

# Association of Metformin with Breast Cancer Incidence and Mortality in Patients with Type II Diabetes: A GRADE-Assessed Systematic Review and Meta-analysis



Grace H. Tang<sup>1</sup>, Meloja Satkunam<sup>1</sup>, Gregory R. Pond<sup>1,2</sup>, Gregory R. Steinberg<sup>3,4</sup>, Giovanni Blandino<sup>2,5</sup>, Holger J. Schünemann<sup>1</sup>, and Paola Muti<sup>1,2</sup>

## Abstract

**Background:** Preclinical data suggest that metformin may reduce breast cancer incidence and improve cancer prognosis. However, the current evidence in observational studies is inconclusive. A systematic review and meta-analysis was conducted to assess the effect of metformin on the incidence of breast cancer and all-cause mortality in patients with type II diabetes (T2D).

**Methods:** A literature search was performed on Medline, EMBASE, and the Cochrane library from inception to November 2016. Outcomes were incidence of breast cancer and all-cause mortality. Risk of bias and overall certainty of evidence was assessed using the Newcastle-Ottawa Scale and Grading of Recommendations Assessment, Development, and Evaluation (GRADE), respectively. Meta-analyses were performed using the most fully adjusted ORs or HRs and 95% confidence intervals (95% CI) as effect measures.

**Results:** A total of 12 observational studies were included for breast cancer incidence and 11 studies for all-cause mortality. No significant association was found between metformin exposure and incidence of breast cancer (OR = 0.93; 95% CI, 0.85–1.03;  $I^2 = 35\%$ ). A 45% risk reduction was observed for all-cause mortality (HR = 0.55; 95% CI, 0.44–0.70;  $I^2 = 81\%$ ). Presence of publication bias is strongly suspected for both outcomes using Egger's funnel plots.

**Conclusions:** The use of metformin may improve overall survival in patients with T2D and breast cancer. No effect of metformin on the incidence of breast cancer was observed. Interpretation of results is limited by the observational nature of the studies and resulting biases.

**Impact:** Clinical trials are warranted to determine the role of metformin in breast cancer risk reduction and prognosis. *Cancer Epidemiol Biomarkers Prev*; 27(6): 627–35. ©2018 AACR.

## Introduction

Type II diabetes (T2D) and cancer are two common chronic diseases that decrease quality of life and survival (1, 2). T2D is an independent risk factor for breast cancer (3–6). A meta-analysis by Boyle and colleagues reported an RR of 1.27 [95% confidence interval (CI), 1.16–1.39] for breast cancer in women with T2D, and no increased risk for women with type I diabetes (3). Preclinical studies have suggested that T2D may play a role in malignant transformation and growth through

hyperglycemia-related oxidative stress, insulin resistance, and chronic low-grade inflammation (1, 7–9). Insulin can also act as a growth hormone and is frequently overexpressed in malignant cells (10–12). Altered glucose metabolism and fasting hyperglycemia have also been associated with breast cancer development and mortality in postmenopausal women (13–15). Furthermore, it has been reported that physical inactivity may contribute to both T2D and breast cancer (11, 16).

Metformin, an inexpensive oral biguanide, is the most commonly prescribed first-line therapy for T2D patients to reduce blood glucose concentrations (17–19). Metformin has an excellent safety profile with minimal side effects and can be administered alongside most cancer therapies without any known drug interactions (17, 20, 21). Over the past decade, the role of metformin as an anticancer therapy has been extensively studied in the preclinical setting, with several proposed direct effects of the drug on suppressing cell growth and proliferation as well as indirect effects involving the lowering of glucose, insulin, and inflammation (7, 17, 18, 22–26). There are also numerous epidemiologic studies indicating the potential use of metformin for breast cancer prevention or as additional treatment for breast cancer. However, the evidence supporting the role of metformin in breast cancer prevention and treatment is inconsistent in the literature, with some studies suggesting no beneficial effects (27, 28).

<sup>1</sup>Department of Health Research Methods, Evidence, and Impact, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada. <sup>2</sup>Department of Oncology, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada. <sup>3</sup>Department of Medicine, Division of Endocrinology, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada. <sup>4</sup>Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada. <sup>5</sup>Oncogenomic and Epigenetic Unit, Italian National Cancer Institute 'Regina Elena,' Rome, Italy.

**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

**Corresponding Author:** Paola Muti, McMaster University, 711 Concession Street, Hamilton, Ontario L8V1C3, Canada. Phone: 905-527-2299, Ext. 42606; Fax: 905-575-2639; E-mail: [muti@mcmaster.ca](mailto:muti@mcmaster.ca)

**doi:** 10.1158/1055-9965.EPI-17-0936

©2018 American Association for Cancer Research.

To date, there are just four published systematic reviews and meta-analyses that have investigated the use of metformin and the incidence of breast cancer and mortality (29–32). Previous meta-analyses did not search gray literature, consider the effects of time-related biases, or use the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system to evaluate overall study certainty of evidence. We therefore conducted a meta-analysis to assess the current literature regarding the use of metformin and the incidence and mortality of breast cancer in patients with T2D compared with other commonly prescribed glucose-lowering medications (GLMs).

## Materials and Methods

The protocol was published on PROSPERO international prospective register of systematic reviews in January 2017 (33). This systematic review and meta-analysis was conducted following the Cochrane Handbook and was reported as per Preferred Reporting for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (34, 35).

### Study selection

The inclusion criteria were the following: (i) females age 18 years or older who were clinically diagnosed with T2D (incidence studies) or females age 18 years or older who were clinically diagnosed with T2D and breast cancer (mortality studies); (ii) comparison of metformin oral therapy with other GLMs; and (iii) outcomes inclusive of incidence of breast cancer or all-cause mortality.

An electronic literature search was conducted from inception to November 2016 on Medline (PubMed), EMBASE, and the Cochrane library with no language restrictions. Search terms were used such as "metformin," "diabetes type II," and "breast neoplasms." In addition, studies were identified through bibliographies of published systematic reviews. Gray literature (abstracts, registered trials) was identified through a manual search from clinical trials registries and conference proceedings.

Two reviewers (G.H. Tang and M. Satkunam) pilot-tested the standardized data collection forms (DCF) and independently screened title, abstract, and full-text of retrieved articles for inclusion. Any disagreement or uncertainty in the broad and secondary screen was resolved by consensus or consultation of a third party. To avoid overlapping patient populations, articles with duplicate datasets were assessed by the most recent publication, and/or larger study duration, and population.

