

Accepted Manuscript

Title: Systematic review and meta-analysis of the effect of metformin treatment on overall mortality rates in women with endometrial cancer and type 2 diabetes mellitus

Author: Faustino R. Perez-Lopez Vinay Pasupuleti Ximena
Gianuzzi Gabriela Palma-Ardiles Wendy
Hernandez-Fernandez Adrian V. Hernandez



PII: S0378-5122(17)30503-0
DOI: <http://dx.doi.org/doi:10.1016/j.maturitas.2017.04.001>
Reference: MAT 6798

To appear in: *Maturitas*

Received date: 31-3-2017

Accepted date: 3-4-2017

Please cite this article as: Perez-Lopez FR, Pasupuleti V, Gianuzzi X, Palma-Ardiles G, Hernandez-Fernandez W, Hernandez AV, Systematic review and meta-analysis of the effect of metformin treatment on overall mortality rates in women with endometrial cancer and type 2 diabetes mellitus, *Maturitas* (2017), <http://dx.doi.org/10.1016/j.maturitas.2017.04.001>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Highlights

- we performed the first meta-analysis to examine the effect of metformin on mortality in endometrial cancer.
 - Metformin use is associated with a significant reduction in overall mortality.
 - The findings suggest that good glycemic control may improve survival in endometrial cancer.
 - Larger observational studies are needed to confirm this finding.
-

Systematic review and meta-analysis of the effect of metformin treatment on overall mortality rates in women with endometrial cancer and type 2 diabetes mellitus

Faustino R. Perez-Lopez^{a, #}, Vinay Pasupuleti^{b, #}, Ximena Gianuzzi^c, Gabriela Palma-Ardiles^c, Wendy Hernandez-Fernandez^c, and Adrian V. Hernandez^{c, d}

^aDepartment of Obstetrics and Gynecology, University of Zaragoza Faculty of Medicine and Lozano Blesa University Hospital, Domingo Miral s/n, Zaragoza 50009, Spain

^bProEd Communications Inc., Cleveland, Ohio, 44122, USA.

^cSchool of Medicine, Universidad Peruana de Ciencias Aplicadas (UPC), Lima 9, Peru.

^dUniversity of Connecticut/Hartford Hospital Evidence-based Practice Center, 80 Seymour St, Hartford, CT 06102, USA.

[#] Contributed equally to the study.

* *Corresponding author:* Adrian V. Hernandez, MD, PhD, University of Connecticut/Hartford Hospital Evidence-based Practice Center, 80 Seymour St, Hartford, CT 06102, USA; +1-860-972-4468; adrianhernandezdiaz@gmail.com

Accepted Manuscript

Abstract

Background: Obesity, insulin resistance and type 2 diabetes mellitus (T2DM) have been associated with endometrial cancer (EC). In this systematic review and meta-analysis we evaluated the effect of metformin on clinical outcomes in patients with EC and insulin resistance or T2DM.

Methods: Four research databases were searched for original articles published in all languages up to 30 October 2016. Outcomes of interest were overall mortality (OM), cancer-specific mortality, disease progression, and metastases. We performed a random effect meta-analysis of adjusted effects expressed as hazard ratios (HR); heterogeneity among studies was described with the I^2 statistic.

Results: Of the 290 retrieved citations, 6 retrospective cohort studies in women with EC (n=4,723) met the inclusion criteria, and 8.9% to 23.8% were treated with metformin; OM data was available from 5 studies. In 4 studies of EC patients (n=4,132), metformin use was associated with a significant reduction in OM in comparison with not using metformin (adjusted HR [aHR] 0.64, 95% CI 0.45-0.89, p=0.009). In three studies evaluating patients with EC and T2DM (n=2,637), metformin use was associated with a significant reduction in OM (aHR 0.50, 95%CI 0.34-0.74, p=0.0006). There was low to moderate heterogeneity of adjusted effects across studies. There was no information about the effect of metformin on cancer-specific mortality, disease progression, or metastases.

Conclusions: Metformin treatment is associated with a significant reduction in OM irrespective of diabetes status in patients with EC. The survival benefit suggests that diabetes screening and maintenance of good glycemic control may improve outcomes in EC.

Keywords: Endometrial cancer; Metformin; Type 2 diabetes mellitus; Overall mortality.

