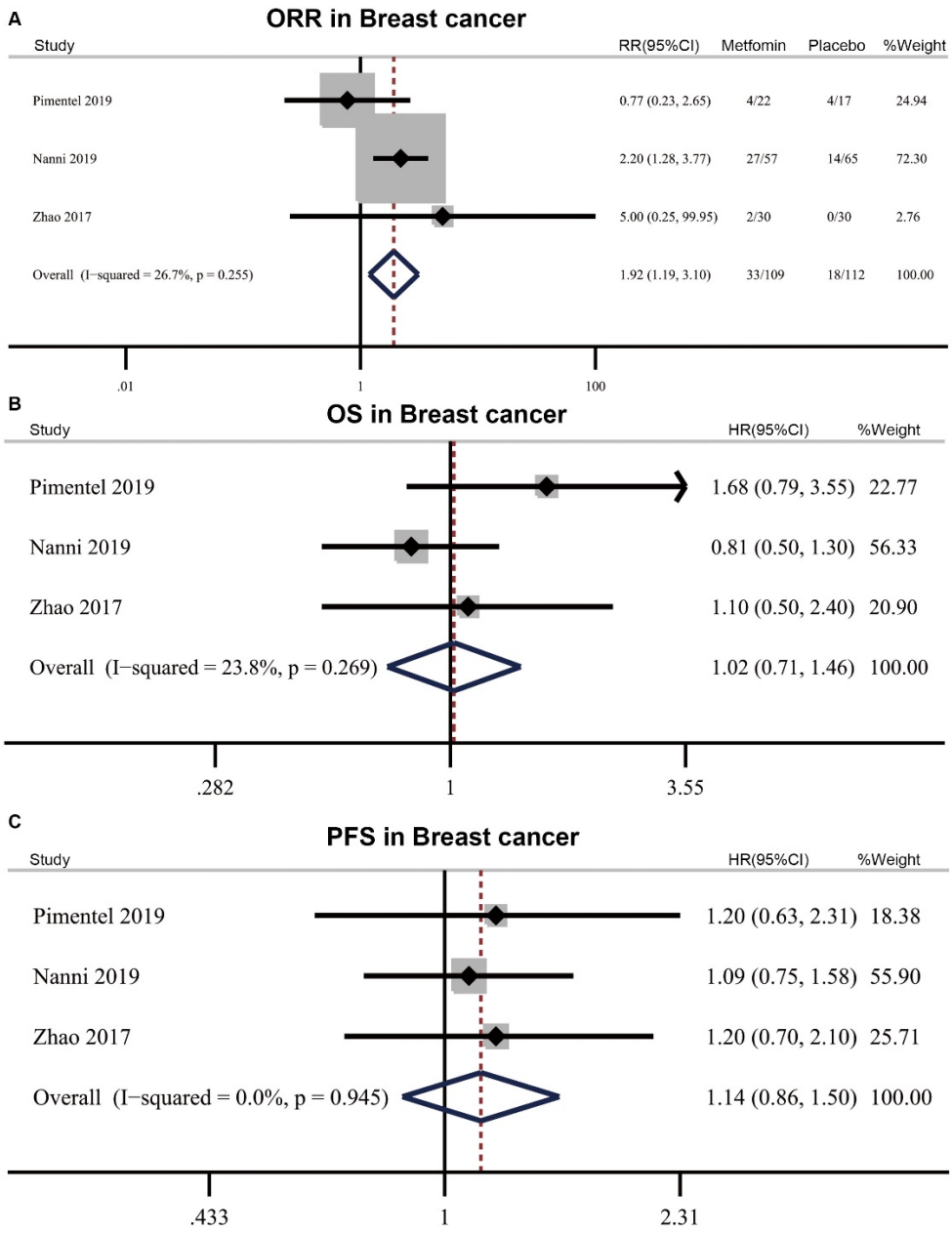

Figure legend: *A. Effect of metformin plus standard treatment on objective response rate in breast cancer. B-C. Effect of metformin plus standard treatment on OS (B) and PFS (C) in breast cancer.*

