Research Article

Cancer Prevention Research

Metformin and Cancer Risk and Mortality: A Systematic Review and Meta-analysis Taking into Account Biases and Confounders

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Abstract

Previous meta-analyses have shown that the antidiabetic agent metformin is associated with reduced cancer incidence and mortality. However, this effect has not been consistently demonstrated in animal models and recent epidemiologic studies. We performed a meta-analysis with a focus on confounders and biases, including body mass index (BMI), study type, and time-related biases. We identified 71 articles published between January 1, 1966, and May 31, 2013, through Pubmed, ISI Web of Science (Science Citation Index Expanded), Embase, and the Cochrane library that were related to metformin and cancer incidence or mortality. Study characteristics and outcomes were abstracted for each study that met inclusion criteria. We included estimates from 47 independent studies and 65,540 cancer cases in patients with diabetes. Overall cancer incidence was reduced by 31% [summary relative risk (SRR), 0.69; 95% confidence interval (CI), 0.52–0.90], although between-study heterogeneity was considerable ($I^2 = 88\%$). Cancer mortality was reduced by 34% (SRR, 0.66; 95% CI, 0.54–0.81; $I^2 = 21\%$). BMI-adjusted studies and studies without time-related biases also showed significant reduction in cancer incidence (SRR, 0.82; 95% CI, 0.70–0.96 with $I^2 = 76\%$ and SRR, 0.90; 95% CI, 0.89–0.91 with $I^2 = 56\%$, respectively), albeit with lesser magnitude (18% and 10% reduction, respectively). However, studies of cancer mortality and individual organ sites did not consistently show significant reductions across all types of analyses. Although these associations may not be causal, our results show that metformin may reduce cancer incidence and mortality in patients with diabetes However, the reduction seems to be of modest magnitude and not affecting all populations equally. Clinical trials are needed to determine if these observations apply to nondiabetic populations and to specific organ sites. *Cancer Prev Res; 7(9); 867–85. ©2014 AACR.*

Introduction

The recognition that hyper-insulinemic states such as metabolic syndrome or type II diabetes mellitus are associated with increased cancer risk has led to intensified interest in the potential of various antidiabetic drugs to prevent cancer (1). Metformin, an oral, well-tolerated biguanide that is used for first-line treatment of diabetes, has been shown to decrease the progression from prediabetes to overt diabetes (2–4). Its multiple actions at both cellular and organismal levels that contribute to anticancer effects include decreased insulin/insulin-like growth factor-1 (IGF-1) signaling, inhibition of the mammalian target of rapamycin (mTOR), inhibition of mitochondrial complex I in the electron transport chain, activation of AMP-activated kinase (AMPK), and reduction of endogenous production of reactive oxygen species (ROS), and associated DNA damage (5).

The evidence for a cancer preventive effect for metformin, however, has not been consistently demonstrated in animal or human studies. Multiple studies examining the effect of metformin on tumor formation in rodent carcinogenesis

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models have shown results ranging from no effect to strong inhibition, albeit using doses that are not always achievable in humans (6–10). Epidemiologic studies comparing the incidence of cancer in diabetics using metformin with those using insulin or other antidiabetic agents have shown somewhat variable results (11–15). Several authors performed meta-analyses to determine if a consistent effect on overall cancer incidence, cancer mortality, or cancer incidence at specific target organs was evident (11–15). A shortcoming of these previous meta-analyses was the inability to identify subgroups that might benefit most (or suffer harm) from metformin. For instance, a recent clinical trial showed that metformin affected breast cancer proliferation differentially according to insulin resistance status and body mass index (BMI), with a trend toward inhibiting proliferation only in women with insulin resistance or high BMI (16). Furthermore, a number of the published studies suffered from time-related biases resulting in inappropriate comparison between metformin users versus nonusers and potentially exaggerated metformin's protective effects (17). Time-related bias in observational studies can produce illusory results in favor of metformin. They are most often a form of differential misclassification bias that can generally be avoided by appropriate accounting of follow-up time and exposure status in the design and analysis of such studies. There are different types of time-related biases. Immortal time bias refers to a period of cohort follow-up time during which a cancer event (that determines end of follow-up) cannot occur. Immortal time bias, for example, can arise when the period between cohort entry and date of first exposure to metformin, during which cancer has not occurred, is either misclassified or simply excluded and not accounted for in the analysis. This is frequently found in studies that compare "users" against "nonusers." The use of a time-dependent approach takes into account this source of bias. In cohort studies where a first-line therapy with metformin is compared with second- or third-line therapies, patients are unlikely to be at the same stage of diabetes, which can induce confounding of the association with an outcome (e.g., cancer incidence) by disease duration. An outcome related to the first-line therapy may also be attributed to the second-line therapy if it occurs after a long period of exposure. Such a situation requires matching on disease duration and consideration of latency time windows in the analysis (17).

Therefore, we performed a systematic review and meta-analysis with emphasis on studies controlling for BMI, prospective studies, and studies without time-related biases.

Materials and Methods

Literature search

The aim of the study was to evaluate the association between metformin use and cancer incidence and mortality in patients with diabetes. The meta-analysis was conducted in accordance with the guidelines for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) and the PRISMA statement (18, 19). The search was carried out on observational studies and trials, without language restrictions. The literature from January 1, 1966, to May 31, 2013, was searched using the following databases: Pubmed, ISI Web of Science (Science Citation Index Expanded), Embase, and the Cochrane library. The following main keywords or corresponding MeSH terms were used: "Metformin," "Biguanides," or "Diabetes Mellitus, Type 2/therapy," and "cancer" or "neoplasms." The search string used for Pubmed is the following: (Metformin and cancer) or ["Metformin"(Mesh) and "Neoplasms"(Mesh) and "epidemiologic studies"(Mesh)] not ["polycystic ovary syndrome" or "Polycystic Ovary Syndrome"(Mesh)]. A manual search was performed for references cited in the selected articles, and in selected reviews or books. This literature search was independently carried out by 2 academic investigators. Group discussion resolved any disagreement with article selection.

Methods of data extraction

Criteria for article inclusion in the analysis were: (i) independence from other studies in order to avoid giving double weight to estimates derived from the same study; when 2 or more studies were not independent, only the study with larger sample size was included; (ii) sufficient information to allow adequate estimation of the hazard ratio (HR)/relative risk (RR)/odds ratios (OR), and 95% confidence intervals (CI; i.e., crude data or adjusted estimates and standard errors, CIs, or *P* values); (iii) comparison of cancer incidence or mortality in patients with diabetes (comparisons with nondiabetic populations were excluded).

We extracted fully adjusted risk estimates for ever use of metformin, alone or in combination with other antidiabetic treatments, compared with antidiabetic treatments other than metformin or no treatment, and we calculated the corresponding variance using the formula proposed by Greenland (20). Association between metformin and cancer incidence/mortality across selected studies was computed as a summary relative risk (SRR) with 95% CIs.

