Incidence of Bladder Cancer in Patients With Type 2 Diabetes Treated With Metformin or Sulfonylureas

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Diabetes Care 2014;37:1910–1917 | DOI: 10.2337/dc13-1489

OBJECTIVE

Previous studies evaluating the effect of metformin on cancer risk have been impacted by time-related biases. To avoid these biases, we examined the incidence of bladder cancer in new users of metformin and sulfonylureas (SUs).

RESEARCH DESIGN AND METHODS

This cohort study included 87,600 patients with type 2 diabetes in The Health Improvement Network database. Use of metformin or an SU was treated as a time-dependent variable. Cox regression–generated hazard ratios (HRs) compared metformin use with SU use, adjusted for age, sex, smoking, obesity, and HbA_{1c} level.

RESULTS

We identified 196 incident bladder cancers in the metformin cohort and 66 cancers in the SU cohort. Use of metformin was not associated with decreased bladder cancer risk (HR 0.81 [95% CI 0.60–1.09]). This association did not differ by sex (*P* for interaction = 0.20). We observed no association with duration of metformin relative to SU use (3 to <4 years of use: 0.57 [0.25–1.34]; 4 to <5 years of use: 0.93 [0.30–2.85; ≥5 years of use: 1.18 [0.44–3.19]; *P* for trend = 0.26).

CONCLUSIONS

Use of metformin is not associated with a decreased incidence of bladder cancer. Similar methods should be used to study other cancers that have previously been identified as potentially preventable with metformin.

Metformin, a biguanide used as first-line treatment for type 2 diabetes mellitus, lowers blood glucose by activating AMP-activated protein kinase (AMPK). AMPK activation, through the reduction of mammalian target of rapamycin (mTOR) signaling, has also been shown to inhibit cancer cell growth and proliferation (1). Thus, it is conceivable that AMPK activators such as metformin may influence human cancer risk.

Bladder cancer is a logical target for metformin chemoprevention. Activation of the mTOR pathway has been detected in bladder cancer, while inhibition of this pathway blocks bladder tumorigenesis (2,3) in animal models and is associated with durable response in some patients with advanced bladder cancer (4). These observations provide the rationale for mTOR-directed therapy with metformin as a potential strategy for bladder cancer chemoprevention.

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Received 24 June 2013 and accepted 24 December 2013.

See accompanying article, p. 1786.

Several observational studies have demonstrated that the use of metformin is associated with a decreased incidence of cancers of the breast (5,6), colon (7–9), liver (7,10,11), lung (9,12), pancreas (13), and prostate (14). Two recent meta-analyses, which included some of these observational studies, reported a 30% reduction in all-cancer incidence in metformin users compared with users of other diabetes therapies (Decensi et al. [15]: relative risk 0.69 [95% CI 0.61–0.79]; Noto et al. [16]: 0.68 [0.53–0.88]). There are limited data, however, examining the effect of metformin on the incidence of bladder cancer (17,18). Given that bladder cancer occurs more commonly in patients with type 2 diabetes (19), it is critically important to determine whether exposure to metformin is associated with a decreased risk of bladder cancer.

Suissa and Azoulay (20) recently reported that many studies of the impact of metformin on subsequent cancer risk suffered from time-related biases, including immortal-time bias from misclassification of metformin exposure time (7,8,12), time-window bias from differential lengths of metformin exposure time (5,6,10,11,14), and time-lag bias inherent to comparisons of first-line metformin treatment with second- or third-line treatments (8,9). Collectively, these biases may have led to the exaggerated reductions in cancer risk observed in these studies. In this study, we aimed to examine the risk of bladder cancer between new users of metformin and sulfonylureas (SUs) among a cohort of type 2 diabetes patients without previous use of these diabetes therapies, thereby avoiding the time-related biases that may have impacted prior studies of metformin and cancer.

RESEARCH DESIGN AND METHODS

Data Source

The Health Improvement Network (THIN) is an electronic medical records database that is representative of the broader U.K. population (http://www.thin-uk.com/). Data available in THIN include demographic information, medical diagnoses, lifestyle characteristics, and other measurements, including glycosylated hemoglobin (HbA_{1c}) and BMI. The software automatically codes medical diagnoses at entry using the Read Coding System (21). THIN also records new and repeat prescriptions written by the general practitioner as the computerized record is used to generate these prescriptions.