Efficacy of metformin in lung cancer

Four RCTs (418 patients) investigated the efficacy of adding metformin to standard treatment in lung cancer. ORR and OS were reported in these four RCTs, and PFS was assessed in three of these RCTs. Meta-analysis results showed adding metformin to standard treatment could benefit ORR (Metformin 65.3% VS placebo 54.6%, RR 1.22, 95% CI 1.03–1.43, P = 0.018) with no significant heterogeneity (I^2 = 30.4%, P_{het} = 0.230) ***(See Figure. 2A below)****. The results showed the addition of metformin to*

standard treatment did not improve OS and PFS in lung cancer patients (OS: HR 0.88, 95% CI 0.65–1.19, P = 0.409, I^2 =49.1%, P_{het} = 0.117; PFS (random effect): HR 0.63, 95% CI 0.32–1.27, P = 0.197, I^2 = 77.5%, P_{het} = 0.012) ***(See Figure. 2B-C below).***

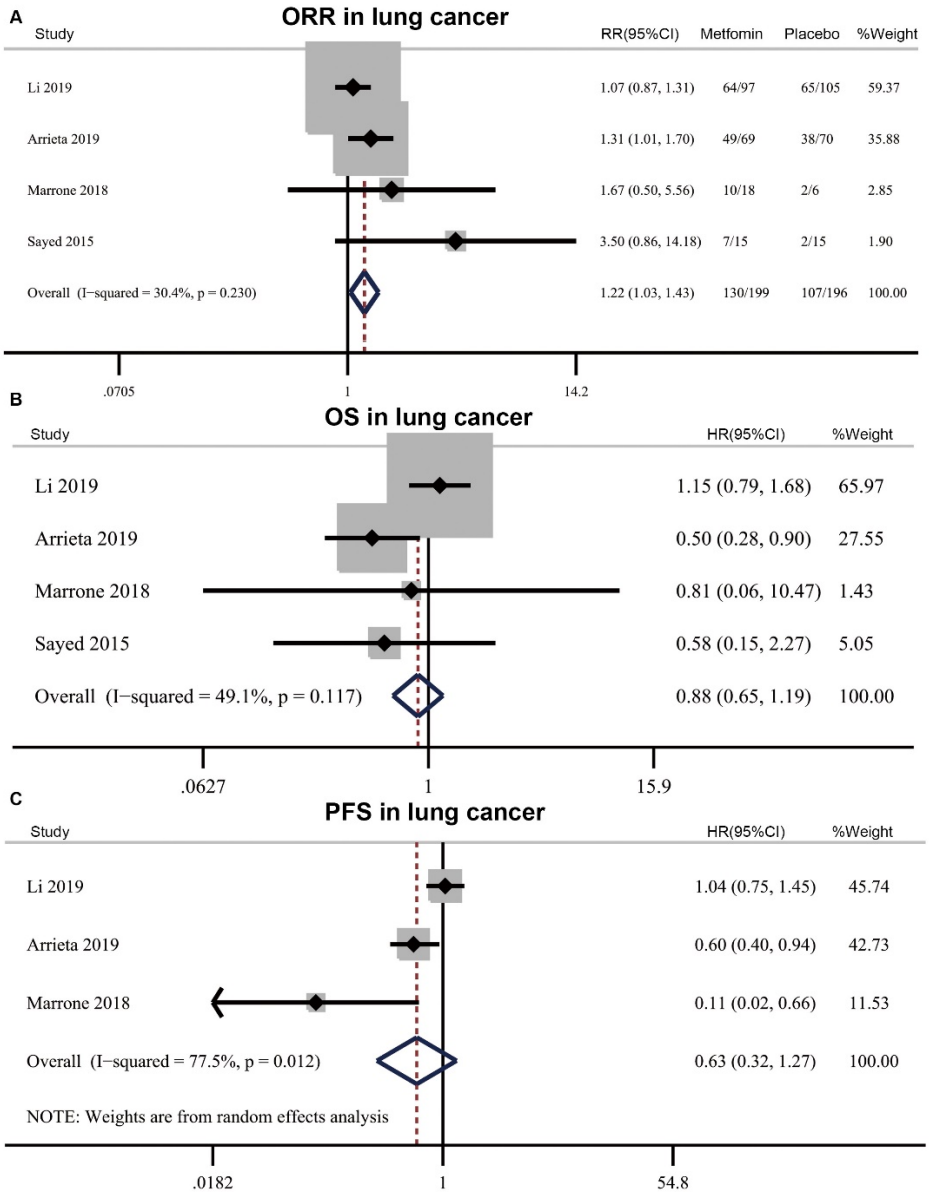

Figure legend: *A. Effect of metformin plus standard treatment on objective response rate in lung cancer. B-C. Effect of metformin plus standard treatment on OS (B) and PFS (C) in lung cancer.*

Efficacy of metformin in pancreatic cancer

There are two RCTs (181 patients) assessing the efficacy of metformin plus standard treatment in pancreatic cancer. The pooled ORR was 17.6% (16/91) in metformin plus standard treatment group and 20% (18/90) in the placebo group, showing metformin did not benefit ORR (RR 0.85, 95% CI 0.49-1.49, P = 0.576) without significant heterogeneity (I^2 = 0.0%, P_{het} = 0.709) ***(See Figure. 3A below).***

Meta-analysis on OS and PFS showed adding metformin to standard treatment did not bring benefit to survival outcome (OS: HR 1.00, 95% CI 0.74–1.37, P = 0.964, I^2=0%, P_{het}=0.680 ; PFS: HR 0.97, 95% CI 0.70–1.35, P = 0.859, I^2 = 49.0%, P_{het} = 0.161) ***(See Figure. 3B-C below)****.*