Statistical analysis

Heterogeneity was evaluated using the I^2 parameter, which represents the percentage of total variation across studies that is attributable to heterogeneity rather than to chance. A threshold below 50% is generally considered acceptable (21). To account for possible sources of bias, we considered the STROBE checklist proposed for observational epidemiologic studies (22). Several sensitivity analyses were considered in this work, taking into account factors presented in the STROBE checklist that could introduce bias. Subgroup and sensitivity analyses and meta-regressions were carried out to investigate between-study heterogeneity and the influence of confounding factors, study design, interaction with other treatments, definitions of disease and population features on the risk estimates. A key factor considered was the adjustment for BMI, given its modifying effect on metformin activity on diabetes incidence (3) and breast cancer proliferation (16).

We also investigated heterogeneity because of study design because retrospective cohort studies could have important sources of bias. Sensitivity analyses were carried out to verify the effect of single studies and inclusion and exclusion criteria on the stability of the summary estimates, such as the use of insulin as treatment comparator. The SRR was estimated by pooling the study-specific estimates by random effects models fitted using SAS (Proc Mixed) with maximum likelihood estimates and CIs based on *t* distribution (23), to be conservative.

To take into account time-related biases that can occur in observational studies (17), we carried out subgroup analyses including only studies that were designed or analyzed to avoid immortal time bias, time-window bias, and time-lagging issues. The summary estimates were based only on studies that specifically used time-dependent techniques needed to avoid immortal time bias and to treat exposures to the different antidiabetic agents as time-dependent variables.

To verify whether publication bias might affect the validity of the estimates, funnel plots were investigated considering regression of Ln(RR) on the sample size, weighted by the inverse of the pooled variance (24). All analyses were performed with SAS software version 8.02 and STATA software version 11.

Results

Meta-analysis

The flow diagram for study inclusion in the meta-analysis is shown in Figure 1. A total of 71 articles were retrieved and checked for relevance in terms of intervention, population studied, and reporting of cancer incidence/mortality data. Twenty-four (25–48) articles were excluded (Supplementary Table S1). Because the UKPDS trials had partially overlapping patient populations, only the risk estimate for the metformin monotherapy trial was included (49).

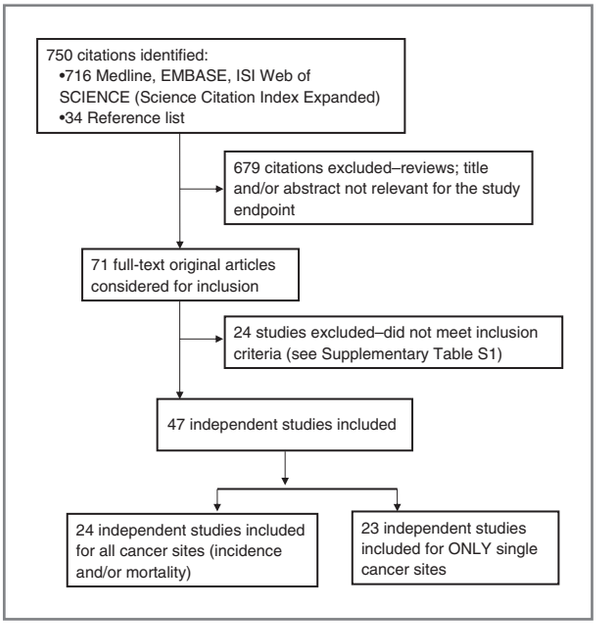

Figure 1. Study flow diagram. Of 750 citations identified, 47 independent studies were included in the analysis.

Overall we included estimates from 47 studies and 65,540 cancer cases: 19 studies (50–67) presented data on overall cancer incidence, 7 studies (38, 49, 54, 68–72) on overall cancer mortality, and 32 studies (45, 48, 50, 52–54, 56, 57, 59, 66, 73–96) reported estimates on single cancer sites. Table 1 shows the characteristics of these 47 studies. There were 11 prospective cohort studies, 16 case–control studies, 14 retrospective cohort studies, and 6 clinical trials of patients with diabetes randomized to metformin versus other treatment published between 1998 and 2013. Treatment comparators were sulfonylureas, insulin, or other antidiabetic treatments. If more than one estimate was presented, the estimate for metformin alone was preferred to metformin combined with other treatments and a comparator other than insulin was chosen.

We also examined SRRs stratified by BMI adjustment and time-related bias. For the latter analysis, 18 studies were judged to have avoided these biases (49, 51, 52, 55, 57, 61, 62, 64, 70, 71, 75, 77, 78, 80, 86, 87). However, the small number of studies may imply lack of robustness of the SRR estimates and where fewer than 3 studies were adjusted for BMI, the BMI-adjusted SRRs are not reported. Estimates from randomized clinical trials were considered to be adjusted for BMI.

Overall cancer incidence and mortality—effects of BMI and study type

The SRRs for metformin and overall cancer incidence (50–59) and mortality (45, 50–54, 56–59, 73–84, 86, 87) are shown in Table 2 and Fig. 2. A risk reduction of 31% (SRR, 0.69; 95% CI, 0.52–0.90), with high heterogeneity ($I^2 = 88\%$), was estimated for overall cancer incidence in subjects taking metformin compared with other antidiabetic compounds. There was a statistically significant, 34% reduction in cancer mortality (0.66, 95% CI, 0.54–0.81), with limited heterogeneity ($I^2 = 21\%$).

A significant reduction in overall cancer incidence in metformin users was also found when the estimates were adjusted for BMI (SRR, 0.82; 95% CI, 0.70–0.96; $I^2 = 76\%$), but not in BMI-unadjusted studies (SRR, 0.58 with 95% CI, 0.31–1.09 and $I^2 = 94\%$; $P = 0.49$ for the difference between estimates). However, no reduction was found when the analysis was restricted to prospective studies (SRR, 0.71; 95% CI, 0.47–1.07; $I^2 = 89\%$) or randomized clinical trials (SRR, 0.95; 95% CI, 0.69–1.30; $I^2 = 5\%$), although the latter studies included only 321 events. Meta-regression also indicates that publication year is not associated with risk estimates ($P = 0.59$), nor was there an association with the use of insulin treatment as comparator ($P = 0.89$).