1. Introduction

Endometrial cancer (EC) is the most common female malignant genital neoplasia in developed countries, and the fourth most common female malignancy overall. It is considered a hormone-dependent cancer, and peak incidence is in women between 50 and 70 years. During the menstrual cycle, estrogen has been postulated to create a pro-carcinogenic environment during the menstrual cycle; pregnancies interrupt such endometrial stimulus.¹ Obese postmenopausal women with endometrioid EC have higher levels of estrogens and related metabolites, increased prevalence of type 2 diabetes mellitus (T2DM) and hyperinsulinemia than women without EC.²⁻⁴ Excessive body weight may contribute to the development of EC since fatty tissue produces large amounts of estrogen. In addition, obesity, T2DM, hyperinsulinemia and insulin resistance have been associated with endometrial carcinogenesis and increased mortality.^{2,4-11}

The biguanide metformin is the first-line treatment for individuals with T2DM. Metformin is known to modulate molecular pathways implicated in several cancers and because of these antitumor properties, metformin may have a role in cancer prevention and treatment.^{12,13} In subjects with T2DM, metformin treatment is associated with reduced risk for cancer development in observational studies, although this finding has not been supported by randomized controlled trials.⁸ In patients with EC, short-term preoperative metformin therapy significantly reduces DNA synthesis, insulin, glucose, insulin-growth factor 1 (IGF-1) and other insulin resistance-related markers.^{14,15} Although, metformin as a cancer therapeutic has generated great interest, metformin effects on stabilizing metabolic status and thereby reducing EC mortality remains controversial.^{8,16,17}

The possibility of prevention of EC recurrence after primary treatment is crucial in long-term patient management. The aim of the current systematic review and meta-analysis was

to determine the effect of metformin treatment on overall mortality, cancer-specific mortality, disease progression, and metastases.

2. Methods

2.1. Data sources and searches

A comprehensive literature search was performed using PubMed-Medline, Embase, Web of Science and Scopus from database inception through 30th October 2016. Database searches were performed independently by two authors (VP and FRPL). The PubMed search strategy is available in the Supplement.

The following pre-determined inclusion criteria were used: (i) cohort studies evaluating the effect of metformin treatment in women with EC, (ii) study population of patients ≥ 18 years, and (iii) study in any language. Our exclusion criteria were: (i) no control group and (ii) data for metformin use and outcomes of interest were not available or could not be extracted for each of the metformin and non-metformin groups.

2.2. Study selection, data extraction, and risk of bias assessment

A list of retrieved articles was reviewed independently by two sets of investigators (Zaragoza and Lima) to choose potentially relevant articles, and disagreements on inclusion/exclusion were discussed and resolved by consensus. Two sets of reviewers (Zaragoza and Lima) independently extracted data from included studies. The following information was extracted: mean age, body mass index, EC histology, and endometrial cancer-related outcomes (duration of follow up, overall mortality, cancer-specific mortality, disease progression, metastases), whenever available. Both unadjusted and adjusted effects evaluating the association between metformin vs non-metformin and the

clinical outcomes and expressed as hazard ratios (HR) were extracted per study. Also, the variables used to adjust the effects were extracted. One author (VP) reviewed the extractions for inconsistencies, and two authors (VP, FRPL) reached consensus.

The Newcastle Ottawa Scale (NOS) for cohort studies was used to evaluate risk of bias,¹⁸ this assessment was done independently by two investigators (VP, FRPL). The NOS evaluates nine items of selection of exposure and non-exposure groups, comparability of exposure and non-exposure groups, and outcome evaluation with a maximum number of 9 stars (i.e. one per item). A total of 7 or more stars implies that the study has low risk of bias.

2.3. Data analysis

Our systematic review and meta-analysis follow the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.¹⁹ As we expected some degree of heterogeneity across studies, the DerSimonian and Laird random effects models and inverse variance method were used for all meta-analyses.²⁰ Associations between metformin use and dichotomous clinical outcomes were expressed as HRs and their 95% confidence intervals (CI). Our primary analyses used adjusted HRs per study. We evaluated statistical heterogeneity using the Cochran chi-square, the I^2 statistic, and the between-study variance using the tau-square (Tau^2).^{21,22} I^2 values of 30-60% represented a moderate level of heterogeneity. A p value <0.1 for the chi-square was defined as indicating the presence of heterogeneity; a $Tau^2 > 1$ suggests the presence of substantial statistical heterogeneity. Publication bias was explored with the funnel plot and tested with the Egger test of funnel plot asymmetry.²³ We performed pre-specified subgroup analyses of effects on T2DM patients only. A sensitivity analyses combining unadjusted HRs was

performed. We used Review Manager (RevMan 5.3, Nordic Cochrane Centre) for statistical analyses.²⁴