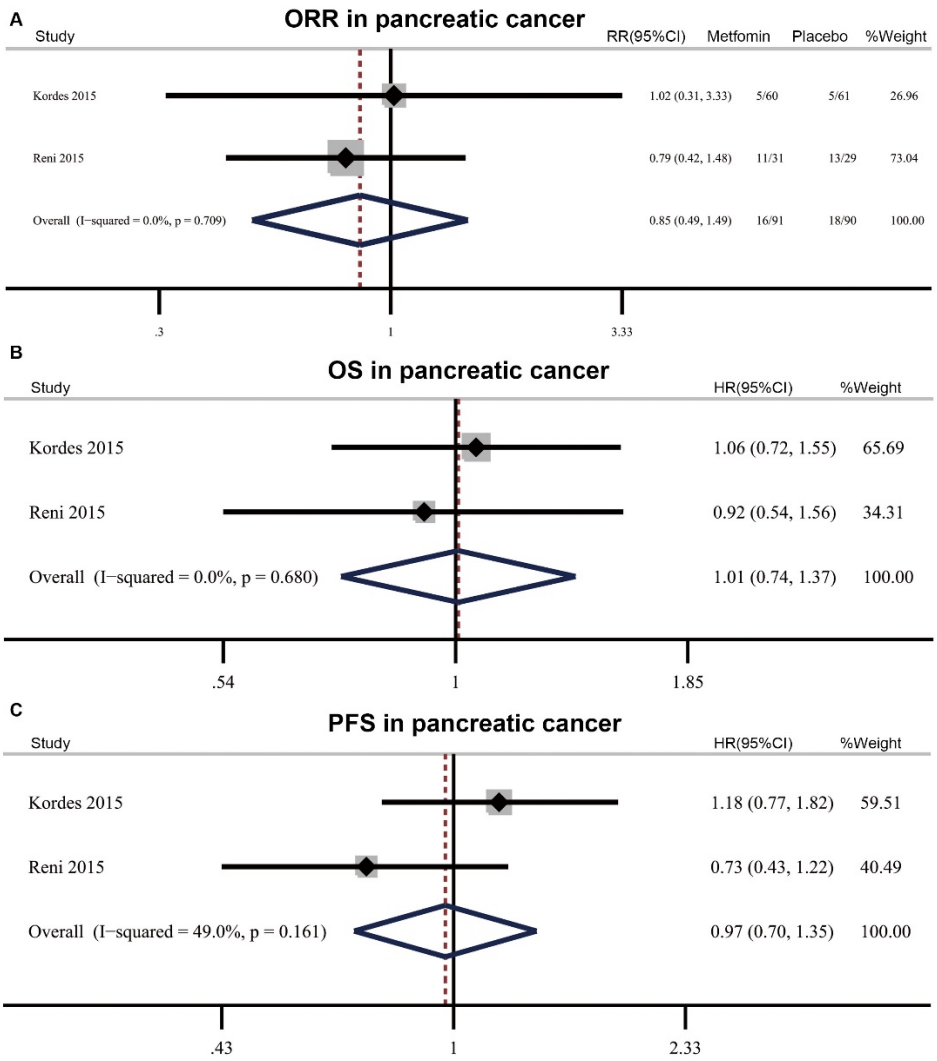

Figure legend: *A. Effect of metformin plus standard treatment on objective response rate in pancreatic cancer. B-C. Effect of metformin plus standard treatment on OS (B) and PFS (C) in pancreatic cancer.*

Comment 2: Perform a scoping literature search for existing meta-analysis investigating metformin use on survival in particular cancers. There are already several published - particularly in pancreatic cancer and lung cancer.

Reply 2: *Thank you very much for your comments. As you suggested, we have searched existing meta-analysis investigating metformin use on survival in particular cancers. Just as you mentioned, there are already several published meta-analyses*

investigating metformin use on survival in several cancers. These meta-analyses were mainly based on observational studies showing metformin use have positive relationship with favorable prognosis. Our current meta-analysis was based on randomized control trials and we focused on exploring the therapeutic role of metformin in combination with standard treatment for cancer patients. In the introduction section of our manuscript, we have mentioned that metformin use was correlated with improve survival in cancer patients. In response to your comments, we have cited some newly searched meta-analysis as our references in the introduction section to further show relationship between metformin use and survival. The revised manuscript is shown as follows ***(see page 6, line 96-98 in the manuscript)****:*