The accuracy and completeness of THIN data are well-documented, and the database has been used for epidemiological studies of several chronic diseases, including diabetes and cancer (22–24). The database currently contains the electronic medical records of >10 million patients, allowing for precise estimates of incidence rates of even rare outcomes, such as those for bladder cancer.

Study Design and Population

Using THIN, we conducted a retrospective cohort study of patients with type 2 diabetes who initiated therapy with either metformin or an SU between 1 July 2000 and 31 August 2010 (Fig. 1). We selected this time frame to avoid missing data on prior treatment with thiazolidinediones (TZDs), which were licensed in the European Union in July 2000 and have been associated with an increased risk of bladder cancer (25,26). From this population, we assembled a study and comparator cohort of new users of metformin or an SU, respectively. A new user was defined as having had a 6-month baseline period in THIN without previous prescriptions for either drug. Those initiating therapy with a combination of metformin and an SU were excluded from the study. Subjects who had received a diagnosis of bladder cancer before cohort entry were excluded to avoid misclassification of prevalent cancers as incident cancers. Subjects <40 years of age at cohort entry were also excluded because bladder cancer is extremely rare prior to age 40 years, and when it occurs, it likely results from a

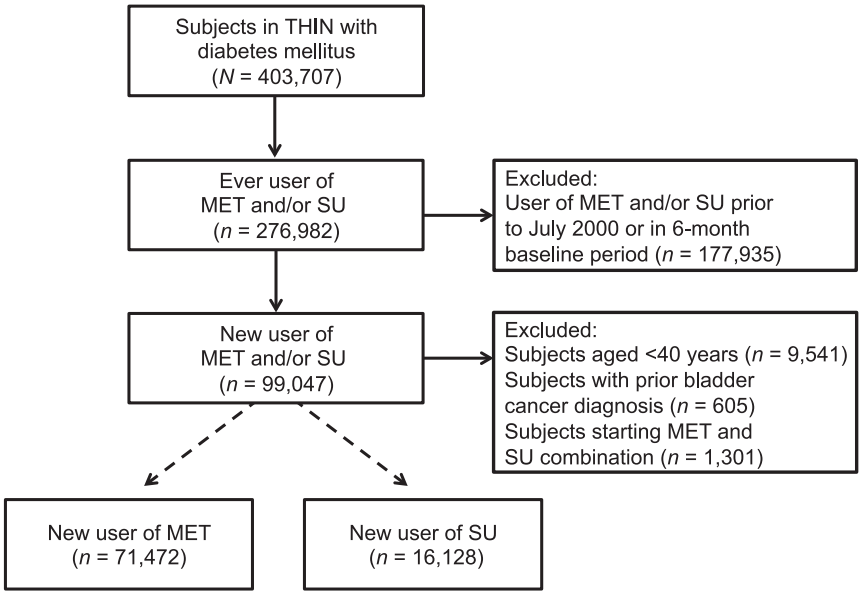

Figure 1—Study flow diagram. A retrospective cohort study was conducted among type 2 diabetes patients in the THIN database. We compared new users of metformin (MET) with new users of SUs and excluded patients with use of these drugs before 1 July 2000 or within 6 months of enrollment in THIN.

different mechanism. The study protocol was approved by the University of Pennsylvania Institutional Review Board and the U.K. Scientific Review Committee.

Exposure Definition

Exposure to metformin was defined as the receipt of two prescriptions for metformin or metformin-containing products within 6 months. Requiring the second prescription within a reasonably brief period helps to exclude the small fraction of patients who may fill a prescription but never return to their general practitioner or who never consumed the medication in the first prescription. The date of the second prescription was taken as the index date. Duration of therapy was calculated by dividing prescription quantity by the prescribed number of units per day, while cumulative duration of therapy was determined by summing the supply of a total day for all prescriptions subsequent to the index prescription.

Identical definitions were used to define exposure to the SUs, including first-generation drugs (acetohexamide, chlorpropamide, tolbutamide, or tolazamide) and second-generation drugs (glipizide, gliquidone, glimepiride, glibenclamide, or gliclazide). SUs were selected as the comparator because, like metformin, the SUs may also be used as first-line treatment for type 2 diabetes (27). Furthermore, in prior studies, no association was observed between the use of SUs and bladder cancer or other cancer incidence (25,26).