3. Results

3.1. Study selection

Our search identified 437 publications (Fig. 1). After removing duplicates, 290 articles were screened by study title/abstract for relevance to study topic and inclusion/exclusion criteria. Thirteen articles were retrieved for full-text analysis (Fig. 1). Six studies (n=4,704 women with EC) reported overall mortality women with EC and were available for qualitative synthesis.²⁵⁻³⁰ Finally, 5 studies (n=4,239 women with EC) reported effects on overall mortality comparing metformin use and non-metformin use groups and were included in the quantitative synthesis.²⁵⁻²⁹ Other pre-specified outcomes (cancer-specific mortality, disease progression, and metastases) were not reported in evaluated studies.

3.2. Characteristics of included studies

Table 1 summarizes the main characteristics of the included studies. The 6 articles (n=4,723) included in the qualitative analysis were retrospective cohort studies. Four studies were conducted in the United States,^{25,26,28,29} one in Poland,²⁷ and one in Austria.³⁰ Sample size in these studies ranged from 107 to 1,495 EC cases identified by clinical records. Four studies enrolled EC patients classified as International Federation of Obstetrics and Gynecology (FIGO) clinical stages I to IV,^{25,26,28,30} one study enrolled patients with FIGO clinical stages I to III,²⁷ while the sixth study enrolled patients with FIGO clinical stages III-IV or recurrent EC.²⁹

The mean age (standard deviation [SD]) ranged from 63.6 (4.1)²⁶ and 64.6 (11.5).²⁸ Overall follow-up ranged from a median of 2.8 years²⁶ to a mean of about 9.3

years.²⁷ Mean body mass index (BMI) were $>30 \text{ kg/m}^2$ in majority of the studies. EC patients treated with metformin ranged from 8.9% to 23.8% in the six cohorts (Table 1).

3.3. Risk of bias assessment

Using the Newcastle-Ottawa Scale (NOS), all 5 studies included in the meta-analysis were identified as having low risk of bias (Table 2). All but one study²⁷ identified important confounders or prognostic factors and were used for adjustment of the association between metformin use and outcomes in EC. There was considerable variation in the selection of confounding variables for adjustment although four studies^{25,26,28,29} had at the least adjusted for age and cancer stage.

3.4. Meta-analyses of overall mortality

In four studies evaluating EC patients and with available adjusted effects (n=4,132), metformin use was associated with a significant reduction in overall mortality in comparison with not using metformin (adjusted HR [aHR] 0.64, 95%CI 0.45 to 0.89, p=0.009) (Fig. 2). In three studies only evaluating EC patients with T2DM and with available adjusted effects (n=2,637), metformin was also associated with a reduction in overall mortality (aHR 0.50, 95%CI 0.34 to 0.74, p=0.0006) (Fig. 3). There was low to moderate heterogeneity of adjusted effects across studies for all analyses. Unadjusted effects from 3 studies (n=1,441) were also meta-analyzed, but metformin was not associated with lower mortality vs not using metformin (unadjusted HR 0.82, 95%CI 0.61-1.09, p=0.17) (Fig. 4).

4. Discussion

Our meta-analysis showed that metformin significantly reduced overall mortality in patients with EC in a wide range of FIGO clinical stages compared to those not treated with metformin. In addition, there was also a reduced overall mortality associated with metformin treatment when only EC patients with T2DM were evaluated. There was low to moderate heterogeneity of effects among evaluated studies.