The SRR for cancer mortality from BMI-adjusted results confirmed a significant reduction with metformin use (SRRs adjusted for BMI: 0.60; 95% CI, 0.45–0.80; $I^2 = 0$), whereas the reduction from unadjusted estimates was not significant (SRR, 0.75; 95% CI, 0.23–2.46; $I^2 = 71\%$). Analysis of prospective studies only showed a statistically significant

Table 1. Epidemiologic studies of metformin and cancer risk

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
UKPD Study Group (49) (UK)	RCT	Mortality	Cases: 139 At risk: 753	Any site: 0.71 (0.29–1.76)[a]	Diet alone ($n = 411$) vs. intensive blood-glucose control policy with metformin ($n = 342$)	n.a.
Schernthaner (61,72) QUARTET M (Europe)	RCT	Incidence	Cases: 9 At risk: 1,194	Any site: 0.51 (0.14–1.90)	Metformin monotherapy ($n = 597$) vs. pioglitazone ($n = 597$)	n.a.
Hanefeld (62,72) QUARTET C (Europe and North America)	RCT	Incidence	Cases: 9 At risk: 639	Any site: 1.99 (0.43–12.32)	Metformin + sulfonylurea ($n = 320$) vs. pioglitazone + sulfonylurea ($n = 319$)	n.a.
Yang (85) (UK)	General practice nested case–control in a retrospective cohort	Incidence	Cases: 125 Controls: 1,195	Colon: 1.00 (0.60–1.70)	3 or more years of metformin therapy vs. noninsulin users	Smoking, history of cholecystectomy, diabetes duration, BMI, sulfonylurea use, aspirin/NSAID use
Bowker (69) (Canada)	Population-based retrospective cohort	Mortality	Cases: 407 At risk: 10,309	Any site: 0.77 (0.63–0.91)	Metformin vs. sulfonylureas use	Insulin use and CDS.
Monami (55) (Italy)	Hospital-based retrospective case–control study	Incidence	Cases: 195 Controls: 195	Any site: 0.28 (0.13–0.57)	Exposure to metformin for more than 36 months vs. other hypoglycemic drugs users	Duration of diabetes, BMI, HbA1c, comorbidity, smoking and alcohol abuse, concomitant hypoglycemic treatment
Oliveria (76) (USA)	Population-based retrospective cohort	Incidence	Cases: 813 At risk: 191,223	Colon: 0.67 (0.52–0.85) Bladder: 0.99 (0.70–1.39) Liver: 0.73 (0.34–1.56) Pancreas: 1.26 (0.80–1.99)	Ever use of metformin monotherapy vs. never use	HBV and HCV infection, cirrhosis, alcoholism, polyps, obesity, ulcerative colitis, Crohn's disease, cystic fibrosis, chronic pancreatitis, dermatomyositis, polymyositis, idiopathic DVT, partial gastrectomy, pelvic radiation, and schistosomiasis.

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Currie (59) (UK)	General practice retrospective cohort	Incidence	Cases: 373 At risk: 7,897	Any site: 0.74 (0.65–0.84); Breast: 1.02 (0.71–1.45); Colon: 0.56 (0.40–0.76); Prostate: 0.93 (0.67–1.32); Pancreas: 0.20 (0.11–0.36)	Metformin monotherapy vs. sulfonylureas monotherapy	Smoking, comorbidity, HbA1c, diabetes duration, weight
Donadon (80) (Italy)	Hospital-based retrospective case–control	Incidence	Cases: 465 Controls: 490	Liver: 0.33 (0.10–0.70)	Metformin users vs. nonusers	No adjusting variables were considered
Home (52) RECORD, (Europe)	RCT	Incidence	Cases: 125 At risk: 2,225	Any site: 1.22 (0.86–1.74) Breast: 1.0 (0.32–3.10)[b] Prostate: 2.0 (0.68–5.82)[b] Pancreas: 5.99 (0.72–49.6)[b] Liver: 4.0 (0.18–88.7)[b]	Metformin ($n = 1,122$) vs. rosiglitazone ($n = 1,103$)	n.a.
Li (86) (USA)	Hospital-based retrospective case–control	Incidence	Cases: 255 Controls: 106	Pancreas: 0.38 (0.22–0.69)	Metformin users vs. nonusers	Race, smoking, alcohol, BMI, family history of cancer, duration of diabetes, and insulin use.
Libby (54) (Scotland, UK)	Population-based retrospective cohort	Incidence and mortality	Cases: 771 At risk: 8,170	Any site incidence: 0.63 (0.53–0.75); Breast: 0.60 (0.32–1.10); Colon: 0.60 (0.38–0.94); Lung: 0.70 (0.43–1.15) Any site mortality: 0.63 (0.49–0.81)	Metformin users vs. nonusers	Smoking, BMI, HbA1c, material deprivation, other drug use (sulfonylureas or insulin)
Wright (79) (USA)	Population-based retrospective case–control	Incidence	Cases: 97 Controls: 101	Prostate: 0.56 (0.32–1.00)	Metformin users vs. nonusers	BMI, statin and aspirin use, other diabetes treatment, PSA screening history, family history of prostate cancer

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Bodmer (73) (UK)	General practice retrospective nested case–control	Incidence	Cases: 17 Controls: 120	Breast: 0.44 (0.24–0.82)	Users of 40+ prescriptions (>5 years) of metformin vs. nonusers[c]	General practice and calendar time by matching, other use of prandial glucose regulators, acarbose, estrogens, smoking, BMI, diabetes duration, and HbA1c
Hassan (81) (USA)	Hospital-based retrospective case–control	Incidence	Cases: 122 Controls: 86	Liver: 0.30 (0.20–0.60)	Biguanide users vs. nonusers	Race, educational level, cigarette smoking, alcohol drinking, HCV, HBV, family history of cancer
Kahn (63) ADOPT (USA)	RCT	Incidence	Cases: 160 At risk: 4,351	Any site: ADOPT-G: 0.78 (0.53–1.14) ADOPT-R: 0.92 (0.63–1.35)Breast: 2.0 (0.60–6.62)[b] Colon: 1.75 (0.51–5.96)[b] Prostate: 1.0 (0.41–2.40)[b] Pancreas: 0.1 (0.005–1.84)[b]	Metformin ($n = 1,454$) vs. glibencamide ($n = 1,441$) vs. rosiglitazone ($n = 1,456$)	n.a.
Landman (70) (Netherlands)	General practice prospective cohort	Mortality	Cases: 122 At risk: 1,353	Any site: 0.43 (0.23–0.80)	Metformin users vs. nonusers	Smoking, diabetes duration, HbA1c, serum creatinine, BMI, blood pressure, total cholesterol/HDL, albuminuria, insulin use, sulfonylurea use and macrovascular complications
Williams-Herman (64) (18 countries worldwide)	RCT	Incidence	Cases: 18 At risk: 543	Any site: 0.61 (0.22–1.79)	Metformin ($n = 364$) vs. Sitagliptin ($n = 179$)	n.a.
Yang (58) (China)	Hospital-based prospective cohort	Incidence	Cases: 271 At risk: 6,103	Any site: 0.99 (0.70, 1.41)	Sulfonylurea use + ever use of metformin vs. never use of metformin	BMI, smoking status, alcohol, HbA1c, SBP, LDL-C–related risk, HDL and triglyceride, statins, RAS inhibitor usage, insulin usage