After final selection of full-text articles for quantitative analysis, the two reviewers (G.H. Tang and M. Satkunam) independently extracted information from each study. A standardized pilot-tested DCF was used to collect: study characteristics, population characteristics, exposure ascertainment, adjustment for confounders, and outcome assessment. The most fully adjusted estimate was recorded if several estimates were reported. When appropriate, effect estimates and 95% CIs were inverted to ensure comparator(s) (i.e., non-metformin) was the reference value. If studies included both nondiabetic patients and diabetics not on metformin treatment as comparators, we extracted only diabetics not on metformin treatment as per our inclusion criteria and to minimize confounding by diabetes status.

### Risk of bias assessment

The Newcastle-Ottawa Scale (NOS) for nonrandomized studies was used to assess risk of bias (RoB) for the included

studies (36, 37). The two reviewers (G.H. Tang and M. Satkunam) independently assessed each study using the NOS using a standardized pilot-tested DCF and provided an overall score based on the assessment criteria (from 0 to 9 stars). The NOS uses a nine-point summative "star system" in which individual studies are based on: (i) selection of study groups (four stars); (ii) comparability of groups (two stars); and (iii) ascertainment of exposure and outcomes (three stars; refs. 36, 37). Disagreements were resolved by consensus or a consultation of a third party. To our knowledge, there are no established cutoffs for a "low," "moderate," or "high" RoB for the NOS. As such, the authors relied on previous literature to determine a high RoB as a score of  $\leq 5$ , moderate RoB as a score between 6 and 7, and low RoB as a score between 8 and 9 (38).

### GRADE

The overall certainty of evidence across studies was assessed using GRADE as outlined by the GRADE Working Group (39). The certainty of evidence was summarized in four categories: high (4), moderate (3), low (2), and very low certainty (1; ref. 40). The rating of the certainty of evidence reflects the confidence that the estimates of effect are correct (40). As per the GRADE criteria, reasons for certainty to be rated downward include RoB, inconsistency, indirectness, imprecision, and publication bias (39, 40). Reasons to raise certainty include large magnitude of effect, dose-response relation, and adjustment of all plausible residual confounding (39, 40).

### Agreement statistics

Agreement statistics were calculated between the two independent reviewers (G.H. Tang and M. Satkunam) for interrater concordance. An unweighted kappa score was calculated after the initial comparison between reviewers for the broad and secondary screen. A weighted kappa was used for the RoB because the NOS uses an ordinal scale (41). The value of the unweighted and weighted kappa was interpreted using Altman and colleagues (42).

### Statistical analysis

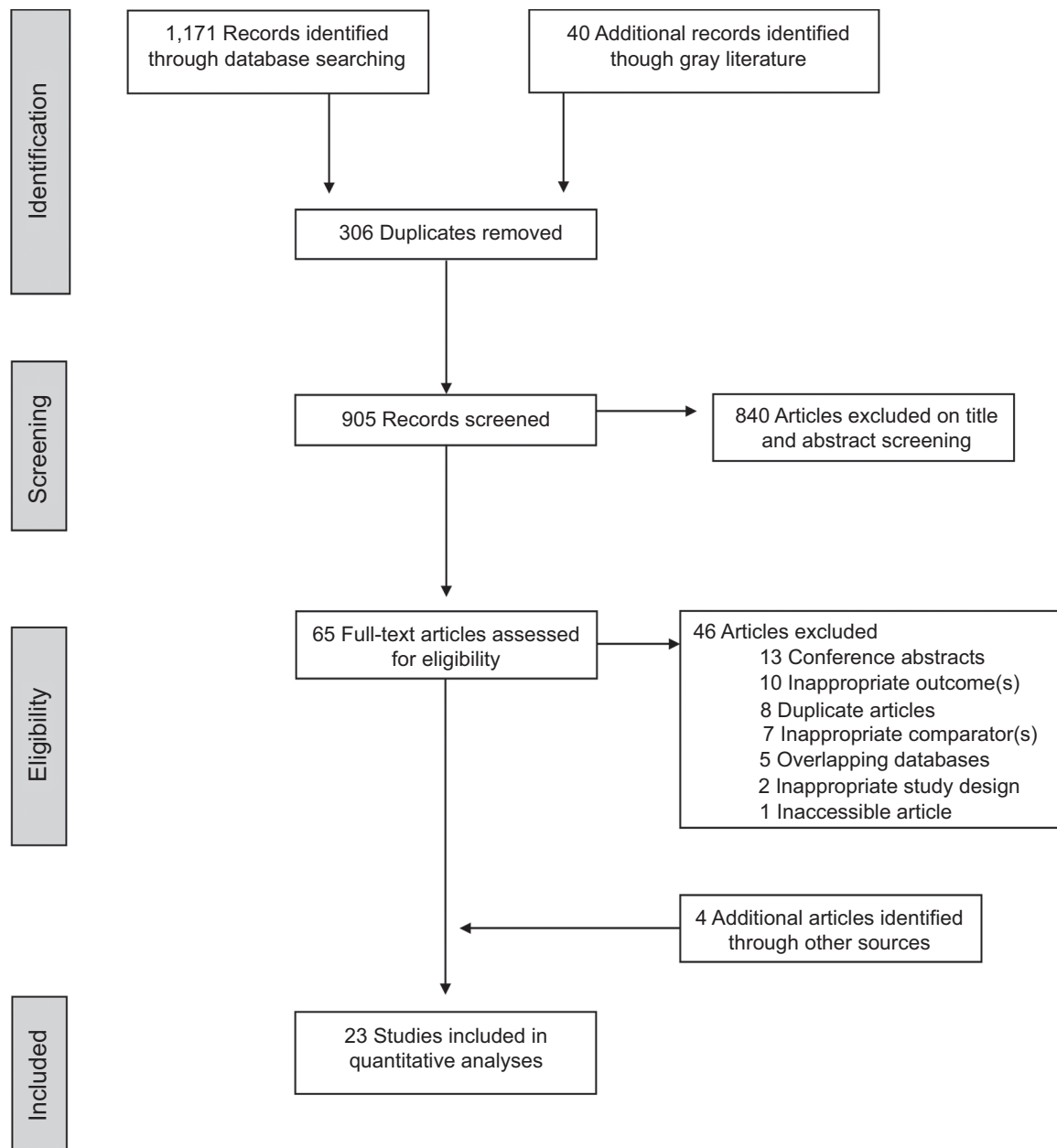
The meta-analyses were performed using Review Manager Software v5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration), if the included studies were sufficiently homogenous in population, used GLMs as interventions, and outcomes provided a meaningful summary of effect. When multiple durations of metformin exposure were reported, the longest duration was pooled in the meta-analysis as the effect may not be seen in shorter durations. The random effects model was chosen as the main model of interest *a priori*. Heterogeneity was assessed using Cochran Q statistic based on a  $\chi^2$  distribution (significance if  $P < 0.10$ ; ref. 34). Degree of heterogeneity was interpreted using the  $I^2$  test. An  $I^2$  value  $\leq 25\%$  was deemed low heterogeneity, 26% to 50% as moderate heterogeneity, and  $>50\%$  as high heterogeneity (34). Publication bias was assessed by using the Egger's funnel plot (43).