Changes in the text:

Meanwhile, many primary researches and meta-analyses reported that metformin use was associated with an improved survival outcome in pancreatic cancer (1-3), lung cancer (4,5), breast cancer (6,7), or colorectal cancer (8) etc.

Reference:

1. Wirunsawanya K, Jaruvongvanich V, Upala S. Survival Benefits From Metformin Use in Pancreatic Cancer: A Systemic Review and Meta-analysis. Pancreas 2018;47:e11-e4.

2. Wan G, Sun X, Li F, et al. Survival Benefit of Metformin Adjuvant Treatment For Pancreatic Cancer Patients: a Systematic Review and Meta-Analysis. Cell Physiol Biochem 2018;49:837-47.

3. Jian-Yu E, Graber JM, Lu SE, et al. Effect of Metformin and Statin Use on Survival in Pancreatic Cancer Patients: a Systematic Literature Review and Meta-analysis. Curr Med Chem 2018;25:2595-607.

4. Lin JJ, Gallagher EJ, Sigel K, et al. Survival of patients with stage IV lung cancer with diabetes treated with metformin. Am J Respir Crit Care Med 2015;191:448-54.

5. Zeng S, Gan HX, Xu JX, et al. Metformin improves survival in lung cancer patients with type 2 diabetes mellitus: A meta-analysis. Med Clin (Barc) 2019;152:291-7.

6. Xu H, Chen K, Jia X, et al. Metformin Use Is Associated With Better Survival of Breast Cancer Patients With Diabetes: A Meta-Analysis. Oncologist 2015;20:1236-44.

7. Yang T, Yang Y, Liu S. Association between Metformin Therapy and Breast Cancer Incidence and Mortality: Evidence from a Meta-Analysis. J Breast Cancer 2015;18:264-70.