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Azoulay (77) (Canada)	Population-based retrospective nested case–control	Incidence	Cases: 739 Controls: 7,359	Prostate: 1.23 (0.99–1.52)	Ever vs. never users of metformin[c]	HbA1c, alcohol use, obesity, smoking, lower urinary tract symptoms, previous cancer, previous use of NSAID, antihypertensive drugs, and statins, use of other antidiabetic agents
Baur (65) (Germany)	Hospital-based prospective cohort	Incidence and mortality	Cases: 66 At risk: 1,308	Any site incidence: 0.66 (0.26–1.64)Any site mortality: 0.71 (0.2–2.59)	Metformin users vs. nonusers	Smoking, BMI, HbA1c
Bosco (74) (Denmark)	Population-based retrospective nested case–control	Incidence	Cases: 393 Controls: 3,930	Breast: 0.81 (0.63–0.96)	Metformin for at least 1 year vs. women not prescribed antidiabetic medication, or used metformin for at least 1 year	Diabetes complications, clinical obesity year of birth, parity, postmenopausal hormone use.
Ferrara (75) (USA)	Population-based prospective cohort	Incidence	Cases: 9,082 At risk: 252,467	Breast: 0.90 (0.80–1.00); Colon: 1.00 (0.90–1.20); Prostate: 1.00 (0.90–1.10); Pancreas: 1.20 (1.00–1.50); Lung: 1.00 (0.80–1.10); NHL: 1.00 (0.80–1.20); Corpus Uteri: 0.90 (0.80–1.20); Kidney/ renal pelvis: 1.30 (1.0–1.6); Rectum: 0.90 (0.70–1.20); Melanoma: 0.80 (0.60–1.10)	Ever use of pioglitazone and metformin vs. never use of metformin	Year of cohort entry, race/ethnicity, income, smoking, glycemic control, diabetes duration, creatinine levels, congestive heart failure, use of other diabetes medications
Hense (51) (Germany)	Population-based prospective cohort	Incidence	Cases: 1,364 At risk: 26,742	Any site: 0.95 (0.90–1.01)	Metformin (only) users vs. nonusers	Diabetes duration, BMI, insulin therapy
Lai (84) (Taiwan)	Population-based retrospective cohort	Incidence	Cases: 129 At risk: 19,624	Lung: 0.55 (0.37–0.82)	Metformin users vs. nonusers	Pulmonary tuberculosis, chronic obstructive pulmonary disease, and propensity score (quintile).

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Lee (53) (South Korea)	Population-based prospective cohort	Incidence	Cases: 339 At risk: 15,717	Any site: 0.12 (0.08–0.19); Colon: 0.36 (0.13–0.98); Liver: 0.06 (0.02–0.16); Esophagus: 0.44 (0.07–2.61); Stomach: 1.41 (0.42–4.73)	At least 2 prescription of metformin vs. any other oral antihyperglycemic medication	Other oral antihyperglycemic medication, Charlson comorbidity index score, metformin dosage and duration
Mellbin (71) (Sweden)	Prospective cohort follow-up analysis from RCT	Mortality	$N = 1{,}073$ N events $= 37$	Any site: 0.25 (0.08–0.83)	Patients using metformin vs. not using at discharge	Smoking habits, previous myocardial infarction or previous congestive heart failure, creatinine at randomization, percutaneous transluminal coronary angioplasty or coronary artery bypass grafting during the hospitalization, and mean updated blood glucose
Morden (56) (USA)	General practice retrospective cohort	Incidence	Cases: 5,466 At risk: 81,681	Any site: 1.01 (0.94–1.08); Breast: 1.28 (1.05–1.57); Colon: 0.94 (0.72–1.22); Prostate: 0.97 (0.76–1.24); Pancreas: 1.25 (0.89–1.75)	Metformin vs. not in insulin-treated patients	Race, low-income subsidy status, comorbidities, tobacco exposure, Charlson, comorbidities excluding malignancy, diabetes, insulin dose quartiles
Bo (68) (Italy)	Hospital-based retrospective cohort	Mortality	Cases: 122 At risk: 3,703	Any site: 0.56 (0.34–0.94)	Metformin use vs. diet control only	Diabetes duration, HbA1c, smoking, BMI, presence of retinopathy, nephropathy, coronary or peripheral artery disease, other co-morbidities and the use of antihypertensive drugs and acetylsalicylic acid

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Bodmer (83) (UK)	General practice retrospective nested case–control	Incidence	Cases: 920 Controls: 5,519	Colon: 1.43 (1.08–1.90)	Metformin users (50+ prescriptions) vs. nonusers	Diabetes duration, BMI, smoking, prior use of aspirin, NSAID, statins, estrogen use (women), sulfonylureas and insulin use
Bodmer (82) (UK)	General practice retrospective case–control	Incidence	Cases: 307 Controls: 1,347	Pancreas: 0.83 (0.57–1.21)	Metformin users vs. nonusers	BMI, smoking, alcohol consumption, diabetes duration, other antidiabetics drugs
Bodmer (88) (UK)	General practice retrospective case–control	Incidence	Cases: 1.029 Controls: 6,174	Lung: 1.09 (0.85–1.38)	Metformin users (40+ prescriptions) vs. nonusers	BMI and smoking
Chlebowski (48,95) (USA)	Prospective Cohort (WHI program)	Incidence	Cases: 233 At risk: 68,019	Breast: 0.65 (0.46–0.91)	Metformin vs. other antidiabetic drugs	Family history, prior breast biopsy, age at menarche, menopause, parity, age at first live birth, breastfeeding, education, smoking, alcohol use, BMI, physical activity, duration of prior estrogen alone, estrogen + progesterone use, bilateral oophorectomy, weight loss
Hsieh (89) (Taiwan)	Population-based prospective cohort	Incidence	Cases: 6.554 At risk: 61,777	Any site: 0.56 (0.44–0.71) Breast: 0.57 (0.33–0.97) Colon: 0.54 (0.39–0.76) Prostate: 0.97 (0.60–1.55) Lung: 0.64 (0.45–0.90)Liver: 0.66 (0.49–0.91) Pancreas: 0.63 (0.28–1.42) Stomach: 0.63 (0.39–1.08)	Metformin vs. sulfonylurea	Only age and sex