Secondary analyses were conducted to account for any significant levels of heterogeneity using the random effects model. If a study contributed  $>50\%$  of the total weight of all studies evaluated, an exploratory analysis was carried out such that the most weighted study will be removed. Subgroup analyses were conducted for the duration of metformin treatment, type of pooled

GLMs, and studies that appropriately accounted for time-related biases (i.e., immortal time bias, time window bias, and time lag bias). Studies that appropriately accounted for time-related biases include using the Cox proportional hazard model with time-varying determinants, intention-to-treat analysis, matching for duration of exposure, and application of time-lag periods as described by Suissa and Azoulay (2012; ref. 44). Sensitivity analyses were conducted to compare RoB of studies as per NOS score [*a priori*; low RoB ( $>7$ ) vs. high RoB ( $\leq 7$ )], obesity/body mass index (BMI), and type of study (breast cancer specific vs. all types of cancer).

## Results

A total of 1,171 records were identified through database searches, and an additional 40 records were identified from gray literature. After deduplication, a total of 905 abstracts/titles were eligible for the broad screen. Of these, 65 full-text articles were retrieved for eligibility screening using the inclusion and exclusion criteria as stated *a priori*. An additional 4 articles were identified through previously published systematic reviews and meta-analyses. A total of 23 studies were included for quantitative synthesis (Fig. 1). An unweighted kappa of 0.46 (moderate



**Figure 1.**

Figure 1 describes the selection of studies. PRISMA diagram for the selection of studies for quantitative analysis.

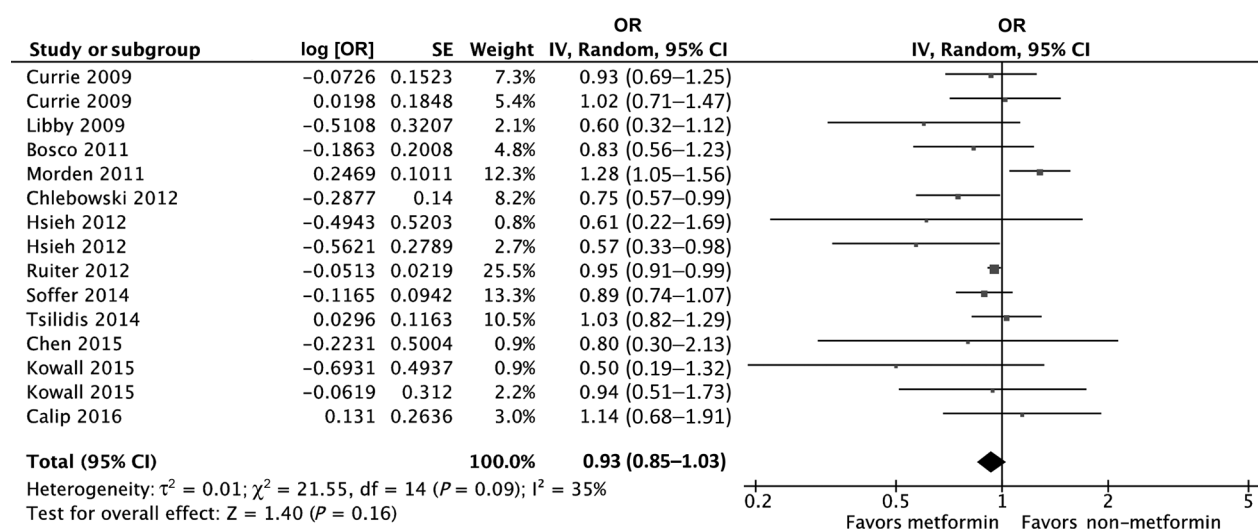


Figure 2.

Forest plot for metformin and the incidence of breast cancer. The squares represent the OR of each single study. Horizontal lines, 95% CIs. The diamond represents the pooled estimate. Duplicate risk estimates from Currie (2009), Hsieh (2012), and Kowall (2015) indicate comparison with two different types of GLMs.

agreement) and 0.78 (good agreement) between reviewers were calculated for the broad and secondary screen, respectively.

### Study characteristics

Summary of study characteristics and main outcomes of the included studies are described in Supplementary Tables S1 to S4 for breast cancer incidence and all-cause mortality (27, 28, 45–65). For breast cancer incidence, comparators used against metformin included sulfonylureas, insulin, "non-metformin," and "other GLMs." An estimated total of 7,506 users of metformin and 8,724 non-metformin users were reported. Of the metformin users, an estimated total of 1,238 (16.5%) were diagnosed with breast cancer compared with the estimated 1,673 (19.2%) from the comparator group. For all-cause mortality studies, "non-metformin" was the comparator used against metformin. An estimated total of 3,400 metformin users and 2,987 non-metformin users were pooled in the analysis. An estimated total

of 491 (14.4%) deaths were reported for metformin users and estimated 1,220 (40.8%) deaths for non-metformin users.

### RoB assessment

The NOS for case–control and cohort studies was used to assess the RoB for individual studies (Supplementary Tables S2 and S4). A weighted kappa of 0.29 was calculated, indicating a fair agreement between reviewers. No consistent themes were identified as sources of disagreement.

### Breast cancer incidence

A total of 12 studies with 15 risk estimates were pooled in the meta-analysis (Fig. 2). The overall effect size using the random effects model did not demonstrate a significant protective effect of metformin against breast cancer (OR = 0.93; 95% CI, 0.85–1.03). A moderate degree of heterogeneity was detected between studies ( $I^2 = 35\%$ ). Egger's funnel plot

Table 1. Summary of secondary analyses for metformin and incidence of breast cancer

		$n^a$	OR (95% CI)	$P$	$I^2$
Duration of treatment	$\geq 3$ years <sup>b</sup>	4	0.95 (0.91–0.99)	0.01	0%
	$< 3$ years	8	0.85 (0.72–1.00)	0.05	35%
Comparators	Sulfonylureas <sup>c</sup>	6	0.94 (0.85–1.04)	0.26	14%
	Insulin	4	1.06 (0.83–1.36)	0.65	39%
	Non-metformin <sup>d</sup>	5	0.85 (0.74–0.97)	0.01	0%
Time-related biases	No bias <sup>e</sup>	8	0.94 (0.91–0.98)	0.005	0%
	Bias	7	0.89 (0.70–1.13)	0.34	59%
RoB of studies	Low RoB	5	0.90 (0.80–1.02)	0.09	1%
	High RoB	10	0.93 (0.80–1.09)	0.38	46%
BMI/obesity	Adjusted	8	0.90 (0.73–1.11)	0.34	56%
	Unadjusted <sup>f</sup>	7	0.95 (0.91–0.98)	0.007	0%
Type of study	Breast cancer specific	3	0.82 (0.67–1.01)	0.07	0%
	All cancers	12	0.95 (0.86–1.06)	0.38	38%

<sup>a</sup>Number of effect sizes pooled.