8. Coyle C, Cafferty FH, Vale C, et al. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. Ann Oncol 2016;27:2184-95.

Comment 3: L62-63, I strongly disagree with this statement, e.g. discovery of monoclonal antibodies has revolutionised cancer care.

Reply 3: *Thank you very much for your comment on our statement. Your point is very right. We have revised the statement according to your suggestion. The revised statement is shown as follows* ***(see page 5, line 78-81 in the manuscript)****:*

Changes in the text:

Currently, the research on chemotherapy and discovery of novel monoclonal antibodies have revolutionized cancer therapy. However, the therapeutic role of conventional non-anti-cancer drug in combination with chemotherapy is not clear.

Comment 4: L70, I believe it is few clinical trials - as there have been several observational studies conducted.

Reply 4: *Thank you very much for your comments. And I respect you very much for your extensive knowledge on research. And your strict and cautious standard on writing inspires me much. We have revised our writhing here according to your suggestion. The revised statement is shown as follows* ***(see page 5, line 88 in the manuscript)****:*

Changes in the text:

However, few clinical trials have investigated the adjuvant therapeutic efficacy of these drugs in cancer patients.

Comment 5: L92-93, the findings are inconsistent.

Reply 5: *Thank you very much for your suggestion. We have revised the writing of this sentence according to your suggestion. The revised sentence is shown as follows* ***(see page 6, line 100 in the manuscript)****:*

Changes in the text:

Although several RCTs have been conducted to assess the efficacy and safety of adding metformin to standard treatment in inoperable cancer patients, the findings are inconsistent.

Comment 6: I would outline which authors carried out which activities of the SR/meta-analysis with their initials.

Reply 6: *Thank you very much for your suggestion. We have outlined the authors who carried out in our study with their initials as you suggested. The revised is as showed below:*

Changes in the text:

(1) *Two investigators (Z.H.W and B.C.Q) independently reviewed all the retrieved studies to identify the eligible studies* ***(see page 7, line 119 in the manuscript).***

(2) Authors' contributions

(I) Conception and design: Z.H.W and Z.N.W; (II) Administrative support: Y.X.S; (III) Provision of study materials or patients: B.C.Q and X.Z.H; (IV) Collection and assembly of data: Z.H.W, B.C.Q, C.Z and P.G; (V) Data analysis and interpretation: Z.H.W, P.G and J.X.S; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors ***(see page 16-17, line 333-338 in the manuscript).***

Comment 7: It is already known that metformin is well tolerated. The risk of hypoglycaemia is minimal.

Reply 7: *Thank you very much for your comment. As you mentioned, metformin is now well tolerated and is very safe during clinical application. According to your suggestion, we have revised our sentence "hypoglycemia or lactic acidosis was rarely reported in the studies included in our meta-analysis, indicating that studies evaluating the therapeutic benefits of metformin are relatively safe and cost-effective" in the discussion section into a more suitable description. The revised sentence is shown as follows* ***(see page 15, line 302-303 in the manuscript)****:*

Changes in the text:

Moreover, metformin is well tolerated during clinical application and the risk of hypoglycemia is minimal.

Comment 8: L263-267 I strongly disagree with this explanation.

Reply 8: *Thank you for your comment. We agree with your point on this explanation. On line 263-267, we mentioned that adding metformin to neoadjuvant therapy showed significant efficacy for ER-positive breast cancer patients. Such conclusion comes from a conference abstract and was not convincing enough to support our explanation. According to your suggestion, we have deleted the explanation on line*

263-267, and rewritten this part. The revised discussion is shown as follows ***(see page 14, line 283-291 in the manuscript)****:*

Changes in the text:

The anti-cancer effects of metformin may be beneficial in other situations as well, for instance, in early-stage cancer or adjuvant therapy settings. A previously published cohort study of type II diabetic patients with NSCLC in the United States' military health system has reported survival benefits for early-stage patients receiving metformin (42). Moreover, a recent meta-analysis (4) of 24178 participants from 27 eligible studies has revealed that metformin is a useful adjuvant, with survival benefits in patients with early-stage colorectal and prostate cancer. Collectively, these findings indicate that further trials investigating the benefits of adding metformin to neoadjuvant or adjuvant therapy for patients with early-stage or resectable cancer are needed.