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Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Lehman (78) (USA)	Population-based retrospective cohort	Incidence	Cases: 360 At risk: 5,042	Prostate: 2.15 (1.83–2.52)	Metformin versus sulfonylurea only (restricted to nonstatin users)[c]	HbA1c, diabetes duration, race/ethnicity, Charlson comorbidity score
Liao (91) (Taiwan)	Population-based prospective cohort	Incidence	Cases: 56 At risk: 49,803	Pancreas: 0.85 (0.39–1.89)	Metformin users vs. nonusers	No adjusting variables were considered
Magliano (66) (Australia)	Community-based longitudinal cohort	Incidence	Cases: 309 At risk: 1,294	Any site: 0.88 (0.67–1.17); Prostate: 2.16 (1.19–3.9)	Metformin users vs. nonusers	No adjusting variables were considered
Mazzone (94) (USA)	Hospital-based retrospective case–control	Incidence	Cases: 507 Controls: 507	Lung: 0.48 (0.28–0.81)	Metformin users vs. nonusers	Medication use, BMI, HbA1C, smoking
Ngwana (50) (Belgium)	General practice retrospective cohort	Incidence	Cases: 221 At risk: 4,012	Any site: 0.20 (0.03–1.64); Breast: 0.46 (0.07–3.10); Colon: 0.11 (0.01–1.07); Prostate: 0.61 (0.31–1.19)	Metformin vs. other antidiabetic treatments and diet only	Weight and initial HbA1c
Redaniel (90) (UK)	General practice retrospective cohort	Incidence	Cases: 873 At risk: 52,657	Breast: 1.02 (0.79–1.3)	Metformin vs. sulfonylurea	Period, region, BMI, year of diagnosis
Ruiter (57) (Netherlands)	Hospital-based prospective cohort	Incidence	Cases: 3,552 At risk: 85,289	Any site: 0.90 (0.88–0.91); Breast: 0.95 (0.91–0.98); Colon: 0.91 (0.88–0.94); Prostate: 0.92 (0.88–0.94); Pancreas: 0.73 (0.66–0.80); Liver: 0.67 (0.53–0.86); Lung: 0.87 (0.84–0.91); Esophagus: 0.90 (0.82–0.97); Stomach: 0.83 (0.76–0.90)	Metformin vs. sulfonylurea derivatives[c]	Age at first oral glucose-lowering drug prescription, number of other drugs used in the year before the start of OGLD, number of hospitalizations in the year before the start of OGLD, calendar time
Becker (96) (UK)	General practice retrospective case–control	Incidence	Cases: 291 Controls: 1,746	Endometrial: 0.88 (0.58–1.32)	Metformin users (25+ prescriptions) vs. no prior use	BMI, smoking, diabetes duration
Chaiteerakij et al. (93) (USA)	Hospital-based retrospective case–control	Incidence	Cases: 105 Controls: 34	Liver: 0.4 (0.2–0.9)	Metformin users vs. nonusers	Ethnicity, and residential area, propensity scores for statin-use

(Continued on the following page)

Table 1. Epidemiologic studies of metformin and cancer risk (Cont'd)

First author (ref.) (country)	Study design	Endpoint	Sample size	Risk estimates (95% CI)	Treatment comparison	Adjusting variables (other than age and sex)
Chen (92) (Taiwan)	Population-based retrospective case–control	Incidence	Cases: 22,047 Controls: 25,773	Liver: 0.79 (0.75– 0.83)	Metformin users vs. nonusers	Cirrhosis, HCV, DM duration, comorbidities, other medications
Chung (67) (South Korea)	Population-based retrospective cohort	Incidence	Cases: 73 At risk: 1,217	Any site: 0.57 (0.39–0.85)	Metformin users vs. nonusers	Not specified
Currie (60) (UK)	General practice retrospective cohort	Incidence	Cases: 4,029 At risk: 84,622	Any site: 0.91 (0.83–1.00)	Metformin vs. sulfonylurea	Systolic blood pressure, HbA1c, total cholesterol, serum creatinine, BMI, smoking status, antihypertensive lipid-lowering, antiplatelet therapy, duration of diabetes, prior history of cancer, LVD, microvascular disease, number of contacts with the general practitioner in the year before the index date, Charlson comorbidity index
Smiechowski (87) (Canada)	Population-based prospective nested case–control	Incidence	Cases: 808 Controls:7,764	Lung: 0.94 (0.76–1.17)	Metformin users vs. nonusers	Diabetes duration, HbA1c, obesity, smoking, excessive alcohol use, previous cancer, chronic obstructive pulmonary disease, asthma, nonsteroidal anti-inflammatory drugs, aspirin, statins, and other antidiabetic drugs

Abbreviations: ADOPT, a diabetes outcome progression trial; %CI, percent CI; CDS, chronic disease score; DVT, deep vein thrombosis; HbA1c, hemoglobin A1c; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; *N*, number; NSAID, nonsteroidal anti-inflammatory drug; OGLD, oral glucose lowering drugs; PSA, prostate-specific antigen; RAS, renin–angiotensin system; RCT, randomized controlled trial; RECORD, rosiglitazone evaluated for cardiac outcomes and regulation of glycaemia in diabetes; SBP, systolic blood pressure; UKPDS, United Kingdom Prospective Diabetes Study.

[a]Risk estimate for users of metformin alone.

[b]ADOPT-G and ADOPT-R, glibenclamide and rosiglitazone arms of ADOPT study. Risk estimates represent multiple comparisons from a single trial, and the analysis takes account of correlation between these comparisons; risk estimates for single cancer sites were calculated from crude data.

[c]Excluded patients on monotherapy with insulin.

Table 2. Summary risk estimates (SRRs) and 95% CIs for all endpoints

Endpoints	Groups	SRR (95% CI)	I^2	Number of studies[a]
Cancer incidence	All studies	0.69 (0.52–0.90)	88	19
	Adjusted for BMI	0.82 (0.70–0.96)	76	11
	Adjusted for time-related bias	0.90 (0.89–0.91)	56	8
	Prospective studies	0.71 (0.47–1.07)	89	12
	Randomized clinical trials	0.95 (0.69–1.30)	5	5
Cancer mortality	All studies	0.66 (0.54–0.81)	21	7
	Adjusted for BMI	0.60 (0.45–0.80)	0	5
	Adjusted for time-related bias	0.45 (0.16–1.26)	0	3
	Prospective studies	0.48 (0.23–0.97)	0	4
Single cancer sites				
Breast	All studies	0.88 (0.75–1.03)	60	13
	Adjusted for BMI	0.82 (0.67–1.00)	48	7
	Adjusted for time-related bias	0.94 (0.90–0.99)	32	6
	Prospective studies	0.94 (0.90–0.99)	44	7
Colon	All studies	0.80 (0.64–1.00)	76	12
	Adjusted for BMI	0.84 (0.50–1.40)	81	6
	Adjusted for time-related bias	0.92 (0.85–0.98)	24	3
	Prospective studies	0.82 (0.57–1.17)	74	5
Prostate	All studies	1.06 (0.80–1.41)	91	12
	Adjusted for BMI	0.98 (0.68–1.40)	55	6
	Adjusted for time-related bias	1.25 (0.87–1.80)	96	6
	Prospective studies	0.93 (0.89–0.97)	59	6
Pancreas	All studies	0.75 (0.49–1.15)	84	11
	Adjusted for time-related bias	0.48 (0.16–1.43)	83	5
	Time-related unbiased	0.77 (0.38–1.55)	40	5
	Prospective studies	0.89 (0.61–1.29)	80	6
Liver	All studies	0.47 (0.28–0.79)	82	9
	Adjusted for time-related bias	0.65 (0.39–1.08)	38	3
	Prospective studies	0.78 (0.72–0.85)	52	5
Lung	All studies	0.82 (0.67–0.99)	57	5
	Adjusted for smoking	0.95 (0.82–1.11)	57	5
	Adjusted for time-related bias	0.88 (0.81–0.95)	36	3
	Prospective studies	0.97 (0.69–1.35)	26	3

[a]Estimates may not correspond to number of studies.

reduction with metformin, in contrast to the effect seen on cancer incidence (SRR, 0.48; 95% CI, 0.23–0.97; $I^2 = 0$).

