New users of metformin are at low risk of incident cancer: A cohort study among people with type 2 diabetes

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STRUCTURED ABSTRACT

Objectives: The anti-diabetic properties of metformin are mediated through its ability to activate the AMP-activated protein kinase (AMPK). Activation of AMPK can suppress tumour formation and inhibit cell growth, in addition to lowering blood glucose levels. We tested the hypothesis that metformin reduces the risk of cancer in people with type 2 diabetes. **Research Design and Methods:** In an observational cohort study using record-linkage databases, based in Tayside, Scotland, UK, we identified people with type 2 diabetes who were new users of metformin in 1994-2003. We also identified a set of diabetic comparators, individually matched to the metformin users by year of diabetes diagnosis, who had never used metformin. In a survival analysis we calculated hazard ratios for diagnosis of cancer, adjusted for baseline characteristics of the two groups using Cox regression. **Results:** Cancer was diagnosed among 7.3% of 4,085 metformin users compared with 11.6% of 4,085 comparators, with median times to cancer of 3.5 years and 2.6 years respectively ($p < 0.001$). The unadjusted hazard ratio for cancer was 0.46 (0.40–0.53). After adjusting for sex, age, BMI, HbA1c, deprivation, smoking and other drug use, there was still a significantly reduced risk of cancer associated with metformin: 0.63 (0.53–0.75). **Conclusions:** These results suggest that metformin use may be associated with a reduced risk of cancer. A randomized trial is needed to assess whether metformin is protective in a population at high risk for cancer.

Recent research suggests that the anti-diabetic drug metformin, which exerts its effects by activating the AMP-activated protein kinase (AMPK) may have potential for the treatment of cancer in humans[1]. The hypothesis that metformin may have anti-cancer effects is supported by laboratory studies, showing that metformin is associated with reduced incidence of pancreatic cancer in hamsters[2], and delays onset of mammary[3] and other tumours[4] in tumour-prone mice. Metformin also inhibits growth of human breast cancer cells[5]. Although the potential for prevention of cancer in humans using metformin has not been explored, we previously reported the results of a pilot case-control study that identified a reduced risk of cancer among patients with type 2 diabetes who had used metformin[6]. However, the outcome was limited to hospital admissions for cancer, and the date of diagnosis was assumed to be date of first hospital admission.

Other diabetic drugs may also have cancer-related effects. An independent epidemiological study found that users of sulphonylureas were at higher risk of cancer-related mortality than metformin users[7]. Sulphonylureas (and insulin) increase circulating insulin levels and hyperinsulinaemia may promote carcinogenesis[8]. Treatments such as metformin and glitazones reduce insulin resistance, with insulin resistance possibly associated with increased risk of cancer[9.] The objective of this study was to test the hypothesis that metformin use is associated with a reduced risk of cancer in people with type 2 diabetes, using a national cancer registry to ensure valid diagnoses of cancer, with precise dates of diagnosis. We also adjusted results for the effects of exposure to other diabetic drugs.

RESEARCH DESIGN AND METHODS

Data Sources

This observational, historical cohort study was carried out using anonymous patient data for the resident population of Tayside Health Board in Scotland, UK (approx. 400,000 people). Data were provided by the Health Informatics Centre (HIC), University of Dundee, which has developed the record-linkage of multiple, routinely collected data sets for research. SCI-DC (formerly known as DARTS) is a validated population-based diabetes register with detailed clinical information[10]. A pharmacoepidemiological database (formerly known as the MEMO drug safety database) holds computerised records of every diabetic drug dispensed to Tayside residents since 1993[11]. Scottish Morbidity Record 6 (SMR6) is a national database of all diagnoses of cancer[12]. Computerised death certification records from the Registrar General, with ICD9/ICD10-coded causes of death[13,14], were also available. All HIC data are anonymised prior to analysis to maintain confidentiality and conform to data protection legislation.

Study design

The DARTS database was used to identify patients who were diagnosed with type 2 diabetes in Tayside before 2004 (patients diagnosed over 35 years are classified as type 2 diabetes, as are younger patients with no insulin requirement).

Metformin users

We identified patients who received a first metformin prescription any time between 1st January 1994 and December 2003 (excluding patients who received metformin in 1993 or before their diabetes diagnosis). We classified these patients as metformin users and defined their index date as date of first metformin prescription. Patients who had a record of cancer on SMR6 at any time between 1980 and their index date were excluded.