Outcome and Follow-up

The primary outcome was an incident diagnosis with bladder cancer, after the index date. Follow-up started on the index date and ended with the first of the following censoring events: incident diagnosis of bladder cancer; transfer out of THIN practice; death; or end of available data. The latter date was taken as 31 August 2010 to minimize the chance of surveillance bias given the U.S. Food and Drug Administration warnings of a possible association between TZDs and bladder cancer in September 2010 (28).

Statistical Analysis

Incidence rates of bladder cancer (per 100,000 person-years) with 95% CIs were calculated for the study (metformin) and comparator (SU) cohorts. Once a patient met the definition of exposure, the patient was considered exposed from that point forward, even if therapy was discontinued. Patients who switched or combined therapy, however, were censored on the date of treatment crossover.

Cox regression models were used to generate relative hazards of bladder cancer in metformin users compared with SU users, adjusted for potential confounders (29). As potential confounders, we considered age, sex, smoking, recurrent urinary tract infections, obesity, congestive heart failure, myocardial infarction, renal impairment, diabetes duration, HbA_{1c} level, other common diabetes medications (insulin and TZDs), and other commonly prescribed medications (ACE inhibitors, statins, aspirin, and nonsteroidal anti-inflammatory drugs). All covariates were measured at baseline during the 6 months immediately prior to the initiation of metformin or SU therapy, with the exception of smoking. Smoking status was measured using data recorded at any time before or during follow-up. Diabetes duration was measured as the time from a first diabetes diagnosis. Obesity was defined using diagnostic codes and BMI records. Covariates changing the hazard ratio (HR) between metformin exposure and outcome by $>$10% were included in the full model. Full models were then reduced by sequentially deleting variables with model P values $\geq$0.10 to arrive at the final model. The final model included age, sex, smoking (ever vs. never), obesity, (BMI $\geq$30), and HbA_{1c} level. Patients with missing baseline BMI or HbA_{1c} values were omitted from fully adjusted analyses (case-wise deletion).

Cox regression was also used to determine whether the risk of bladder cancer decreased with increasing duration of metformin therapy. In these analyses, metformin and SU use was entered into the model as time-varying exposures. During the follow-up period, each user was categorized according to their cumulative duration of therapy ($<$1, 1–2, 2–3, 3–4, 4–5, and $\geq$5 years). To test for trend, duration of exposure was included as a continuous variable in the Cox regression model. The duration analyses were planned a priori given previous studies suggesting that the antitumor effect of metformin increased with each year of metformin use (15).

The proportional hazards assumptions were met for all Cox models. All statistical tests were two-sided and conducted at the 5% significant level. STATA version 12.0 was used for all statistical analyses (StataCorp, College Station, TX).

Subgroup and Sensitivity Analyses

A subgroup analysis limited the cohort to patients with no documented use of any diabetes medication prior to the start of follow-up, allowing for comparison between new users of metformin and SUs as the initial therapy. We also performed a series of sensitivity analyses to test the robustness of our results. To assess the potential impact of misclassification bias, we extended the baseline period used to define new user from 6 months to 1 year and redefined exposure as the receipt of two prescriptions in 3 months rather than 6 months. To address the possibility of detection bias, we conducted a 1-year lagged analysis in which patients with bladder cancers occurring in the first year of follow-up were excluded. To assess for residual confounding by use of other diabetes therapies during follow-up, we repeated the primary analysis after adjustment for the use of TZDs and insulin as time-updating variables. Finally, we also considered the effects of missing data in two ways. First, we entered mean BMI and mean HbA_{1c} level in our final model using data recorded throughout the entire follow-up period. Second, linear regression was used to impute missing data on baseline BMI and HbA_{1c} levels using all descriptive variables included in the primary analysis. To account for the variability among imputations, SEs were adjusted according to the method proposed by Rubin (30).