Organ specific analyses—effects of BMI and study type on cancer incidence

The SRR estimates for breasts, prostate, colon, pancreas, liver, and lungs are illustrated in Fig. 3 and Table 2. The risk reduction with metformin use in unadjusted analyses reached statistical significance only for liver (9 studies, SRR, 0.47; 95% CI, 0.28–0.78; $I^2 = 82\%$) and lung cancer (8 studies, SRR, 0.82; 95% CI, 0.67–0.99; $I^2 = 66\%$; Table 2). Analysis of prospective studies confirmed this association for liver but not lung cancer. Too few liver or lung cancer studies were available to address the effect of BMI. Most notably, the summary estimate for lung cancer adjusted for smoking showed no significant association (SRR, 0.95; 95% CI, 0.82–1.11; $I^2 = 58\%$).

The meta-analysis of the 13 studies on breast cancer risk showed a nonsignificant trend (SRR, 0.88; 95% CI, 0.75–1.03; $I^2 = 60\%$). However, BMI adjustment showed borderline significance in 5 studies (SRR, 0.82; 95% CI, 0.67–1.00; $I^2 = 48\%$). Analysis of 7 prospective studies showed statistical significance (SRR, 0.94; 95% CI, 0.90–0.99; $I^2 = 44$). Metformin treatment and prostate cancer risk did not show any association in 12 studies (SRR, 1.06; 95% CI, 0.80–1.41; $I^2 = 91\%$), even upon BMI adjustment. However, in the subgroup of 6 prospective studies, the reduction became significant (SRR, 0.93; 95% CI, 0.89–0.97; $I^2 = 59\%$), albeit with low magnitude. For colon cancer, the SRR suggested borderline significant risk reduction (12 studies, SRR, 0.80, 95% CI, 0.64–1.00; $I^2 = 76\%$). The SRRs from subgroups of studies adjusted for BMI and with prospective designs did not suggest a significant reduction in cancer risk. No risk reduction was found for metformin use in

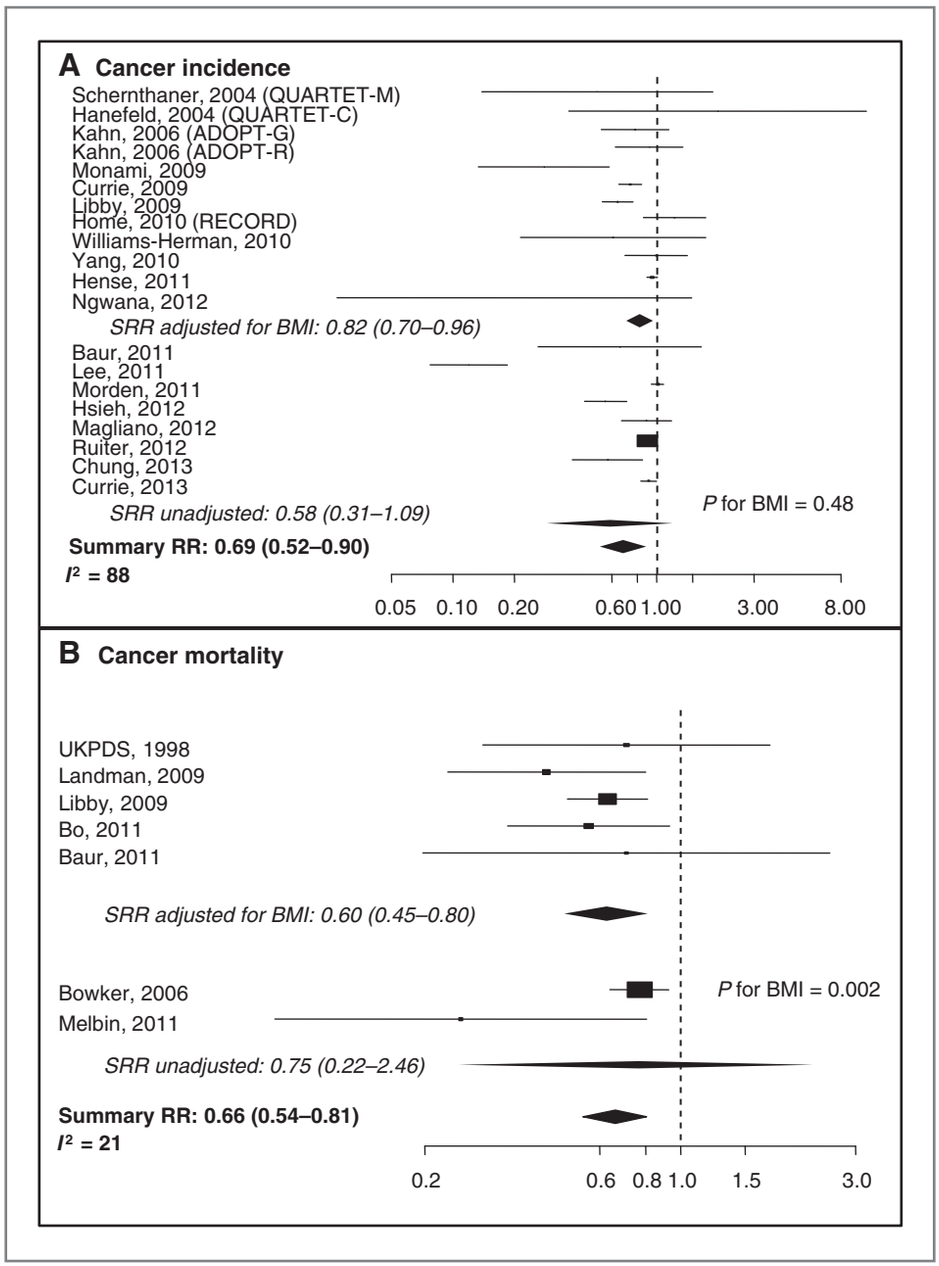

Figure 2. Forest plot of the association between metformin and cancer incidence or cancer mortality. Forest plots of risk estimates from observational studies and randomized controlled trials of metformin use and cancer incidence (A) or cancer mortality (B). Black boxes indicate HRs, and horizontal lines represent 95% CIs. Black diamonds, SRR estimates. The vertical dotted line represents a risk estimate of 1.00.

pancreatic cancer (SRR, 0.75; 95% CI, 0.49–1.15; $I^2 = 84\%$) even after BMI adjustment or when the analysis was limited to 6 prospective studies (SRR, 0.89; 95% CI, 0.61–1.29; $I^2 = 80\%$).