Comparator cohort

Comparator patients individually matched to the metformin users were generated at random from a pool of patients with type 2 diabetes who had no record of metformin use. We used a computer algorithm that identified comparators for each metformin user (listed in random order). For each metformin user, a potential comparator was identified with the same year of diagnosis and assigned the same index date. Year of diagnosis was chosen as a matching variable to control for effects of treatment patterns which could vary over time but not be measured directly. However, if there was a record of cancer on SMR6 prior to the index date, or if they had died, the comparator was discarded (but was potentially available for a different metformin user). This process was repeated until suitable comparators were identified. The comparators that were identified for metformin users were therefore diagnosed with diabetes in the same year, and survived until the index date without cancer (the date that their corresponding metformin user started metformin). Any potential survival bias (meformin users surviving to go on to metformin) was thus eliminated.

Baseline data were collated for all metformin users and comparators: age at index date, age at diagnosis of diabetes, sex, smoking status, mean body mass index (BMI) and HbA1c during the study period, use of sulphonylureas or insulin within 3 months or 1 year of the index date respectively. An area-based measure of material deprivation (Carstairs score[15]) based on four variables from the national census (car ownership, unemployment, overcrowding and head of household job classification) was also used.

Main outcome measures

We followed up all patients from their index dates for pre-defined outcomes. The primary outcome was diagnosis of cancer (as recorded on SMR6). Time to outcome was defined as the period from index date to (a) date of diagnosis of cancer on SMR6 (b) date of death if no cancer diagnosis (c) end of the study (31st December 2003) if no cancer diagnosis.

Secondary outcomes

We evaluated the risks of the following secondary outcomes: diagnosis with bowel cancer (ICD9 153-154, ICD10 C18-C20), lung cancer (ICD9 162, ICD10 C33-C34) or breast cancer in women (ICD9 174, ICD10 C50), all-cause mortality and mortality from cancer (any mention on death certificate).

Statistical methods

Time from index date to outcome in the cohorts was shown using Kaplan Meier plots. The proportional hazards assumption was examined using log (-log) survival plots, parallel lines indicating that the assumption was reasonable. The relationship between metformin use and diagnosis of cancer was assessed in a Cox regression unadjusted, and then in a multivariable model adjusted for age at index date, sex, smoking status, deprivation, mean body mass index (BMI) and HbA1c during the study period, and use of sulphonylureas and insulin. These were all treated as categorical variables (as defined in Table 1), with the exception of HbA1c and BMI which were treated as continuous variables. The analyses were stratified by year of diagnosis (the matching variable).

Dose-response analyses

For each metformin user, we identified the maximum metformin dose prescribed during follow-up. We then categorised these as low dose (less than 50% of maximum prescribable dose), medium dose (50-80% of maximum prescribable dose), and high dose (more than 80%). The risks associated with each dose level were determined in a stratified analysis, with adjustments for all covariates. However, in case of confounding by duration of follow-up, we further stratified patients according to length of time in the study.

Ethical Approval

Ethical approval was obtained from the Multi-Centre Research and Ethics Committee for Scotland.

Role of funding source

RESULTS

There were 13,344 patients alive in January 1993 who were diagnosed with type 2 diabetes in Tayside before 2004, of which 12,255 were eligible for the study. We identified 5,183 patients who received a first prescription for metformin after 1994, and selected 4,944 for whom there was no cancer diagnosis prior to metformin use. The remaining patients were 5,883 patients who received no metformin prescriptions between 1993 and 2004, and 1,189 who received a prescription for metformin in 1993. These latter patients were excluded as their date of starting metformin was unknown (no prescribing data available prior to 1993). Fig 1 shows how patients were selected for the study.

Metformin users and comparators

The computer algorithm was used to identify comparators for the 4,804 metformin users who were aged 35 years or over at their index date. The comparators were identified from the 5,773 patients who were over 35 years and had no record of metformin use. A comparator was identified for 4,364 users (the remaining 440 metformin users for whom comparators could not

be found were excluded). However, all further analyses were restricted to the 4,085 metformin users who had more than one prescription for metformin, and their respective comparators.

The baseline characteristics of the two groups are presented in Table 1. Metformin users were younger than their comparators and slightly more likely to be current smokers (although smoking status was unavailable for about a quarter of study subjects). BMI and HbA1c values were also unavailable for 3% and 9% of study subjects respectively, who were therefore assigned the mean values. Metformin users had higher mean values of BMI and HbA1c. There was a much higher proportion of metformin users who were treated with sulphonylureas within 3 months, but a lower proportion who used insulin within a year. These differences were all statistically significant.