Comment 9: Consider why some subgroups may benefit from metformin. You identify there may be a role in locally advanced cancer. This may be related to the direct/indirect anti-cancer effects of metformin and it's function in the liver.

Reply 9: *Thank you for your comment. As you mentioned, we identify that metformin may play a role in locally advanced cancer. This may be related to its direct or indirect anti-cancer effect. The direct anti-cancer properties of metformin may result from its inhibitory effect on cancer cells, particularly through acting on the AMPK pathway [1]. The indirect anti-cancer effects of metformin may be consequence of its blood glucose-lowering properties and anti-inflammatory effects [2-3]. In addition, there is population-based evidence suggesting that metformin requires long-term use to exert its anti-cancer effect [4]. This evidence can partially explain the potential role of metformin in locally advanced cancer patients compared with metastatic cancer patients. Such metastatic cancer patients are usually with shorter survival time and may not be able to receive metformin therapy enough for a therapeutic effect to emerge. We have added this explanation in the discussion section of our manuscript* ***(see page 15, line 291-297 in the manuscript).***

Reference:

1. Dowling RJ, Zakikhani M, Fantus IG et al. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res 2007;67: 10804–10812.

2. Fidan E, Onder Ersoz H, Yilmaz M et al. The effects of rosiglitazone and metformin on inflammation and endothelial dysfunction in patients with type 2 diabetes mellitus. Acta Diabetol 2011; 48: 297–302.

3. Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. BMC Med 2011; 9: 33.

4. Bodmer M, Meier C, Krahenbuhl S et al. Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care 2010; 33: 1304–1308.

Response to Reviewer #B

Comment 1: Introduction section should be substantially shortened.

Reply 1: *Thank you very much for your comment. According to your suggestion, we have substantially shortened our introduction section. We mainly shorten the paragraph 2 and 3 of introduction. The revised paragraph 2-3 is shown as follows* ***(see page 5-6, line 82-100 in the manuscript):***

Changes in the text:

In recent years, several conventional non-anti-cancer drugs, such as non-steroid anti-inflammatory drugs (NSAIDs), statin, metformin, have gained much attention for their anti-cancer properties. Several epidemiological studies (3-5) have reported significant survival benefits in cancer patients using these drugs. Other studies (6-9) have revealed that these drugs exert anti-cancer effects both in vitro and in vivo, and could work synergistically with chemotherapeutic drugs to inhibit tumor growth. However, few clinical trials have investigated the adjuvant therapeutic efficacy of these drugs in cancer patients. Whether adding these non-anti-cancer drugs to standard clinical treatments offers adjunctive benefit to cancer patients is still unclear.

As a conventional anti-diabetic drug, metformin has been used in the treatment of type II diabetes mellitus for over 30 years (10). Several experimental studies have reported that metformin has anti-cancer effects in lung cancer (7), pancreatic cancer (11) and gastric cancer (12) etc. In addition, population-based studies have indicated

that metformin use correlated with a reduced incidence rate of various cancers (13-16). Meanwhile, many primary researches and meta-analyses reported that metformin use was associated with an improved survival outcome in pancreatic cancer (17-19), lung cancer (20,21), breast cancer (22,23), or colorectal cancer (4) etc. Although several RCTs have been conducted to assess the efficacy and safety of adding metformin to standard treatment in inoperable cancer patients, the findings are inconsistent.

Comment 2: Please delete the sentence "Therefore, it is necessary to perform a meta-analysis to systematically assess the efficacy and safety of metformin combined with standard treatments in inoperable cancer patients."