Previous studies and meta-analyses have demonstrated that long term exposure to metformin (but not sulfonylurea) reduces the risk of colorectal and non-small cell lung cancer in patients with T2DM.^{31,32} In contrast, metformin use did not have a significant effect on the incidence of endocrine-dependent cancers such as breast or prostate cancer.^{8,33-35} However, some of these observations have been limited by inadequate follow-up of patients in these studies and lack of confirmation from randomized controlled trials.^{36,37} On the other hand, metformin use may substantially improve cancer survival, whilst insulin exposure is associated to increases in other-than-cancer mortality. For these reasons metformin has been recommended as first-line treatment in T2DM patients with cancer.³⁸

EC risk factors including postmenopausal age, obesity, hypertension and impaired glucose tolerance, are associated with hyperinsulinemia and insulin resistance. Hence, circulating insulin and C-peptide levels and the HOMA-IR values are higher in women with EC.⁴ The relationship between insulin resistance, DM and EC seems to be causal as has been shown by Mendelian randomization studies.³⁹ *In vitro* studies have demonstrated that metformin inhibits insulin action, reduces migration capacity of endometrial epithelial cells without reduction of proliferation, and the effect may be globally considered as an anti-metastatic effect in conditions of high as well as normal glucose levels.⁴⁰ Metformin also induces apoptosis on endometrial cancer cells,⁴¹ and antidiabetic doses of metformin produces

hypermethylation of tumor-promoting pathway genes and inhibition of cell proliferation in both normal and cancer cells.⁴²

In our systematic review and meta-analysis, available studies were heterogeneous with respect to studied populations which included EC patients in FIGO stages I to IV and recurrent cases. In general, one may consider that early stages of the disease (stages I and II) have high rates of curation with surgery and the mortality risk is very low and thus can be challenging to detect a benefit for metformin treatment. The available studies did not allow to assess the effect of metformin according to clinical stages, duration of metformin of treatment, and years of evolution of T2DM. A second source of heterogeneity may be therapeutic approaches which may differ from one institution to another; in addition, clinical practice for management of the EC and guidelines include a wide variety of treatment options and follow-up recommendations in stages III, IV and recurrences.⁴³⁻⁴⁵

Although only few observational studies have studied the relationship between metformin use and EC mortality, our meta-analysis findings demonstrate a clear association between them.

EC is a prevalent disease among postmenopausal women and in those with either excessive weight or BMI > 30 kg/m². Most of included studies in the current review reported an average BMI of more than 30 kg/m² in patients with EC. This profile of women is also overlapping with those suffering with T2DM. Furthermore, in the current meta-analysis postmenopausal women with T2DM and EC had a larger mortality risk reduction with metformin in comparison to those diabetics without metformin. Further well-designed, large studies can vastly improve our understanding on how best to utilize metformin in the management of patients with EC. Future research should report information on duration of T2DM and metformin treatment and dosages, analyze patients by FIGO clinical stages and histological types (type I endometrioid EC versus type II non-endometrioid EC), provide

details about surgical and other complementary treatments and have longer follow-up times.

Our study has several limitations. First, the observational nature of cohort studies cannot exclude that the effect of metformin on mortality may be associated to other patient characteristics. Second, we expected substantial heterogeneity of study characteristics among studies such as EC stage, duration of metformin use, or metformin dose; to accommodate this methodological heterogeneity we used random effects models. Third, variability of effects across studies (i.e. statistical heterogeneity) was expected; however, we found a low to moderate heterogeneity of effects. Fourth, the number of studies was low, and we could not evaluate the probability of publication bias. Finally, the set of variables used to adjust effects was different among studies.

In conclusion, this systematic review and meta-analysis demonstrated that metformin treatment reduced overall mortality in postmenopausal women with EC. Large observational studies are necessary to further elucidate the protective effects of metformin in this population.

Contributors

FRP-L and AVH designed the study.

FRP-L and VP did the literature searches and designed the data-extraction form.

FRP-L, XG, GP-A and WH-F extracted data.

VP and FRP-L cross-checked the data extraction.

VP did the statistical analyses.

AVH supervised the statistical analyses.

All authors wrote the paper, and read and approved the submitted version.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

There was no specific funding for this review.

Provenance and peer review

This article has undergone peer review.

Accepted Manuscript

REFERENCES

- [1] Felix AS, Yang HP, Bell DW, Sherman ME. Epidemiology of endometrial carcinoma: Etiologic importance of hormonal and metabolic influences. *Adv Exp Med Biol* 2017;943:3-46.
- [2] Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. *Diabetologia* 2007;50:1365-74.
- [3] Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, et al. Serum estrogens and estrogen metabolites and endometrial cancer risk among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2016;25:1081-9.
- [4] Hernandez AV, Pasupuleti V, Benites-Zapata VA, Thota P, Deshpande A, Perez-Lopez FR. Insulin resistance and endometrial cancer risk: A systematic review and meta-analysis. *Eur J Cancer* 2015;51:2747-58.
- [5] Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008;371:569–78.
- [6] Fader AN, Arriba LN, Frasure HE, von Gruenigen VE. Endometrial cancer and obesity: epidemiology, biomarkers, prevention and survivorship. *Gynecol Oncol* 2009;114:121–7.
- [7] Campbell PT, Newton CC, Patel AV, Jacobs EJ, Gapstur SM. Diabetes and cause-specific mortality in a prospective cohort of one million U.S. adults. *Diabetes Care* 2012;35:1835-44.
- [8] Thakkar B, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism* 2013;62:922-34.
- [9] Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JP. Type 2 diabetes and cancer: Umbrella review of metaanalyses of observational studies. *BMJ* 2015;350:g7607.