Main outcome measures

Cancer was diagnosed among 297 (7.3%) of the metformin users during follow-up, compared with 474 (11.6%) of the comparators. Median time to cancer was 3.5 years (IQR 2.1 – 5.8 years) for metformin users, compared with 2.6 years (IQR 1.2 – 4.1 years) for comparators. Fig 2 shows the Kaplan-Meier plot for diagnosis of cancer (log rank test $p < 0.001$). The proportional hazards assumption was met for all study subjects.

The unadjusted Cox regression showed a statistically significant reduction in the risk of cancer in the new metformin users with a hazard ratio of 0.46 (0.40 – 0.53) (Table 1). Increased cancer

risk was observed among males and with increasing age. It also appeared that higher BMI and HbA1c were associated with a reduced risk of cancer. This is difficult to explain, but may be a diagnostic bias, with high BMI and HbA1c indicative of less frequent health care seeking behaviour.

In the multivariable analysis, the adjusted hazard ratio increased to 0.63 (0.53 - 0.75) for metformin use. Never and ex smokers were at reduced risk of cancer, but the reduced risks associated with increased BMI and HbA1c were less marked. No statistically significant effects were observed for use of sulphonyureas or insulin.

Secondary outcomes

The unadjusted and adjusted risks of the secondary outcomes are presented in Table 2. Metformin users were at much lower risk of overall mortality and cancer-related mortality than their comparators. Overall, 14.9% of metformin users died, compared with 34.8% of the comparators. The median survival times were 3.6 years (IQR 2.2 – 5.9 years) and 2.8 years (1.4 – 4.3 years) respectively. 3.0% of metformin users died from cancer, compared with 6.1% of comparators. Reduced risks of a similar magnitude to that for all cancers were observed for bowel cancer, lung cancer and breast cancer, although the results were not all statistically significant.

Dose-response analysis

The stratified analysis for maximum dose is also presented in Table 2. Although none of the risks for low, medium and high doses stratified by length of follow-up were significantly different from each other (as indicated by overlapping confidence intervals), there was a clear trend for metformin users to have a higher risk of cancer in the first two years of follow-up. However, after this, among patients with the same duration of follow-up, the risk appeared to be lower with the highest metformin doses.

CONCLUSIONS

This study supports the hypothesis that users of metformin are at lower risk of cancer compared with people with type 2 diabetes on other treatments. Fewer than 8% of a cohort of metformin users were diagnosed with cancer during a maximum of 10 years of follow-up, compared with 11% of a comparator cohort of non-users. The median time to cancer was 3.6 years among metformin users, compared to 2.5 years among comparators, and they also had reduced overall and cancer-related mortality.

This was an observational study therefore we could not control for all differences between study groups. Users of metformin could have been at lower baseline risk of cancer than the comparators. Indeed, they were younger than their comparators (but mean BMI and HbA1c were higher). Metformin users did seem to be a different group clinically from non users, with a much lower rate of mortality (some of which could be explained by their lower risk of cardiovascular mortality[16]). While this limitation is inherent in the observational nature of the study, we adjusted results for known potential confounders and there were sizable changes to the risk estimates.

There may still have been residual confounding, or unknown confounders, but it is unlikely that this could account for the entire 37% reduced risk of cancer observed.

Adjusting for use of other diabetic drugs was necessary because there was a higher proportion of metformin users who were treated with sulphonylureas compared with the comparators, but a lower proportion treated with insulin. This probably reflects the heterogeneity of the pool of potential comparators. Patients not treated with metformin will encompass those who do not yet require oral therapy, and also those who have progressed to insulin after treatment with sulphonylureas only. However, we found no statistically significant independent effects of sulphonylureas and insulin on risk of cancer in the Cox regression analysis. In contrast, males and older people were at increased risk of cancer, as might be expected. The results were similar for specific cancer types.

In a dose-response analysis, metformin users appeared to have a higher risk of cancer during the first two years of follow-up. This may be because patients who begin treatment with metformin are more likely to have cancer diagnosed as they have increasing contact with health care professionals. In later years of follow-up, high maximum doses of metformin were associated with the greatest reduction in risk of cancer. Metformin dose usually increases with increasing duration of use, therefore dose variables can be confounded by duration. This could produce a survival bias, with higher doses spuriously associated with reduced cancer because patients have survived to receive a higher dose. This is the reason for stratification by length of follow-up (although residual confounding may still be present).