Reply 2: *Thank you very much for your suggestion. We have deleted the sentence* ***"Therefore, it is necessary to perform a meta-analysis to systematically assess the efficacy and safety of metformin combined with standard treatments in inoperable cancer patients."*** *as you suggested* ***(see page 6, line 100 in the manuscript)****.*

Comment 3: Did the authors register your protocol in advance in a publicly available repository?

Reply 3: *Thank you for your comment. We agree with you very much on the registration of our protocol in a publicly available repository. Honestly speaking, i am sorry that our protocol was not registered in a publicly available repository. Our meta-analysis is performed strictly in accordance with the process of systematic review and meta-analysis. Meanwhile, in order to eliminate potential bias, the literature search strategy, study inclusion and exclusion criteria, data extraction, and statistical analysis were all prospectively defined. According to your suggestion, we also added this point to the limitation part of our manuscript. The revised limitation is shown as follows* ***(see page 16, line 324-326 in the manuscript)****:*

Changes in the text:

Thirdly, our meta-analysis was not registered in a publicly available repository. However, our meta-analysis is performed strictly in accordance with the process of systematic review and meta-analysis.

Comment 4: How do the authors interpret the fact that metformin 500 mg provided greater benefit regarding ORR compared to metformin 1000 mg?

Reply 4: *Thank you very much for your comment. Firstly, as was reported in many clinical studies, the metformin doses varying from 500 to 2000mg daily were commonly used dosages with well safety. In our study, our subgroup analysis results showed both 500mg metformin and 1000mg metformin daily could benefit the objective response rate, indicating that these metformin doses applied in these studies were suitable for cancer patients. From the results, we showed that the 500mg provided a better effect compared with 1000mg. As was known to us, metformin plays an anti-cancer effect mainly through acting on AMPK/mTOR pathway. A previous study showed metformin inhibited mTOR signaling via a dose-dependent mechanism, revealing that low-dose metformin directly inhibited mTOR through AMPK and TSC pathway, while high-dose metformin may work through other ways [1]. The findings of this study may partially explain that low metformin dose provided better effect. We have also added this discussion in our manuscript* ***(See page 13-14, line265-271 in the manuscript)****.*

Reference:

1. Howell JJ, Hellberg K, Turner M, et al. Metformin Inhibits Hepatic mTORC1 Signaling via Dose-Dependent Mechanisms Involving AMPK and the TSC Complex. Cell Metab. 2017;25(2):463-471.

Changes in the text:

From the results, we showed that the 500mg provided a better effect compared with 1000mg. As was known to us, metformin plays an anti-cancer effect mainly through acting on AMPK/mTOR pathway. A previous study showed metformin inhibits mTOR signaling via a dose-dependent mechanism, which revealed that low-dose metformin directly inhibited mTOR through AMPK and TSC pathway, while high-dose metformin may through other ways. The finding of this study may partially explain that low metformin dose provided better effect.

Comment 5: Any statement regarding publication bias?

Reply 5: *Thank you for your suggestion. We have added the results of publication bias analysis. The results of Begg's and Egger's tests showed that no significant publication bias was found in the overall analysis of OS ($P_{Begg's}$ =0.602, $P_{egger's}$ = 0.632) and PFS ($P_{Begg's}$ =0.711, $P_{egger's}$ = 0.191) (See figure below). The results were shown as follows*

(See page 12, line 237-240 in the manuscript):