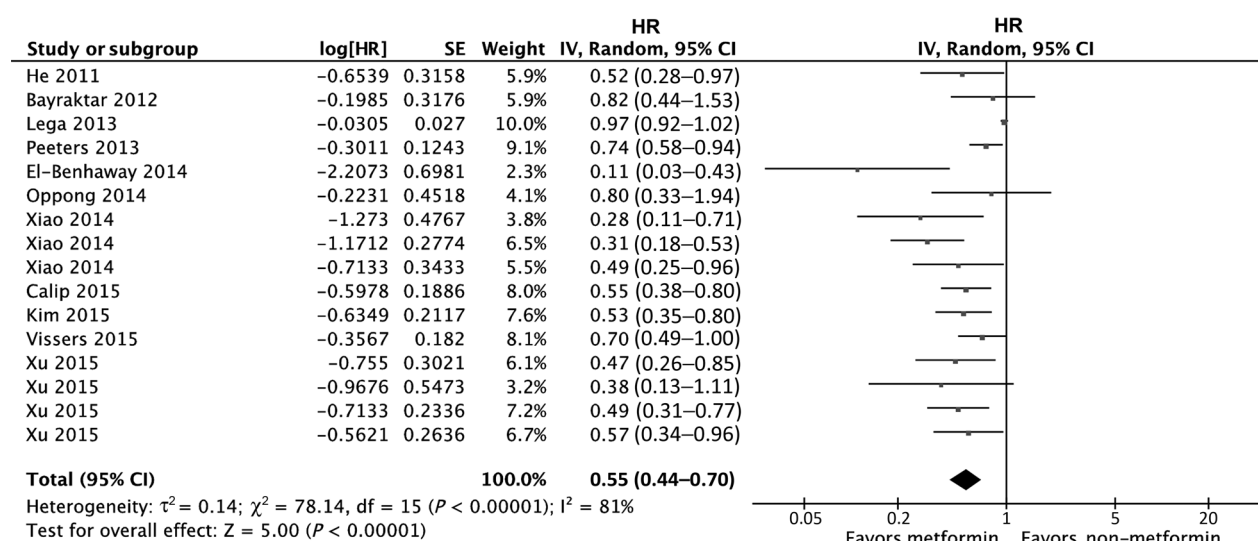
<sup>b</sup>Exploratory analysis conducted to remove Ruiter et al. (98% of overall weight), OR = 0.88; 95% CI, 0.62–1.23;  $I^2 = 15\%$ .

<sup>c</sup>Exploratory analysis conducted to remove Ruiter et al. (71.2% of overall weight), OR = 0.88; 95% CI, 0.68–1.13;  $I^2 = 31\%$ .

<sup>d</sup>Exploratory analysis conducted to remove Soffer et al. (53% of overall weight), OR = 0.80; 95% CI, 0.66–0.97;  $I^2 = 0\%$ .

<sup>e</sup>Exploratory analysis conducted to remove Ruiter et al. (88.5% of overall weight), OR = 0.90; 95% CI, 0.80–1.02;  $I^2 = 0\%$ .

<sup>f</sup>Exploratory analysis to remove Ruiter et al. (90.6% of overall weight), OR = 0.90; 95% CI, 0.79–1.03;  $I^2 = 0\%$ .



**Figure 3.**

Forest plot for metformin and all-cause mortality. The squares represent the HR of each single study. Horizontal lines, 95% CIs. The diamond represents the pooled estimate. Duplicate estimates from Xiao (2014) indicate different subtypes of breast cancer. Duplicate estimates from Xu (2015) indicate results from two different administrative databases.

was performed, which suggested the presence of publication bias (Supplementary Fig. S1).

A summary of secondary and exploratory analyses is described in Table 1. For those who received metformin treatment  $\geq 3$  years, there was a significant protective effect (OR = 0.95; 95% CI, 0.91–0.99). There was a stronger protective effect for those who received metformin  $< 3$  years, but the effect was not significant (OR = 0.85; 95% CI, 0.72–1.00). No protective effect of metformin was reported when compared with sulfonylureas or insulin. A protective effect of metformin was identified in comparison with the "non-metformin" group (OR = 0.85; 95% CI, 0.74–0.97). Of the 7 studies that adjusted for time-related biases, there was a protective effect of metformin (OR = 0.94; 95% CI, 0.91–0.98). No effect was observed when comparing study RoB, type of study, and studies that adjusted for obesity/BMI.

#### All-cause mortality

A total of 11 studies with 16 estimates were pooled in the final meta-analysis (Fig. 3). The overall effect using the random effects model demonstrated a statistically significant protective effect of metformin against all-cause mortality (HR = 0.55; 95% CI, 0.44–0.70). A significant degree of heterogeneity ( $I^2 = 81\%$ ) was identified. We performed Egger's funnel plot, which suggested presence of publication bias (Supplementary Fig. S2).

A summary of secondary analyses is described in Table 2. Studies that did not adjust for time-related biases demonstrated

a stronger benefit of metformin therapy for overall survival compared with studies that appropriately accounted for time-related biases (HR = 0.48; 95% CI, 0.40–0.59 and HR = 0.75; 95% CI, 0.58–0.98, respectively). High and low RoB studies demonstrated a significant protective effect (HR = 0.58; 95% CI, 0.47–0.71 and HR = 0.55; 95% CI, 0.36–0.83, respectively). A significant protective effect was observed for studies that were adjusted for BMI/obesity (HR = 0.50; 95% CI, 0.43–0.59).

#### GRADE certainty of the body of evidence

GRADE evidence profile for summary of findings is presented in Table 3. For incidence of breast cancer, the overall certainty was "very low," due to RoB and the presence of publication bias. For all-cause mortality, the overall certainty was "very low" due to high RoB, unexplained heterogeneity, and presence of publication bias.