- [10] Arnold M, Jiang L, Stefanick ML, Johnson KC, Lane DS, LeBlanc ES, et al. Duration of adulthood overweight, obesity, and cancer risk in the Women's Health Initiative: A longitudinal study from the United States. *PLoS Med* 2016;13:e1002081.
- [11] Aarestrup J, Gamborg M, Tilling K, Ulrich LG, Sørensen TI, Baker JL. Childhood body mass index growth trajectories and endometrial cancer risk. *Int J Cancer* 2017;140:310-5.
- [12] Franciosi M, Lucisano G, Lapice E, Strippoli GF, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: Systematic review. *PLoS One* 2013;8:e71583.
- [13] Zhang P, Li H, Tan X, Chen L, Wang S. Association of metformin use with cancer incidence and mortality: A meta-analysis. *Cancer Epidemiology* 2013;37:207-18.
- [14] Mitsuhashi A, Kiyokawa T, Sato Y, Shozu M. Effects of metformin on endometrial cancer cell growth in vivo: a preoperative prospective trial. *Cancer* 2014;120:2986-95.
- [15] Sivalingam VN, Kitson S, McVey R, Roberts C, Pemberton P, Gilmour K, et al. Measuring the biological effect of presurgical metformin treatment in endometrial cancer. *Br J Cancer* 2016;114:281-9.
- [16] Perez-Lopez FR. Metformin treatment and evolution of endometrial cancer. *Climacteric* 2014;17:207-9.
- [17] Zhang ZJ, Li S. The prognostic value of metformin for cancer patients with concurrent diabetes: a systematic review and meta-analysis. *Diabetes Obes Metab* 2014;16:707-10.
- [18] Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [21 December 2016]

- [19] Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and metaanalyses: the PRISMA statement. *J Clin Epidemiol* 2009;62:1006-12.
- [20] DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177-88.
- [21] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557-60.
- [22] Higgins JP. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* 2008;37:1158-60.
- [23] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple graphical test. *BMJ* 1997;315:629-34.
- [24] Review Manager (RevMan) [Computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration; 2011.
- [25] Ko EM, Walter P, Jackson A, Clark L, Franasiak J, Bolac C, et al. Metformin is associated with improved survival in endometrial cancer. *Gynecol Oncol* 2014;132:438-42.
- [26] Nevadunsky NS, Van Arsdale A, Strickler HD, Moadel A, Kaur G, Frimer M, et al. Metformin use and endometrial cancer survival. *Gynecol Oncol* 2014; 132:236-40.
- [27] Lemanska A, Zaborowski M, Spaczynski E, Nowak-Markwitz E. Do endometrial cancer patients benefit from metformin intake? *Ginekologia Polska* 2015;86:419-23.
- [28]. Al Hilli MM, Bakkum-Gamez JN, Mariani A, Cliby WA, Mc Gree ME, Weaver AL, et al. The effect of diabetes and metformin on clinical outcomes is negligible in risk-adjusted endometrial cancer cohorts. *Gynecol Oncol* 2016;140:270-6.
- [29] Ezewuiro O, Grushko TA, Kocherginsky M, Habis M, Hurteau JA, Mills KA, et al. Association of Metformin Use with Outcomes in Advanced Endometrial Cancer Treated with Chemotherapy. *PLoS One* 2016;11:e0147145.