RESULTS

The final cohort included 87,600 patients with type 2 diabetes who were initiators of therapy with metformin (n = 71,472) or an SU (n = 16,128). Approximately 8% of initiators (n = 7,352) left a THIN practice during follow-up. The median follow-up time was ~2 years for both cohorts (metformin cohort: median 2.1 years [interquartile range {IQR} 0.8–4.0 years] vs. SU cohort: median 2.0 years [IQR 0.7–3.9 years]), and $>$4 years in 25% of subjects (Table 1). Metformin initiators were younger (median [IQR] age 62 years

Table 1—Demographics of the study and comparator cohorts

Characteristics	Metformin initiators* (*n* = 71,472)	SU initiators* (*n* = 16,128)
Age (years)		
<60	29,277 (41.0)	4,102 (25.4)
60–69	21,121 (29.5)	4,186 (26.0)
≥70	21,074 (29.5)	7,840 (48.6)
Median (IQR)	62 (54–71)	69 (59–78)
Male sex	39,886 (55.8)	8,878 (55.0)
Ever smoker	47,171 (66.0)	10,451 (64.8)
BMI (kg/m^2)		
<30	28,390 (39.7)	10,581 (65.6)
≥30	40,035 (56.0)	4,079 (25.3)
Missing	3,047 (4.3)	1,468 (9.1)
Median (IQR)	31.1 (27.7–35.3)	26.8 (24.1–30.4)
HbA_{1c} level		
<7%	9,113 (12.7)	1,888 (11.7)
7–7.9%	18,732 (26.3)	3,296 (20.4)
8–8.9%	13,248 (18.5)	2,682 (16.6)
≥9%	20,616 (28.8)	4,982 (31.0)
Missing	9,763 (13.7)	3,280 (20.3)
Median (IQR)	8.1 (7.3–9.6)	8.3 (7.4–10.0)
Diabetes duration (years)		
0–<1	39,904 (54.5)	9,073 (56.3)
1–5	21,415 (29.9)	4,458 (27.6)
≥5	11,153 (15.6)	2,597 (16.1)
Median (IQR)	8.1 (1.6–39.1)	7.0 (1.5–39.3)
Other diabetes drugs		
Insulin	3,865 (5.4)	369 (2.3)
Insulin after index†	4,169 (5.8)	2,160 (13.4)
TZD	589 (0.8)	259 (1.6)
TZD after index†	11,048 (15.4)	2,453 (15.2)
Other drug treatment		
ACE inhibitors	31,959 (44.7)	6,386 (39.6)
ARB	8,980 (12.5)	1,625 (10.1)
Aspirin	28,168 (39.4)	6,339 (39.3)
NSAIDs	29,435 (41.2)	6,040 (37.4)
Statins	41,586 (58.2)	6,896 (42.7)
Congestive heart failure	1,765 (2.5)	918 (5.7)
Renal impairment	3,992 (5.6)	1,530 (9.5)
Recurrent urinary tract infection	3,940 (5.5)	1,039 (6.4)
Myocardial infarction	4,924 (6.9)	1,534 (9.5)
Duration of follow-up, median (IQR), years	2.1 (0.8–4.0)	2.0 (0.7–3.9)

Values are given as *n* (%), unless otherwise stated. ARB, angiotensin-receptor blocker; NSAID, nonsteroidal anti-inflammatory drug. *All comparisons have *P* values <0.01 except sex (*P* = 0.08), use of aspirin (*P* = 0.80), and use of a TZD after the index date (*P* = 0.43). *P* values were calculated using the Wilcoxon rank sum test or χ^2 test. †Measured after the index date (date of metformin or SU initiation).

[54–71 years] vs. 69 years [59–78 years]), more obese, and less likely to have renal impairment than SU initiators (Table 1). There were small differences in other baseline characteristics including sex, smoking status, HbA_{1c} level, and diabetes duration between the metformin and SU cohorts. HbA_{1c} levels were missing in 14% and 20% of metformin and SU members, respectively, and BMI was missing in 4% and 9% (Table 1). During the follow-up period, the proportion of patients exposed to a TZD was nearly identical in both cohorts (15.4% among metformin initiators vs. 15.2% among SU initiators).

We identified 262 incident bladder cancers during 221,406 person-years: 196 cancers in the metformin cohort and 66 cancers in the SU cohort (Table 2). The unadjusted bladder cancer incidence rates among metformin and SU initiators were 107.8 (95% CI 93.2–123.9) and 166.7 (95% CI 129.0–212.1) per 100,000 person-years. In the fully adjusted model (adjusted for age, sex, smoking, obesity, and HbA_{1c} level), the use of metformin was not associated with bladder cancer risk (HR 0.81 [95% CI 0.60–1.09]). This association did not differ by sex (*P* for interaction = 0.20). Of note, the association did not change appreciably after adjustment for other therapies that may influence bladder cancer risk, including insulin, TZDs, angiotensin receptor blockers, statins, aspirin, and nonsteroidal anti-inflammatory drugs (31–34).