## Discussion

In the current study, we conducted a systematic review and two separate meta-analyses on the association of metformin on the risk of breast cancer incidence and all-cause mortality in patients with T2D. We did not find a significant relationship between metformin therapy and incidence of breast cancer in our main analysis (OR = 0.93; 95% CI, 0.85–1.03;  $I^2 = 35\%$ ). In our meta-analysis regarding metformin exposure and all-cause mortality,

**Table 2.** Summary of secondary analyses for metformin and all-cause mortality

		$n^a$	HR (95% CI)	P	$I^2$
Time-related biases	No bias	4	0.75 (0.58–0.98)	0.04	81%
	Bias	12	0.48 (0.40–0.59)	$< 0.00001$	17%
RoB of studies	Low RoB	7	0.55 (0.36–0.83)	0.004	85%
	High RoB	9	0.58 (0.49–0.71)	$< 0.00001$	35%
BMI/obesity	Adjusted	11	0.50 (0.43–0.59)	$< 0.00001$	0%
	Unadjusted	5	0.74 (0.55–1.01)	0.06	77%

<sup>a</sup>Number of effect sizes pooled.

**Table 3.** Association of metformin with breast cancer incidence and all-cause mortality – GRADE evidence profile

No. of studies	Certainty assessment					No. of patients			Effect		Certainty
	Study design	RoB	Inconsistency	Indirectness	Imprecision	Other considerations	Metformin	Non-metformin	Relative (95% CI)	Absolute (95% CI)	
12	Breast cancer incidence Observational studies	Serious <sup>a,b,c</sup>	Not serious <sup>d,e</sup>	Not serious	Not serious <sup>f</sup>	Publication bias strongly suspected <sup>g</sup>	1,238/7,506 (16.5%)	1,673/8,724 (19.2%)	OR 0.93 (0.85–1.03)	11 fewer per 1,000 (from 5 more to 24 fewer)	⊕○○○ Very low
11	All-cause mortality Observational studies	Serious <sup>h,i,j</sup>	Serious <sup>k,l</sup>	Not serious	Not serious <sup>m</sup>	Publication bias strongly suspected <sup>g</sup>	491/3,400 (14.4%)	1,220/2,987 (40.8%)	HR 0.55 (0.44–0.70)	158 fewer per 1,000 (from 101 fewer to 202 fewer)	⊕○○○ Very low

<sup>a</sup>Presence of time-related biases in 42% (5/12) of the included studies.

<sup>b</sup>Five studies [Hsieh (2009), Morden (2011), Ruiter (2012), Soffer (2014), Chen (2015)] failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale assessment.

<sup>c</sup>Of the included studies, 58% (7/12) were rated high or moderate RoB using the Newcastle-Ottawa Scale (i.e., score < 8).

<sup>d</sup>Moderate degree of heterogeneity ( $I^2 = 35\%$ ).

<sup>e</sup>P value is marginally statistically significant for heterogeneity ( $P = 0.09$ ).

<sup>f</sup>Optional information size is met; summary RR does cross 1.0; upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR = 0.75).

<sup>g</sup>Asymmetry identified in Egger's funnel plot.

<sup>h</sup>Four studies [Peeters (2013), El-Benhawy (2014), Oppong (2014), Kim (2015)] failed to adjust for additional confounding factors as described in the Newcastle-Ottawa Scale.

<sup>i</sup>Presence of time-related biases in 64% (7/11) of the included studies.

<sup>j</sup>Of the included studies, 55% (6/11) studies were rated high or moderate RoB using the Newcastle-Ottawa Scale (i.e., score < 8).

<sup>k</sup>High degree of heterogeneity ( $I^2 = 81\%$ ).

<sup>l</sup>P value is statistically significant for heterogeneity ( $P < 0.00001$ ).

<sup>m</sup>Optional information size is met; summary HR does not cross 1.0; upper and lower limit of the 95% CI does not include appreciable harm or benefit (RR = 0.75).

we identified a 45% risk reduction in 11 pooled studies (HR = 0.55; 95% CI, 0.44–0.70;  $I^2 = 81\%$ ). In both meta-analyses, presence of publication bias was strongly suspected, and time-related biases (e.g., immortal time bias, time-window bias, time-lag bias) were present in several studies. The overall quality of evidence using GRADE was "very low"; therefore, any estimate of effect is uncertain.

In the literature, there are few meta-analyses that exclusively investigated the effect of metformin on breast cancer. The first was published in 2012 by Col and colleagues who pooled a total of 7 studies regarding the incidence of breast cancer. The authors' analyses supported the protective effect of metformin (OR = 0.83; 95% CI, 0.71–0.97;  $I^2 = 51\%$ ; ref. 29). Furthermore, the authors reported a stronger association for studies that had a longer duration (>3 years) of metformin use (OR = 0.75; 95% CI, 0.62–0.91; ref. 29). In the current study, a similar trend of a stronger effect when metformin treatment was  $\geq 3$  years (OR = 0.95; 95% CI, 0.91–0.99;  $I^2 = 0\%$ ) was found. However, the exploratory analysis concluded that this effect was mostly driven by the weight of Ruiter and colleagues and was stronger but insignificant after omission (OR = 0.88; 95% CI, 0.62–1.23;  $I^2 = 15\%$ ). In parallel to our findings, Yang and colleagues and Moradi-Joo and colleagues did not report a protective effect of metformin against breast cancer or when compared with only sulfonylureas therapy, respectively (31, 32). For all-cause mortality, previous meta-analyses by Yang and colleagues and Xu and colleagues reported a significant beneficial effect of metformin therapy in diabetic patients (RR = 0.65; 95% CI, 0.48–0.87 and HR = 0.53; 95% CI, 0.39–0.71, respectively; refs. 30, 31). These findings are similar to our results, which suggest a significant risk reduction for all-cause mortality in diabetic breast cancer patients on metformin therapy (HR = 0.55; 95% CI, 0.44–0.70).

Subgroup analyses were conducted to account for the presence of time-related biases in our meta-analyses as described by Suissa and Azoulay (Tables 1 and 2; ref. 44). For breast cancer incidence, there was a significant protective effect of metformin in studies that adjusted for time-related biases (OR = 0.94; 95% CI, 0.91–0.98;  $I^2 = 0\%$ ). After removal of Ruiter and colleagues, the effect was stronger and no longer significant (OR = 0.90; 95% CI, 0.80–1.02;  $I^2 = 0\%$ ). Our findings support Suissa and Azoulay's review that studies that utilized time-dependent techniques tend to find no association between metformin and cancer incidence because the presence of these biases can greatly exaggerate the protective effect of metformin (44).