Within the known limitations of observational data, we are confident in our study design and data sources. The data sources used were independent of each other, and they provided objective measures of exposure and outcome. The diabetic population of Tayside is well-defined, and the MEMO database used to identify metformin users has been widely used for drug safety research[11]. The likelihood of misclassification of metformin exposure due to data error is low because we ensured that all patients had multiple metformin prescriptions. We were otherwise unable to judge whether patients actually took the metformin as prescribed, although we know that the drug was collected from the pharmacies[11]. We are confident that we eliminated survival bias in our choice of comparators. The national cancer registry (SMR6) was used to identify cancer diagnoses. Specificity is likely to be higher than sensitivity in this register, but if any cancer diagnoses were missed, this would not occur differentially with respect to metformin status.

This study has produced sufficient epidemiological evidence that metformin reduces the risk of cancer to make further investigation a high priority. A plausible biological mechanism mechanism hinges on the discovery that the upstream LKB1 regulator of AMPK is a tumour suppressor, and that activation of AMPK by LKB1 plays an important role in inhibiting cell growth when cellular energy levels are low[17]. Metformin activates AMPK by inhibiting mitochondrial respiration and increasing 5'-AMP which enhance activation of AMPK by LKB1[1]. The blood glucose lowering properties of metformin are mediated through AMPK restoring cellular energy levels by phosphorylating regulatory proteins that lead to stimulation of glucose

uptake into muscle tissues and inhibition of gluconeogenesis in the liver. The anti-cancer properties of metformin are likely to be mediated by AMPK ability to preserve cellular energy levels by phosphorylating proteins such as p27KIP and TSC2 that lead to inhibition of cell growth and proliferation signalling networks[18,19].

Prior to the discovery that the LKB1 tumour suppressor activated AMPK, there was little interest in the role of AMPK in cancer. However, the ability of AMPK to gauge and control cellular energy places it in an ideal position to ensure that cell growth and proliferation is coupled to the availability of a sufficient supply of nutrients and energy. Recent laboratory evidence showing that three distinct drugs activate AMPK delayed tumourigenesis in tumour prone mice, suggests that activators of AMPK could have therapeutic benefit for the treatment of cancer in humans[4]. The protective effects of metformin on cancer development could potentially be rapid, and may occur at quite a late stage of cancer development. Treatment of animal cells with metformin significantly activates the AMPK pathway within 30 minutes[20-22]. Metformin also inhibits growth of cancer cells[5] or mouse embryonic stem cells[4] within 1-2 days. We believe that there is now a strong case to conduct a randomized trial to establish whether metformin is protective in a population at high risk for cancer.

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DISCLOSURE

No Conflicts of Interest.

REFERENCES

1. Hardie DG. AMP-activated protein kinase as a drug target. *Annu Rev Pharmacol Toxicol* 2007; 47:185-210.

2. Schneider MB, Matsuzaki H, Haorah J, Ulrich A, Stabdop J, Ding XZ, Adrian TE, Pour PM. Prevention of pancreatic induction in hamsters by metformin. *Gastroenterology* 2001; 120:1263-1270.

3. Anisimov VN, Egormin PA, Bershtein LM, Zabezhinskii MA, Piskunova TS, Popovich IG, Semenchenko IV. Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice. *Bull Exp Biol Med* 2005; 139:721-723.

4. Huang X, Wellschleger S, Shapiro N, McGuire VA, Sakamoto K, Woods YL, McBurnie W, Fleming S, Alessi DR. Important role of the LKB1-AMPK pathway in suppressing tumourigenesis in PTEN deficient mice. Submitted to *Biochem J;* 2008: http://www.biochemj.org/bj/imps/abs/BJ20080557.htm.

5. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Research* 2006; 66:10267-73.

6. Evans JMM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Reduced risk of cancer identified in patients with type 2 diabetes who use metformin: Results from a pilot case-control study. *Br Med J* 2005; 330: 1304-1305.

7. Bowker SL, Majumdar SR, Veugelers P, Johnson JA**.** Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 2006; 29:254-258.

8. Moore MA, Park CB, Tsuda H. Implications of the hyperinsulinaemia-diabetes-cancer link for preventive efforts. *Eur J Cancer Prev* 1998; 7:89-107.

9. Garmendia ML, Pereira A, Alvarado ME, Atalah E. Relation between Insulin Resistance and Breast Cancer among Chilean Women. *Ann Epidemiol* 2007; 17:403-409.

10. Morris AD, Boyle DIR, MacAlpine R, Emslie-Smith A, Jung RT, Newton RW, MacDonald TM for the DARTS/MEMO Collaboration. The diabetes audit and research in Tayside Scotland (DARTS) study: electronic record-linkage to create a diabetes register. *Br Med J* 1997; 315:524-528.