In analyses that accounted for the duration of therapy (Table 2), we observed no association between increasing duration of metformin therapy and the risk of bladder cancer. For example, there was no decreased risk of bladder cancer among metformin users with ≥5 years of use compared with <1 year of use (≥5 years of use: HR 1.02 [95% CI 0.59–1.75]; P_{trend} = 0.99). Similarly, among SU users, there were no clear patterns between increasing duration of SU therapy and bladder cancer risk (P_{trend} = 0.21).

When we compared bladder cancer risk in the metformin users relative to SU users, there was likewise no pattern of decreasing relative risk of bladder cancer associated with metformin use with increasing duration of metformin therapy (metformin vs. SU therapy: 3 to <4 years of use: HR 0.57 [95% CI 0.25–1.34]; 4 to <5 years of use: 0.93 [0.30–2.85]; ≥5 years of use: 1.18 [0.44–3.19]; P_{trend} = 0.26).

In a subgroup analysis, we restricted the new user cohort to include only those having had a 6-month baseline period in THIN without previous prescriptions for metformin, SU, or any other diabetes therapy (*n* = 82,570). In this analysis, we obtained similar results to our primary analysis for the overall (HR 0.81 [95% CI 0.60–1.09]) and duration of therapy analyses (metformin vs. SU: 3 to <4 years of use, 0.53 [0.22–1.26]; 4 to <5 years of use, 0.91 [0.29–2.83]; ≥5 years of use, 1.14 [0.41–3.13]; P_{trend} = 0.36). We also observed similar results to our primary analysis for all sensitivity analyses described in RESEARCH DESIGN AND METHODS (Table 3).

CONCLUSIONS

In this cohort study, we found no difference in the incidence of bladder cancer among new users of metformin compared with new users of an SU, the

Table 2—Incidence rate and relative risk of bladder cancer in the metformin and SU cohorts

Characteristics	Cancers, *n*	PYS	IR (95% CI), per 100,000 PYS	Unadjusted (HR, 95% CI)	Fully adjusted* (HR, 95% CI)
SU initiators	66	39,588	166.7 (129.0–212.1)	1.00 (referent)	1.00 (referent)
Metformin initiators	196	181,818	107.8 (93.2–123.9)	0.63 (0.47–0.83)	0.81 (0.60–1.09)
Duration of metformin therapy (years)					
<1	75	62,841	119.3 (93.9–149.6)	1.00 (referent)	1.00 (referent)
1 to <2	38	46,426	81.8 (57.9–112.4)	0.66 (0.45–0.99)	0.68 (0.46–1.02)
2 to <3	33	28,493	115.8 (79.7–162.6)	0.92 (0.61–1.40)	0.97 (0.64–1.48)
3 to <4	17	18,513	91.8 (53.5–147.0)	0.71 (0.42–1.22)	0.76 (0.45–1.30)
4 to <5	13	11,773	110.4 (58.8–188.8)	0.84 (0.46–1.52)	0.91 (0.50–1.66)
≥5	20	13,770	145.2 (88.7–224.3)	0.99 (0.59–1.66)	1.02 (0.59–1.75)
P_{trend}†	—	—	—	0.82	0.99
Duration of SU therapy (years)					
<1	31	13,749	225.5 (153.2–320.0)	1.00 (referent)	1.00 (referent)
1 to <2	12	9,972	120.3 (62.2–210.2)	0.52 (0.27–1.02)	0.55 (0.28–1.09)
2 to <3	5	6,068	82.4 (267.5–192.3)	0.35 (0.14–0.91)	0.37 (0.14–0.95)
3 to <4	8	3,943	202.8 (87.6–399.7)	0.84 (0.38–1.84)	0.85 (0.39–1.89)
4 to <5	5	2,507	199.4 (64.7–465.3)	0.79 (0.30–2.06)	0.63 (0.22–1.82)
≥5	5	3,348	149.3 (48.5–348.5)	0.53 (0.20–1.41)	0.56 (0.21–1.47)
P_{trend}†	—	—	—	0.25	0.21
Duration of therapy, metformin vs. SU (years)					
<1	—	—	—	0.51 (0.33–0.77)	0.64 (0.41–1.00)
1 to <2	—	—	—	0.65 (0.34–1.25)	0.79 (0.41–1.53)
2 to <3	—	—	—	1.34 (0.52–3.43)	1.70 (0.66–4.39)
3 to <4	—	—	—	0.43 (0.19–1.00)	0.57 (0.25–1.34)
4 to <5	—	—	—	0.54 (0.19–1.51)	0.93 (0.30–2.85)
≥5	—	—	—	0.94 (0.35–2.50)	1.18 (0.44–3.19)
P_{trend}†	—	—	—	0.36	0.26