We also evaluated the effect of the BMI adjustment within studies (not only between studies) when the data were available. For 12 observational studies, we were able to extract risk estimates adjusted for BMI (or a proxy such as obesity) and crude estimates in order to measure the size of this confounding (Supplementary Table S2). Overall, the data show similar RR estimates between fully adjusted and crude RR estimates, suggesting limited confounding effect.

Summary risk estimates for individual organs were obtained only for breast cancer, for which we had at least 4 studies. SRRs were very similar: 0.79 (0.54, 1.16) and 0.72 (0.48, 1.07) for adjusted and unadjusted estimates, respectively.

These analyses focused on patients with diabetes. In some studies, the diagnosis of diabetes was not verified and the comparator was "antidiabetic drug users" (57, 69, 73, 82, 83). A sensitivity analysis excluding those studies did not modify the results except for colorectal cancer, which became statistically significant (SRR, 0.73; 95% CI, 0.58–0.92) after excluding the paper by Ruiter and colleagues (83). When the potential bias because of insulin

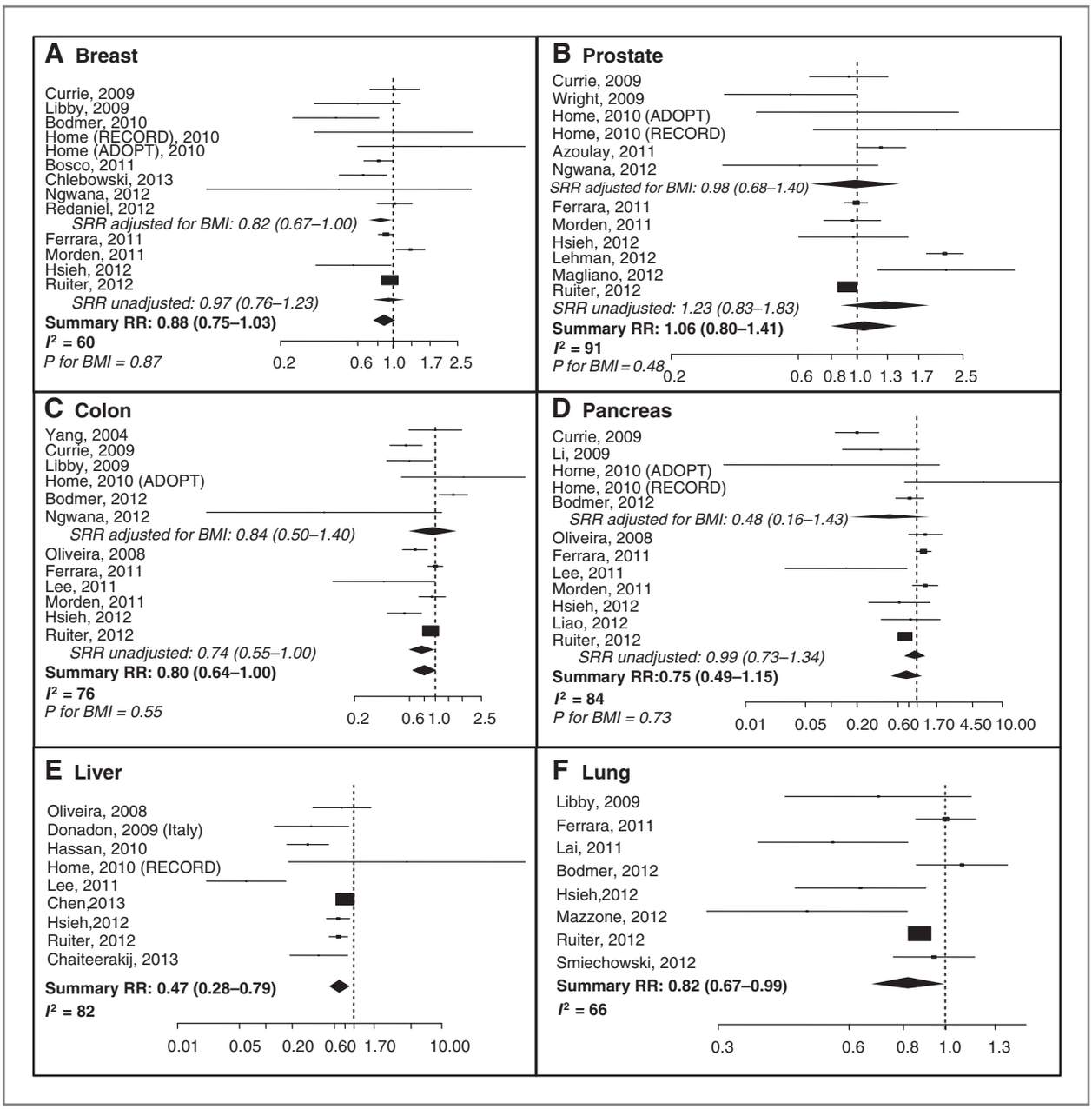

Figure 3. Forest plots of the association between metformin use and individual site cancer incidence. Forest plots of risk estimates from observational studies and randomized controlled trials of metformin use and breast cancer (A), prostate cancer (B), colon cancer (C), pancreas cancer (D), liver cancer (E), and lung cancer (F). Black boxes indicate HRs, and horizontal lines represent 95% CIs. Black diamonds, SRR estimates. The vertical dotted line represents a risk estimate of 1.00. *P* for BMI is the *P* value for the interaction between BMI-adjusted analysis and unadjusted analysis.

treatment as comparator was taken into account, the conclusions did not change. No indication for publication bias was found for any of the summary estimates.

Analysis of studies without time-related biases

The SRRs for overall cancer incidence, organ-specific cancer incidence, and overall cancer mortality obtained from analysis of studies that avoided time-related biases are shown in Table 2. The SRR for overall cancer incidence was statistically significant in 8 studies (SRR, 0.90; 95% CI, 0.89–0.91; $I^2 = 56\%$). The SRR for breast and colorectal cancer also became statistically significant: SRR, 0.94 (95% CI, 0.90–0.99; $I^2 = 32\%$) and SRR, 0.92 (95% CI, 0.85–0.98; $I^2 = 24\%$), respectively. On the other hand, the risk reduction for overall cancer mortality and liver cancer incidence lost statistical significance (SRR, 0.45; 95% CI, 0.16–1.26 and SRR, 0.65; 95% CI, 0.39–1.08, respectively). For lung cancer, the SRR

suggested significant risk reduction, but adjustment for smoking eliminated the effect.