- [30] Seebacher V, Bergmeister B, Grimm C, Koelbl H, Reinthaller A, Polterauer S. The prognostic role of metformin in patients with endometrial cancer: a retrospective study. *Eur J Obstet Gynecol Reprod Biol* 2016;203:291-6.
- [31]. Mei ZB, Zhang ZJ, Liu CY, Liu Y, Cui A, Liang ZL, Wang GH, Cui L. Survival benefits of metformin for colorectal cancer patients with diabetes: a systematic review and meta-analysis. *PLoS One* 2014;9:e91818.
- [32] Arrieta O, Varela-Santoyo E, Soto-Perez-de-Celis E, Sánchez-Reyes R, De la Torre-Vallejo M, Muñoz-Hernández S, et al. Metformin use and its effect on survival in diabetic patients with advanced non-small cell lung cancer. *BMC Cancer* 2016;16:633.
- [33] Ruitter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, Geelhoed-Duijvestijn PH, et al. Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study. *Diabetes Care* 2012;35:119-24.
- [34] Soranna D, Scotti L, Zambon A, Bosetti C, Grassi G, Catapano A, et al. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. *Oncologist* 2012;17:813-22.
- [35] Häggström C, Van Hemelrijck M, Zethelius B, Robinson D, Grundmark B, Holmberg L, et al. Prospective study of Type 2 diabetes mellitus, anti-diabetic drugs and risk of prostate cancer. *Int J Cancer* 2017;140:611-7.
- [36] Tsilidis KK, Capothanassi D, Allen NE, Rizos EC, Lopez DS, van Veldhoven K, et al. Metformin does not affect cancer risk: a cohort study in the U.K. *Clinical Practice Research Datalink analyzed like an intention-to-treat trial. Diabetes Care* 2014;37:2522-32.
- [37] Kowall B, Stang A, Rathmann W, Kostev K. No reduced risk of overall, colorectal, lung, breast, and prostate cancer with metformin therapy in diabetic patients: database analyses from Germany and the UK. *Pharmacoepidemiol Drug Saf* 2015;24:865-74.

- [38] Bo S, Ciccone G, Rosato R, Villosio P, Appendino G, Ghigo E, et al. Cancer mortality reduction and metformin: a retrospective cohort study in type 2 diabetic patients. *Diabetes Obes Metab* 2012;14:23-9.
- [39] Nead KT, Sharp SJ, Thompson DJ, Painter JN, Savage DB, Semple RK, et al. Evidence of a causal association between insulinemia and endometrial cancer: A mendelian randomization analysis. *J Natl Cancer Inst* 2015;107: pii: djv178.
- [40] de Barros Machado A, Dos Reis V, Weber S, Jauckus J, Brum IS, von Eye Corleta H, et al. Proliferation and metastatic potential of endometrial cancer cells in response to metformin treatment in a high versus normal glucose environment. *Oncol Lett* 2016;12:3626-32.
- [41] Xie Y, Wang JL, Ji M, Yuan ZF, Peng Z, Zhang Y, et al. Regulation of insulin-like growth factor signaling by metformin in endometrial cancer cells. *Oncol Lett* 2014;8:1993-1999.
- [42] Zhong T, Men Y, Lu L, Geng T, Zhou J, Mitsuhashi A, et al. Metformin alters DNA methylation genome-wide via the H19/SAHH axis. *Oncogene*. 2016 Oct 24. doi: 10.1038/onc.2016.391. [Epub ahead of print]
- [43] SGO Clinical Practice Endometrial Cancer Working Group, Burke WM, Orr J, Leitao M, Salom E, Gehrig P, Olawaiye AB et al. for the Society of Gynecologic Oncology Clinical Practice Committee. Endometrial cancer: A review and current management strategies. *Gynecologic Oncology* 2014;134:385-92.
- [44] Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al; The ESMO–ESGO–ESTRO Endometrial Consensus Conference Working Group. ESMO–ESGO–ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. *Radiotherapy Oncology* 2015;117:559-81.

[45] Fotopoulou C, Kraetschell R, Dowdy S, Fujiwara K, Yaegashi N, Larusso D, et al. Surgical and systemic management of endometrial cancer: An international survey. Arch Gynecol Obstet 2015;291:897-905.

Accepted Manuscript

Figure legends

Figure 1: Flow chart of the study selection process for eligible studies

Figure 2: Forest plot showing overall mortality risk (after adjustment) with metformin use in patients with endometrial cancer.

Figure 3: Forest plot showing overall mortality risk (after adjustment) with metformin use in patients with endometrial cancer and diabetes mellitus.

Figure 4: Forest plot showing overall mortality risk (before adjustment) with metformin use in patients with endometrial cancer.

Contributors

FRPL and AVH designed the study.