PYS, person-years; IR, incidence rate. *Adjusted for age (<60, 60–69, and ≥70 years), sex, smoking (ever vs. never), HbA_{1c} level (<7%, 7–7.9%, 8–8.9%, and ≥9%), and obesity (BMI ≥30 kg/m^2). †The test of trend was calculated by entering the duration categories in a Cox regression model as a continuous variable, whereas for analysis of discrete duration intervals, the variable was included as a categorical variable.

common alternative first-line therapy for type 2 diabetes. We also observed no association with risk of bladder cancer by the duration of metformin relative to SU use, or metformin or SU use alone. Although these results do not support prescribing metformin in preference to SUs for the purpose of reducing bladder cancer incidence, the results are important for interpreting prior studies of bladder cancer risk with TZDs. Specifically, many of these studies included patients treated with metformin and SUs as comparators. Furthermore, our results help to clarify existing controversy over the potential cancer prevention effects of metformin, focusing on a cancer that is more common in patients with type 2 diabetes (17,19), has been associated with exposure to another class of diabetes medications (25,26), and is potentially sensitive to the mechanism of action of metformin.

Our findings differ from previous observational studies that have reported dramatic reductions in cancer risk associated with the use of metformin, ranging from 19 to 94% (6–18). These contrasting results could be explained if metformin is only biologically active against tumors other than bladder cancer, or perhaps more likely if the methods used in the previous epidemiological studies systemically biased results toward a protective effect of metformin on cancer risk. Suissa and Azoulay (20) have recently described several time-related biases (immortal-time, time-window, and time-lag biases) believed to affect the validity of the previous studies. Most relevant to cohort studies are immortal-time and time-lag biases. Misclassification of immortal time, time during which the outcome under study (cancer) could not have occurred, as metformin-exposed time, leads to immortal-time bias (35). Time-lag bias arises in cohort studies comparing second- or third-line treatments (SUs or insulin) with first-line metformin treatment (20). Inclusion of treatment cohorts with different durations of previous therapy or durations of different diabetes may have differential effects on cancer risk. For example, longer duration of diabetes or use of medications associated with longer duration of diabetes may increase the risk of bladder cancer, as has been demonstrated in some studies (31).

To avoid the time-related biases that may have impacted the results of previous studies, there are several unique features of our study. We used an incident user design comparing metformin to the common alternative first-line therapy, SUs, for patients with type 2 diabetes. The new user design reduces the risk of time-lag and time-window bias by excluding treatment groups with different durations of previous therapy, allowing for direct comparisons between predominantly first-line treatment cohorts. As such, we observed similar durations of diabetes between the metformin and SU treatment cohorts (median diabetes duration: metformin users, 8.1 months [IQR 1.6–39.1] vs. SU users, 7.0 months [IQR 1.5–39.3]). The new user design also assigns a chronological reference point, time zero, for each member of the study

Table 3—Subgroup and sensitivity analyses of the relative risk of bladder cancer in the metformin relative to SU cohorts

Description	Total cancers, *n*	Total patients, *n*	Unadjusted, HR (95% CI)	Fully adjusted, HR (95% CI)*
Original analysis	262	87,600	0.63 (0.47–0.83)	0.81 (0.60–1.09)
Subgroup analysis of first-line therapy†	250	82,570	0.63 (0.47–0.84)	0.81 (0.60–1.09)
Sensitivity analyses				
Use of 1-year baseline period to define new user	252	84,031	0.66 (0.49–0.88)	0.84 (0.61–1.14)
Receipt of two prescriptions in 3 months to define exposure	262	87,277	0.64 (0.48–0.85)	0.81 (0.60–1.09)
Exclusion of bladder cancers during first year of follow-up	185	87,600	0.69 (0.49–0.97)	0.87 (0.60–1.25)
Multiple imputation of BMI and HbA_{1c} level‡	262	87,600	0.64 (0.48–0.85)	0.84 (0.62–1.12)
Use of mean BMI during follow-up	262	87,600	0.63 (0.47–0.83)	0.90 (0.67–1.22)
Use of mean HbA_{1c} level during follow-up	262	87,600	0.63 (0.47–0.83)	0.82 (0.61–1.11)
Use of insulin and TZDs as time-updating variables	262	87,600	0.63 (0.47–0.83)	0.86 (0.64–1.15)
Adjustment for diabetes duration	262	87,600	0.63 (0.47–0.83)	0.81 (0.60–1.10)
Adjustment for prior use of other diabetes medications	262	87,600	0.63 (0.47–0.83)	0.81 (0.60–1.10)
Initial treatment carried forward§	385	87,600	0.78 (0.62–0.98)	0.89 (0.70–1.13)