When only studies without time-related biases and adjusted for BMI were analyzed, the SRR for overall cancer incidence and breast cancer lost significance: SRR, 0.94 (95% CI, 0.88–1.01) and SRR = 0.89 (95% CI, 0.56–1.41), respectively. These numbers, however, were small.

Discussion

Research on metformin use and cancer risk and mortality has expanded considerably over recent years, with conflicting data arising from different epidemiologic, human, and animal carcinogenesis studies. Several previous meta-analyses have concluded that patients with diabetes who use metformin have significantly lower risk of overall cancer incidence (30%–40%), mortality, and site-specific cancer incidence than those who use other antidiabetic medications (11–14). However, the studies included in these meta-analyses are susceptible to several confounders and biases. Here we focused for the first time on 2 critical issues with potential to skew the literature, the effect of BMI, and time-related biases in observational studies. The main results from our study show that metformin use is associated with decreased overall cancer incidence even after adjustment for BMI or time-related biases, but the magnitude of this effect is considerably smaller than observed without such adjustments (10%–18% vs. 31%). Simultaneous adjustment for both BMI and time-related biases results in loss of statistical significance, albeit based on few studies. This is reminiscent of results from Thakkar and colleagues, who showed a diminution in metformin's effect when considering cohort studies (30%) versus case–control studies (10%) versus randomized controlled trials (no effect; ref. 14). Examination of individual organ sites, which is limited by fewer available studies for analysis, shows nonsignificant associations or similarly smaller effects after adjustment. Taken together, these data underscore the importance of understanding the limitation in the current literature and suggest that if metformin use is associated with a reduced risk of cancer, the effect may be smaller than previously shown.

Obesity and its surrogate, high BMI, are intimately linked to increased risk of several cancer types (97, 98). Potential mechanisms include both direct and indirect effects of obesity on insulin, IGF-1, sex hormones, adipokines, and inflammation, many of which are directly impacted by metformin. In our analysis, BMI-adjusted studies showed statistically significant reduction in cancer incidence and mortality whereas unadjusted studies showed no effect. In 12 prospective studies where it was possible to compare BMI-adjusted versus crude estimates within each study, similar RR estimates were noted, suggesting limited confounding effect of BMI. Likewise, summary risk estimates within 4 breast cancer studies were similar. BMI adjustment did not significantly affect the cancer risk estimates for individual organ sites, although the risk estimates for breast cancer became borderline significant. A direct correlation between BMI and inflammation, adipocyte size, and aromatase expression has been shown in breast tissue from women undergoing breast cancer surgery, pointing to inflammation as a potential biologic basis for the cancer–obesity connection (99). However, BMI and insulin resistance had a modifying effect on the metformin modulation of breast cancer cell proliferation in a presurgical trial (16). Furthermore, metformin is the drug of choice in obese patients with diabetes because it reduces weight (3, 100), so its use is associated with obesity. Thus, modification of the cancer–obesity relationship by metformin is likely complex and requires extensive study.

A recent review by Suissa and Azoulay underscored the prevalence of time-related biases in observational studies, potentially leading to inflated estimates of the protective effect of metformin (17). These biases include immortal time bias (unexposed time is misclassified as drug-exposed time), time-window bias (differential exposure opportunity time windows between exposed and unexposed subjects), and time-lag bias (comparison of treatment given during different stages of the disease). Of note, exclusion of time-biased studies from our analysis resulted in statistically significant 10% risk reduction in overall cancer incidence, although the magnitude is substantially smaller than the previously reported 30% to 40%. In organ-specific analyses, reduction in colorectal cancer incidence became significant (8%), whereas liver cancer risk reduction became nonsignificant. Exclusion of time-biased studies in the analysis of cancer mortality resulted in loss of statistical significance.

The effect of metformin use on cancer mortality may result from different mechanisms than the effect on incidence. Retrospective analyses suggest that diabetics treated with metformin during chemotherapy have better survival than those treated with other antidiabetic agents (28, 101). Interestingly, mouse xenograft models show that metformin targets breast cancer stem cells and synergizes with doxorubicin to prevent relapse (102). If metformin increases the effectiveness of chemotherapy, then its inclusion in chemotherapeutic regimens may exert a favorable impact on survival.

This study has several limitations. These include heterogeneity of study designs and treatment comparators. More than two thirds of the studies had a retrospective design, which is prone to important sources of bias. However, our analyses of prospective studies generally found similar SRRs, although for breast, liver, and prostate cancer, these results became statistically significant. A second limitation is the nature of the comparator group, which mainly included treatment with insulin and insulin secreatagogues. These classes of agents increase insulin levels and have been associated with increased cancer risk (14, 55, 69, 103). Thus, the potential protective effect of metformin in an untreated or noninsulin using population cannot be precisely estimated. A third factor to consider is allocation bias, with metformin users being at different stage of diabetes than comparators, as discussed previously with regard to time-lag bias. Generally, treatment with metformin starts at a younger age, likely because of treatment guidelines (104) and in subjects with higher BMI, possibly because of its

weight lowering effects (105). Although the majority of studies adjusted for confounders such as age, we here presented the analyses adjusting for BMI and excluding time-biased studies. However, BMI is dynamic, and weight gain is an important risk factor for mortality of several cancers (106). Therefore, adjustment for a single BMI value might be inadequate to account for confounding by BMI dynamics over time. Finally, the effect of other confounders, both known (but not adjusted for) or heretofore unrecognized, should not be underestimated. This is best illustrated by lung cancer, where overall and time-unbiased analyses point to a protective effect, whereas adjustment for smoking, which is by far the most important cause of lung cancer, leads to loss of significance.

A critical question emerging from this meta-analysis of studies in diabetic patients is the generalizability to nondiabetic populations. Our data demonstrate a cancer preventive signal, albeit of lesser magnitude once the appropriate adjustments are made than previously reported. This signal now needs to be studied in controlled clinical trials focusing on carefully defined populations, such as the prediabetic population in the Diabetes Prevention Program Trial (3, 4), for which long-term follow-up to ascertain the effects of metformin on cancer incidence is currently ongoing. However, it needs to be emphasized that existing data about metformin use in nondiabetic populations are severely limited.

Clinical trials are needed to determine if the observations seen in diabetic populations can be expanded to prediabetic or nondiabetic populations and to whom they should be expanded for the best benefit/risk ratio. Although some of these early phase trials are ongoing, additional information is needed before making general recommendations or launching large-scale clinical efforts.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Gandini, M. Puntoni, B.M. Heckman-Stoddard
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Gandini, M. Puntoni, B.M. Heckman-Stoddard, L. Ford
Writing, review, and/or revision of the manuscript: S. Gandini, M. Puntoni, B.M. Heckman-Stoddard, B.K. Dunn, A. DeCensi, E. Szabo
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Study supervision: M. Puntoni, A. DeCensi, E. Szabo

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