In addition to time-related biases, the presence of time-dependent confounders may also affect results. A review by Farmer and colleagues described BMI and its surrogate obesity, as a time-dependent confounder (66). When time-dependent confounders are detected, standard statistical models cannot estimate the true causal effect of time-varying treatment (66, 67). To explore BMI as a time-dependent confounder, we conducted sensitivity analyses that adjusted BMI or obesity. For incidence, our findings are similar to a previous meta-analysis that did not identify a significant protective effect (summary RR = 0.82; 95% CI, 0.67–1.00;  $I^2 = 48\%$ ; ref. 68). These results support Farmer and colleagues' claim that studies least likely to be affected by bias do not support a causal effect between metformin and cancer risk (66). For all-cause mortality, studies that adjusted for BMI/obesity suggest that there is a protective effect of metformin (HR = 0.50; 95% CI, 0.43–0.59;  $I^2 = 0\%$ ). A similar protective effect was reported by Xu and colleagues, with a 57% reduction in

all-cause mortality (HR = 0.43; 95% CI, 0.34–0.55;  $I^2 = 0\%$ ; ref. 30). These results must be interpreted cautiously as the relationship between BMI and cancer by metformin in a time-dependent context is complex.

There are a few potential explanations for the observed effect between metformin use and overall survival in breast cancer patients with diabetes. A study by Jiralerspong and colleagues reported that patients with T2D and breast cancer receiving metformin and neoadjuvant chemotherapy have a higher pathologic complete response compared with patients with T2D not taking metformin (69). This further suggests that metformin may have antitumor effects in patients with breast cancer.

From a physiologic perspective, mechanisms of action for metformin as anticancer therapy have been extensively studied in the preclinical setting (7, 17, 18, 22–25). Although the exact mechanisms have yet to be elucidated, the most widely accepted mechanism involves the activation of AMPK (7, 18, 70). AMPK is an established molecular regulator of cell metabolism to suppress tumor growth (7, 18, 70). The activation of AMPK has been shown to inhibit the mTOR signaling pathway, which is known to promote cell growth and tumorigenesis (7, 17, 18, 71, 72). AMPK increases tuberous sclerosis complex protein 2 activity, leading to the inactivation of mTOR and thereby decreasing protein synthesis and cell growth of cancer cells (7, 17, 73). It has also been proposed that metformin can indirectly reduce the level of circulating insulin and insulin-like growth factor-1 (7).

Although the current analyses are based on the best available evidence, there are several limitations. The included studies were mostly retrospective and varied in study population, diabetes duration, length of metformin exposure, type of glucose-lowering therapy as comparator, and adjustment of confounding factors. Metformin dose and adherence were not closely evaluated as the dose at baseline may not represent dose during follow-up, as disease severity can change (29). In addition, the potential impact of less easily detectable biases (i.e., time-related biases and time-dependent confounders) can contribute to the largest protective effects of metformin (44, 66). The ethnic diversity may also affect the results because of the different treatment guidelines for breast cancer and diabetes, and cancer screening protocols (e.g., mammogram screening) in different countries. In our meta-analyses, we did not investigate the association of metformin in other oncologic outcomes, such as cancer recurrence and breast-specific cancer mortality. We were also unable to stratify by the type of breast cancer classification, glycated hemoglobin levels, or evaluate the levels of circulating androgen and estrogen levels in our subgroup analyses due to limited information. Results from secondary analyses must be interpreted cautiously, as some studies were weighted more than others in the pooled analysis using the inverse variance method. Finally, publication bias is strongly suspected due to the asymmetrical shape of the funnel plots. Both funnel plots for breast cancer incidence and all-cause mortality suggest that the absent studies show that metformin is harmful. Therefore, if these studies were not published, this could greatly affect the overall interpretation of our results.

Despite the limitations of this study, there are also several strengths worthy of mention. A comprehensive literature search was conducted using a broad search strategy and through gray literature. The screening of abstracts, full-text articles, and abstraction were completed in duplicate with a second author. Multiple secondary analyses to account for heterogeneity, presence of

time-related biases were conducted. To our knowledge, this is also the first systematic review and meta-analysis in metformin and breast cancer to use the GRADE system.

Currently, there is an ongoing phase III randomized control trial (RCT), the PLOTINA study, conducted at the Italian National Cancer Institute comparing metformin versus placebo in postmenopausal women at high risk of T2D ( $n = 16,000$ ; ref. 74). The aim of this RCT is to evaluate the effect of metformin on the incidence of breast cancer incidence and cardiovascular diseases (74). An additional second RCT with similar eligibility criteria will further add a diet intervention (based on reduction of high caloric food, increase in vegetable intake) to evaluate the effects of metformin (74).

Another ongoing large multicenter phase III RCT is the NCIC CTG MA.32, for metformin versus placebo in early breast cancer patients (75). This trial will compare invasive disease-free survival of patients with node-positive or high-risk node-negative breast cancer who are receiving standard therapy (75). Secondary outcomes include overall survival, distant disease-free survival, breast cancer-free survival, BMI changes, adverse events, and quality of life (75). Interim data monitoring and safety results reported that metformin in breast cancer patients demonstrated beneficial effects on body weight, insulin, glucose, leptin, and C-reactive protein, supporting the continuation of this large trial to determine the effects of metformin on cancer outcomes and noncancer outcomes (76).

In conclusion, we observed a protective effect of metformin for all-cause mortality in breast cancer patients with T2D. We did not observe a significant effect for the incidence of breast cancer. Results from our meta-analyses must be interpreted cautiously due to the presence of methodologic bias, publication bias, and very low rating using GRADE. Observational studies in the context of metformin and cancer therapy are complex as management for diabetes changes over time, and the presence of time-related biases is difficult to detect. Further studies are warranted to determine the role of metformin in breast cancer risk reduction and prognosis.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** G.H. Tang, G.R. Pond, P. Muti  
**Development of methodology:** G.H. Tang, G.R. Pond, P. Muti  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** G.H. Tang  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** G.H. Tang, G.R. Pond, G. Blandino, H.J. Schünemann, P. Muti  
**Writing, review, and/or revision of the manuscript:** G.H. Tang, M. Satkunam, G.R. Pond, G.R. Steinberg, G. Blandino, H.J. Schünemann, P. Muti  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** M. Satkunam, P. Muti  
**Study supervision:** G.R. Pond, P. Muti

### Acknowledgments

We would like to thank Avery Longmore for serving as a third-party reviewer.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 11, 2017; revised December 15, 2017; accepted March 30, 2018; published first April 4, 2018.