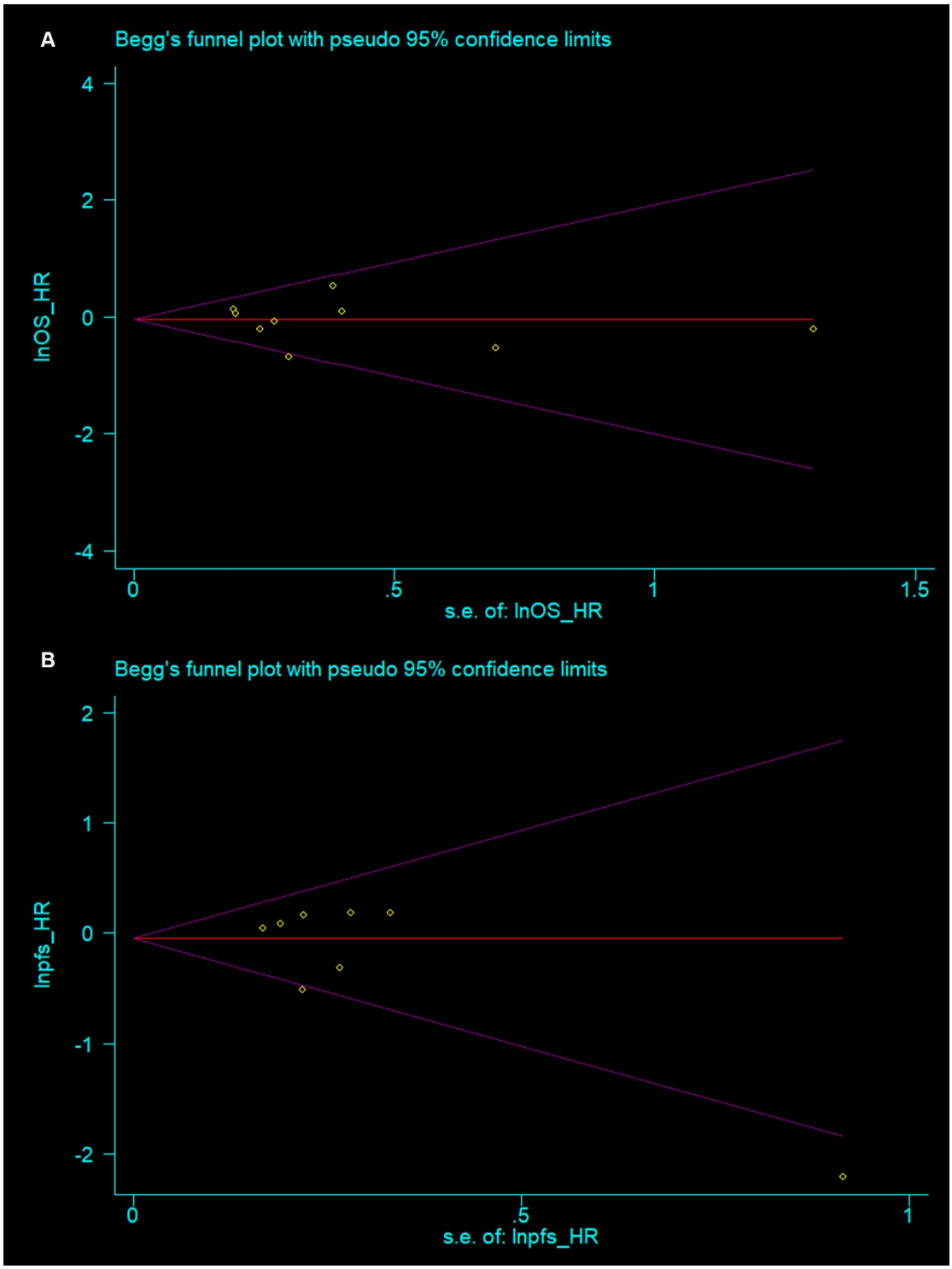

Figure legend: *The results of The results of Begg's and Egger's tests*

Changes in the text:

Publication bias

Begg's and Egger's tests were performed to evaluate the publication bias. The results of Begg's and Egger's tests showed that no significant publication bias was

found in the overall analysis of OS (PBegg's =0.602, Pegger's = 0.632) and PFS (PBegg's =0.711, Pegger's = 0.191) (Supplementary Figure 3).

Comment 6: Overall, the paper is interesting and falls within the scope of the journal. It represents the first relevant meta-analysis in the field, despite the fact that involved population is quite heterogeneous and results should be interpreted with caution.

Reply 6: *Thank you very much for your positive affirmation on our research. Thank you again for your valuable comments on improving the quality of our study.*