FRPL and VP did the literature searches and designed the data extraction form.

FRPL, XG, GPA and WHF extracted data

VP and FRPL cross-checked the data extraction.

VP did the statistical analyses.

AVH supervised the statistical analyses.

All authors wrote the paper, and read and approved the submitted version.

Funding

There was no specific funding for this manuscript.

Table 1 Basic characteristics of included studies

| First author, year published | Study location | Study population, FIGO stages | Sample size | Histology, n (%) | EC + DM cases treated with metformin, % | Age, Mean (SD) | BMI, Mean (SD) | Overall duration of follow-up, median [IQR] | Primary analysis: metformin use vs no metformin use & HRs with adjustment | Secondary analysis: diabetic with metformin use vs diabetic with no metformin use & HRs with adjustment | Sensitivity analysis: same as primary analysis with HRs without adjustment | Sensitivity analysis: same as secondary analysis with HRs without adjustment |
|------------------------------|----------------|-------------------------------|-------------|---|---|-------------------|----------------|--|---|---|--|--|
| Nevadunsky NS, 2013 | U.S. | Stages I-IV EC | 985 | Endometrioid 593 (60.2) Non-endometrioid 392 (39.8) | 11.6 | 63.9 (11.4) | 32.3 (8.3) | 3.3 years [1.6–6.3] years | yes | yes | yes | no |
| Ko EM, 2014 | U.S. | Stage I-IV EC | 1,495 | Histology 363 diabetic women: endometrioid 280; non-endometrioid 83 | 13.3 [#] | 63.6 (4.1) | 37.7 (3.3) | 33 months | yes | no | no | no |
| Lemanska A, 2014 | Poland | Stage I-III EC | 107 | Histology 96: Endometrioid 71, non endometrioid 25 | 23.8 | 64.3, range 40-91 | 32.8 | 9.3 years | no | no | yes | no |
| Al Hilli MM, 2016 | U.S. | Stage I-IV EC | 1,303 | Histology available in 1,058: details NA | 8.9 | 64.6 (11.5) | 33.4 (9.4) | 4.3 [2.7–6.9] years for diabetic patients, and 5.2 [3.2–7.8] years for nondiabetic patients. | yes | yes | no | no |
| Ezewuiro O, 2016 | U.S. | Stage III-IV or recurrent EC | 349 | 140 endometrioid or adenocarcinoma NOS; other types 209 | 8.9 | 64 (11) | 31.4 (9.2) | 37.0 [0.6-168.7] months | yes | yes | yes | yes |
| Seebacher V, 2016 | Austria | Stages I-IV EC | 465 | Endometrioid 421 (90.5) Non-endometrioid 44 (9.5) | 9.9 | 65.3* | 29.0* | 51 months | no | no | no | no |

*median; EC = endometrial cancer; DM = diabetes mellitus; SD = standard deviation; HR = hazard ratio; NOS = not otherwise specified; NA = not available

[#]34% of metformin users were also using sulfonylureas, 18% thiazolidinediones, 15% insulin, and 7% other anti-diabetic agents. Nearly one-third (29%) of non-metformin user diabetics used insulin based regimens.

Table 2 Newcastle-Ottawa Scale for risk of bias assessment

| First author, year published | Selection criteria | | | | Comparability criteria | | Outcome criteria | | | Number of stars |
|------------------------------|--|-------------------------------------|---------------------------|--|---|----------|-----------------------|---|----------------------------------|-----------------|
| | Representativeness of the Exposed Cohort | Selection of the Non-Exposed Cohort | Ascertainment of Exposure | Demonstration That Outcome of Interest Was Not Present at Start of Study | Comparability of Cohorts on the Basis of the Design or Analysis | | Assessment of Outcome | Was Follow-Up Long Enough for Outcomes to Occur | Adequacy of Follow Up of Cohorts | |
| Nevadunsky NS, 2013 | * | * | * | * | *(age) | *(stage) | * | * | * | 9 |
| Ko EM, 2014 | * | * | * | * | *(age) | *(stage) | * | * | * | 9 |
| Lemanska A, 2014 | * | * | * | * | - | - | * | * | * | 7 |
| Al Hilli MM, 2016 | * | * | * | * | *(age) | *(stage) | * | * | * | 9 |
| Ezewuiro O, 2016 | * | * | * | * | *(age) | *(stage) | * | * | * | 9 |