## References

- Giovannucci E, Harlan DM, Archer MC, Bergental RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. *Diabetes Care* 2010;33:1674–85.
- Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. *Endocr Relat Cancer* 2009;16:1103–23.
- Boyle P, Boniol M, Koehlin A, Robertson C, Valentini F, Coppens K, et al. Diabetes and breast cancer risk: a meta-analysis. *Br J Cancer* 2012;107:1608–17.
- Liao S, Li J, Wei W, Wang L, Zhang Y, Li J, et al. Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature. *Asian Pac J Cancer Prev* 2011;12:1061–5.
- Tsilidis K, Kasimis J, Lopez D, Ntzani E. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. *BMJ* 2015;350:g7607.
- Hardefeldt PJ, Edirimanne S, Eslick GD. Diabetes increases the risk of breast cancer: a meta-analysis. *Endocrine-related cancer* 2012;19:793–803.
- Kasznicki J, Sliwiska A, Drzewoski J. Metformin in cancer prevention and therapy. *Ann Transl Med* 2014;2:57.
- Drzewoski J, Drodzowska A, Sliwiska A. Do we have enough data to confirm the link between antidiabetic drug use and cancer development? *Pol Arch Med Wewn* 2011;121:81–7.
- Azar M, Lyons TJ. Diabetes, insulin treatment, and cancer risk: what is the evidence? *F1000 Med Rep* 2009;4:1–4.
- Lipscombe LL, Fischer HD, Austin PC, Fu L, Jaakkimainen RL, Ginsburg O, et al. The association between diabetes and breast cancer stage at diagnosis: a population-based study. *Breast Cancer Res Treat* 2015;150:613–20.
- Michels K, Solomon C, Hu F, Rosner B, S H, G C, et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. *Diabetes Care* 2003;26:1752–8.
- Lawlor DA, Smith GD, Ebrahim S. Hyperinsulinaemia and increased risk of breast cancer: findings from the British Women's Heart and Health Study. *Cancer Causes Control* 2004;15:267–75.
- Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. *Cancer Epidemiol Biomarkers Prev* 2002;11:1361–8.
- Agnoli C, Grioni S, Sieri S, Sacerdote C, Ricceri F, Tumino R, et al. Metabolic syndrome and breast cancer risk: a case-cohort study nested in a multi-centre Italian cohort. *PLoS One* 2015;10:1–12.
- Bjørge T, Lukanova A, Jonsson H, Tretli S, Ulmer H, Manjer J, et al. Metabolic syndrome and breast cancer in the Me-Can (metabolic syndrome and cancer) project. *Cancer Epidemiol Biomarkers Prev* 2010;19:1737–45.
- Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res* 2010;3:1451–61.
- Chae YK, Arya A, Malecek M-K, Shin DS, Carneiro B, Chandra S, et al. Repurposing metformin for cancer treatment: current clinical studies. *Oncotarget* 2016;7:40767–80.
- Hatoum D, McGowan EM. Recent advances in the use of metformin: Can treating diabetes prevent breast cancer? *Biomed Res Int* 2015;2015:548436.
- Dowling RJO, Niraula S, Stambolic V. Metformin in cancer: Translational challenges. *J Mol Endocrinol* 2012;48:R31–43.
- The Diabetes Prevention Program Research Group. Long-term safety, tolerability, and weight loss associated with metformin in the diabetes prevention program outcomes study. *Diabetes Care* 2012;35:731–7.
- Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. *Ann Oncol* 2016;27:2184–95.
- Dowling RJO, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. *BMC Med* 2011;9:33.
- Fidan E, Onder Ersoz H, Yilmaz M, Yilmaz H, Kocak M, Karahan C, 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.
- Blandino G, Valerio M, Cioce M, Mori F, Casadei L, Pulito C, et al. Metformin elicits anticancer effects through the sequential modulation of DICER and c-MYC. *Nat Commun* 2012;3:865.
- Kourelis TV, Siegel RD. Metformin and cancer: new applications for an old drug. *Med Oncol* 2012;29:1314–27.
- Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. *Cell Metab* 2014;20:953–66.
- Lega IC, Austin PC, Gruneir A, Goodwin PJ, Rochon PA, Lipscombe LL. Association between metformin therapy and mortality after breast cancer: a population-based study. *Diabetes Care* 2013;36:3018–26.
- 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.
- Col NF, Ochs L, Springmann V, Aragaki AK, Chlebowski RT. Metformin and breast cancer risk: a meta-analysis and critical literature review. *Breast Cancer Res Treat* 2012;135:639–46.
- Xu H, Chen K, Jia X, Tian Y, Dai Y, Li D, et al. Metformin use is associated with better survival of breast cancer patients with diabetes: a meta-analysis. *Oncologist* 2015;20:1236–44.
- 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.
- Moradi-joo M, Mohabbat-bahar S, Heidari S, Davoodi SH, Sheyklo G, Akbari M. Metformin versus sulfonylurea in breast cancer risk of diabetic patients: a systematic review and meta-analysis. *Iran J Cancer Prev* 2016;9:e5971.
- Tang G, Satkunam M, Schünemann H, Steinberg G, Pond G, Muti P. Association of metformin with breast cancer incidence and mortality: a systematic review and meta-analysis. Available from: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.asp?ID=CRD42017054888](http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017054888).
- Higgins JPT, Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0. 2011. Available from: <http://training.cochrane.org/handbook>.
- Moher D, Liberati A, Tetzlaff J, Altman DG, Grp P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement (Reprinted from *Annals of Internal Medicine*). *Phys Ther* 2009;89:873–80.
- 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. 2016. Available from: [http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).
- Lo CK-L, Mertz D, Loeb M. Newcastle-Ottawa Scale: comparing reviewers' to authors' assessments. *BMC Med Res Methodol* 2014;14:1–5.
- Ahmed S, Shahid RK, Leis A, Haider K, Kanthan S, Reeder B, et al. Should noncurative resection of the primary tumour be performed in patients with stage iv colorectal cancer? A systematic review and meta-analysis. *Curr Oncol* 2013;20:e420–41.
- Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction - GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 2011;64:383–94.
- Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol* 2011;64:401–6.
- Jakobsson U, Westergren A. Statistical methods for assessing agreement for ordinal data. *Scand J Caring Sci* 2005;19:427–31.
- Altman DG. Inter-rater agreement. *Pract Stat Med Res* 1991;5:403–9.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Br Med J* 1997;315:629–34.
- Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. *Diabetes Care* 2012;35:2665–73.
- Currie CJ, Poole CD, Gale EAM. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009;52:1766–77.
- Hsieh M-C, Lee T-C, Cheng S-M, Tu S-T, Yen M-H, Tseng C-H, et al. The influence of type 2 diabetes and glucose-lowering therapies on cancer risk in the Taiwanese. *Exp Diabetes Res* 2012;2012:413782.
- Libby G, Donnelly LA, Donnan PT EA. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009;32:1620–5.
- Bosco JLF, Antonsen S, Sorensen HT, Pedersen L, Lash TL. Metformin and incident breast cancer among diabetic women: a population-based case-control study in Denmark. *Cancer Epidemiol Biomarkers Prev* 2011;20:101–11.