*Adjusted for age (<60, 60–69, and ≥70 years), sex, smoking (ever vs. never), HbA_{1c} level (<7%, 7–7.9%, 8–8.9%, and ≥9%), and obesity (BMI ≥30 kg/m^2). †Exclusion of subjects with use of diabetes therapies (*n* = 5,030; 5.7%) prior to start of follow-up. ‡Linear regression was used to impute missing data on HbA_{1c} levels and BMI. To account for the variability between imputations, SEs were adjusted according to the method proposed by Rubin (30). §Follow-up time continued for metformin or SU users who switched therapy or started combination therapy.

and comparator cohorts from which follow-up time begins, thereby minimizing bias from immortal time. We further reduced this bias by adopting a Cox model with time-dependent drug exposures, and by censoring patients if they had switched or combined therapy between metformin and an SU.

Our findings remained consistent in several sensitivity analyses designed to assess the impact of other common biases in pharmacoepidemiologic studies of diabetes therapies. For example, we assessed the potential misclassification of use by redefining exposure as receiving two prescriptions for therapy within a 3-month period, and redefining new user as requiring a therapy-free baseline period of 1 year. Results from these sensitivity analyses were similar to the primary analysis, indicating minimal misclassification of unexposed patients as exposed and prevalent users as new users. We also considered the impact of detection bias among initiators of metformin or SUs by excluding cancers occurring in the first year after the initiation of therapy. Compared with the results of the primary analysis, this 1-year lagged analysis did not alter our results appreciably, suggesting that detection bias, even if present, was likely minimal.

There are several additional strengths of this study. THIN data allowed for adjustment for important covariates, most notably smoking history. Tobacco use is strongly associated with bladder cancer incidence (36). Many studies of diabetes therapies and bladder cancer have relied on administrative data sources that lack information on tobacco use. The diagnosis of bladder cancer in this study is likely highly accurate. Recorded information on cancer has been validated in a related database (General Practice Research Database), which shares some practices with THIN and uses the same electronic software for data collection (37). Observed cancer incidence within THIN is comparable to that reported in U.K. cancer registry data, particularly since 2001 (24). The incidence rate of bladder cancer in the U.K. population aged ≥65 years was 80 per 100,000 person-years in 2008 (38). The incidence of bladder cancer in our cohort was relatively high (113 per 100,000 person-years), which is consistent with the estimated 40% increase in the risk of bladder cancer observed among patients with type 2 diabetes (17,19).

Diabetes is a chronic disease requiring treatment with several medication classes over time, such as the TZDs. Several studies have recently shown that treatment with pioglitazone, a TZD, may increase the risk of bladder cancer. Because the proportion of patients exposed to a TZD during follow-up was nearly identical in metformin compared with SU users (15.4% vs. 15.2%), the possible effects of TZDs on the risk of bladder cancer are unlikely to have impacted our results. This is evidenced by a sensitivity analysis producing results similar to our primary analysis after adjustment for the use of TZD and insulin as time-updating variables (primary analysis: HR 0.81 [95% CI 0.60–1.09] vs. sensitivity analysis: HR 0.86 [95% CI 0.64–1.15]). Furthermore, given that our study ended prior to publicity of the TZD-bladder cancer warnings, surveillance for bladder cancer in response to these warnings would be nondifferential between the metformin and SU cohorts.