49. Morden NE, Liu SK, Smith J, Mackenzie TA, Skinner J, Korc M. Further exploration of the relationship between insulin glargine and incident cancer: a retrospective cohort study of older Medicare patients. *Diabetes Care* 2011;34:1965–71.
50. Chlebowski RT, McTiernan A, Wactawski-Wende J, Manson JE, Aragaki AK, Rohan T, et al. Diabetes, metformin, and breast cancer in postmenopausal women. *J Clin Oncol* 2012;30:2844–52.
51. Ruitter R, Visser LE, Van Herk-Sukel MPP, Coebergh JWW, 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.
52. Soffer D, Shi J, Chung J, Schottinger JE, Wallner LP, Chlebowski RT, et al. Metformin and breast and gynecological cancer risk among women with diabetes. *BMJ Open Diabetes Res Care* 2015;3:e000049.
53. Chen Y-C, Kok V, Chien C-H, Horng J-T, Tsai J. Cancer risk in patients aged 30 years and above with type 2 diabetes receiving antidiabetic monotherapy: a cohort study using metformin as the comparator. *Ther Clin Risk Manag* 2015;11:1315–23.
54. 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.
55. Calip GS, Yu O, Elmore JG, Boudreau DM. Comparative safety of diabetes medications and risk of incident invasive breast cancer: a population-based cohort study. *Cancer Causes Control* 2016;27:709–20.
56. He X, Esteva FJ, Ensor J, Hortobagyi GN, Lee MH, Yeung SCJ. Metformin and thiazolidinediones are associated with improved breast cancer-specific survival of diabetic women with HER2+ breast cancer. *Ann Oncol* 2012;23:1771–80.
57. Bayraktar S, Hernandez-Aya LF, Lei X, Meric-Bernstam F, Litton JK, Hsu L, et al. Effect of metformin on survival outcomes in diabetic patients with triple receptor-negative breast cancer. *Cancer* 2012;118:1202–11.
58. Peeters PJHL, Bazelier MT, Leufkens HGM, de Vries F, De Bruin ML. The risk of colorectal cancer in patients with type 2 diabetes: associations with treatment stage and obesity. *Diabetes Care* 2015;38:495–502.
59. El-Benhawy SA, El-Sheredy HG. Metformin and survival in diabetic patients with breast cancer. *J Egypt Public Health Assoc* 2014;89:148–53.
60. Oppong BA, Pharmed LA, Oskar S, Eaton A, Stempel M, Patil S, et al. The effect of metformin on breast cancer outcomes in patients with type 2 diabetes. *Cancer Med* 2014;3:1025–34.
61. Xiao Y, Zhang S, Hou G, Zhang X, Hao X, Zhang J. Clinical pathological characteristics and prognostic analysis of diabetic women with luminal subtype breast cancer. *Tumour Biol* 2014;35:2035–45.
62. Calip GS, Yu O, Hoskins KF, Boudreau DM. Associations between diabetes medication use and risk of second breast cancer events and mortality. *Cancer Causes Control* 2015;26:1065–77.
63. Kim HJ, Kwon H, Lee JW, Kim HJ, Lee SB, Park HS, et al. Metformin increases survival in hormone receptor-positive, HER2-positive breast cancer patients with diabetes. *Breast Cancer Res* 2015;17:64.
64. Xu H, Aldrich MC, Chen Q, Liu H, Peterson NB, Dai Q, et al. Validating drug repurposing signals using electronic health records: a case study of metformin associated with reduced cancer mortality. *J Am Med Informatics Assoc* 2014;22:179–91.
65. Vissers PAJ, Cardwell CR, van de Poll-Franse LV, Young IS, Pouwer F, Murray LJ. The association between glucose-lowering drug use and mortality among breast cancer patients with type 2 diabetes. *Breast Cancer Res Treat* 2015;150:427–37.
66. Farmer RE, Ford D, Forbes HJ, Chaturvedi N, Kaplan R, Smeeth L, et al. Metformin and cancer in type 2 diabetes: a systematic review and comprehensive bias evaluation. *Int J Epidemiol* 2016;46:745.
67. Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000;11:550–60.
68. Gandini S, Puntoni M, Heckman-Stoddard BM, Dunn BK, Ford L, DeCensi A, et al. Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev Res* 2014;7:867–85.
69. Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009;27:3297–302.
70. Morales DR, Morris AD. Metformin in cancer treatment and prevention. *Annu Rev Med* 2015;66:17–29.
71. Mei Z-B, Zhang Z-J, Liu C-Y, Liu Y, Cui A, Liang Z-L, et al. Survival benefits of metformin for colorectal cancer patients with diabetes: a systematic review and meta-analysis. *PLoS One* 2014;9:e91818.
72. Kim I, He Y-Y. Targeting the AMP-activated protein kinase for cancer prevention and therapy. *Front Oncol* 2013;3:1–12.
73. Yoshida S, Hong S, Suzuki T, Nada S, Mannan AM, Wang J, et al. Redox regulates mammalian target of rapamycin complex 1 (mTORC1) activity by modulating the TSC1/TSC2-Rheb GTPase pathway. *J Biol Chem* 2011;286:32651–60.
74. Muti P, Berrino F, Krogh V, Villarini A, Barba M, Strano S, et al. Metformin, diet and breast cancer: an avenue for chemoprevention. *Cell Cycle* 2009;8:2661.
75. Goodwin PJ, Stambolic V, Lemieux J, Chen BE, Parulekar WR, Gelmon KA, et al. Evaluation of metformin in early breast cancer: A modification of the traditional paradigm for clinical testing of anti-cancer agents. *Breast Cancer Res Treat* 2011;126:215–20.
76. Goodwin PJ, Parulekar WR, Gelmon KA, Shepherd LE, Ligibel JA, Hershman DL, et al. Effect of Metformin vs. Placebo on weight and metabolic factors in NCIC CTG MA.32. *J Natl Cancer Inst* 2015;107:1–8.