There are several potential limitations of this study. Our study could be subject to confounding by indication due to group differences in diabetes severity or duration. To minimize confounding by indication, we selected a reference group of new users of the alternative first-line antidiabetes drug, the SUs, and adjusted for baseline HbA_{1c} level. Importantly, diabetes duration was comparable among the new user cohorts and was not observed to confound the association between metformin use and risk of bladder cancer. To avoid bias from treatment crossover, we censored patients if had they started combination therapy. Ending follow-up time at this point, regardless of diabetes duration, may exclude patients with more advanced stage of diabetes. In a sensitivity analysis allowing follow-up time to continue in users who switched therapy or started combination therapy (i.e., initial treatment carried forward), we observed results that were not

substantially different from the censor at switch approach. Importantly, while research has shown positive associations between type 2 diabetes and the incidence of bladder cancer, data on the association between diabetes duration or severity (i.e., HbA_{1c}) and bladder cancer risk remain controversial (39,40).

Although our cohort consisted predominantly of patients receiving initial therapy, a small subset (~6%) had been previously treated with other diabetes medications. Time-lag bias can result from unequal distribution of disease duration. Exclusion of subjects with previous use of diabetes therapy in a subgroup analysis produced nearly identical results to the primary analysis. Thus, time-lag bias is unlikely to have substantially impacted our results.

Our cohorts had incomplete data on several important variables. HbA_{1c} levels and BMI were missing in 14% and 20%, and 4% and 9%, respectively, of metformin and SU cohort members. When we conducted a sensitivity analysis using multiple imputation for the missing HbA_{1c} and BMI values, our results were similar to the primary analysis (Table 3). Likewise, we also observed similar results after adjustment for mean HbA_{1c} level and mean BMI using data recorded during the entire follow-up period, strongly suggesting that our results are unlikely to be biased from missing data on these confounders. THIN lacks information on some bladder cancer risk factors including race and occupational exposures. However, black race accounts for <5% of the U.K. population, and occupational exposures are not strongly associated with bladder cancer (41). Thus, it is unlikely for variables such as race and occupation to account for residual confounding in this study.

The median follow-up time for our cohort was 2 years. A post hoc analysis demonstrates that we were able to detect a relative HR of bladder cancer in metformin relative to SU users of 0.65 with 80% power, consistent with the 95% CIs (0.61–1.09) observed in our primary analysis. Although we were underpowered to detect smaller differences in effect size (i.e., HR > 0.65), we were adequately powered to detect large differences (i.e., HR < 0.65), such as those reported in the previous studies examining the impact of metformin on subsequent cancer risk (6,8–15). Similarly, these data cannot exclude a protective effect of even longer-term therapy with metformin.

Despite not observing a cancer prevention effect, our results should not detract others from investigating the therapeutic potential of metformin on bladder cancer progression among patients with known bladder cancer. Metformin inhibits cancer cell growth via AMPK activation and subsequent downstream inhibition of the mTOR. Dysregulation of mTOR has been implicated in the progression of several cancers, including bladder cancer (2). Recently, inhibition of mTOR has demonstrated antitumor activity in a subset of patients with advanced bladder cancer (4), suggesting that this pathway is active in bladder cancer and could serve as a therapeutic target in this disease.

In summary, in the U.K. THIN patient population, we found no evidence for a decreased risk of bladder cancer in type 2 diabetes patients using metformin. There was also no association with bladder cancer risk by duration of metformin use. To avoid time-related biases, future studies investigating the effect of metformin on cancer incidence should use time-dependent analyses to accurately classify and measure metformin exposure. Likewise, it will be important to use the same methods to study other cancers that have previously been identified as potentially preventable with metformin therapy.

Funding. This research was supported by the National Institutes of Health (grant K12-CA-076931 to R.M.; grant UL1-RR-024134 to K.H. and J.D.L.; grant 1F30-HL-115992-01 to B.S.F.; and grant K24-DK-078228 to J.D.L.).

Duality of Interest. K.H. has received research funding from AstraZeneca and Bristol-Myers Squibb. J.D.L. has served as a paid consultant for Abbott/AbbVie, Prometheus, Lilly, Shire, Nestle, Janssen Pharmaceuticals, Amgen, and AstraZeneca. J.D.L. also has received research funding from Takeda, Centocor, and Shire. No other potential conflicts of interest relevant to this article were reported.

The funder had no role in the design of the study, analysis and interpretation of data, or writing of and decision to submit the report for publication.

Author Contributions. R.M. analyzed the data and wrote the manuscript. X.W. assisted with statistical analysis. N.P., K.H., B.S.F., S.M.K., N.B.H., D.J.V., and J.D.L. reviewed and edited the manuscript. R.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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