11. Evans JMM, McDevitt DG, MacDonald TM. The Tayside Medicines Monitoring Unit (MEMO): A record-linkage system for pharmacovigilance. *Pharmaceut Med* 1995; 9:177-184.

12. Information and Statistics Division Scotland. Scottish Cancer Registry. *Accessed online.* http://www.isdscotland.org/isd/3535.html

13. World Health Organization. Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death. Ninth revision. Geneva: World Health Organization 1978.

14. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. Tenth revision. Geneva: World Health Organization 1994.

15. Carstairs V. Deprivation and health in Scotland. *Health Bull (Edinb)* 1990;48: 162-175.

16. Evans JMM, Ogston SA, Emslie-Smith A, Morris AD. Risks of mortality and adverse cardiovascular outcomes in type 2 diabetes: A comparison of patients treated with sulphonylureas and metformin. *Diabetologia* 2006; 49:930-936.

17. Alessi D R, Sakamoto K Bayascas JR. LKB1-Dependent signaling pathways. *Annu Rev Biochem* 2006; 75: 137-163

18. Inoki K, Guan K-l. Complexity of the TOR signalling network. *Trends in Cell Biology* 2006; 16:206-212.

19. Carling D. The role of the AMP-activated protein kinase in the regulation of energy homeostasis. *Novartis Found Symp* 2007; 286:72-81.

20. Fryer LG, Parbu-Patel A, Carling D. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 2002; 277:25226-25232.

21. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* 2003; 144, 5179-5183.

22. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear L J, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; 108, 1167-1174.

Table 1: Baseline characteristics and results of Cox regression analysis for incidence of cancer, among metformin users and comparators

	Metformin users (n = 4,085) N (%)	Comparators (n = 4,085) N (%)	No. of diagnosed cancers	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio[*] (95% CI)
Metformin use					
Comparators	4,085	-	474	1.00	1.00
Metformin users	-	4,085	297	0.46 (0.40 – 0.53)	0.63 (0.53 – 0.75)
Sex					
Females	1848 (45.9)	1875 (45.2)	315	1.00	1.00
Males	2237 (54.1)	2210 (54.8)	456	1.26 (1.09 – 1.45)	1.37 (1.18 – 1.59)
Age (yrs)					
35-55	1001 (24.5)	533 (13.1)	51	1.00	1.00
56-63	964 (23.6)	647 (15.8)	138	2.72 (1.97 – 3.75)	2.66 (1.92 – 3.68)
64-69	865 (21.2)	691 (16.9)	160	3.40 (2.48 – 4.67)	3.13 (2.27 – 4.32)
70-76	781 (19.1)	939 (23.0)	205	4.61 (3.39 – 6.28)	4.09 (2.98 – 5.61)
77-100	474 (11.6)	1275 (31.2)	217	5.95 (4.37 – 8.12)	4.86 (3.51 – 6.73)
Smoking status					
Current	577 (14.1)	558 (13.7)	109	1.00	1.00
Ex	1015 (24.9)	866 (21.2)	177	0.99 (0.78 – 1.26)	0.77 (0.60 – 0.98)
Never	1637 (40.1)	1411 (34.5)	271	0.87 (0.69 – 1.09)	0.75 (0.60 - 0.94)
Not known	856 (21.0)	1250 (30.6)	214	1.19 (0.94 – 1.51)	0.91 (0.72 – 1.16)
Carstairs deprivation category					
1 (least deprived)	224 (5.5)	206 (5.0)	46	1.00	1.00
2	805 (19.7)	902 (22.1)	162	0.75 (0.56 – 1.01)	0.74 (0.55 – 0.99)
3	1129 (27.6)	1171 (28.7)	223	0.80 0.61 – 1.07)	0.82 (0.62 – 1.09)

4	458 (11.2)	488 (12.0)	99	0.78 (0.56 – 1.08)	0.81 (0.58 – 1.12)
5	521 (12.8)	457 (11.2)	77	0.66 (0.47 – 0.92)	0.69 (0.49 – 0.97)
6	603 (14.8)	579 (14.2)	106	0.71 (0.52 – 0.98)	0.79 (0.58 – 1.09)
7 (most deprived)	345 (8.5)	282 (6.9)	58	0.72 (0.50 – 1.04)	0.83 (0.58 – 1.19)
BMI					
Mean (sd)	30.7 (3.5)	28.6 (3.1)	-	0.93 (0.91 – 0.95)	0.98 (0.96 – 1.00)
HbA1c					
Mean (sd)	7.9 (1.0)	7.2 (1.2)	-	0.77 (0.72 – 0.82)	0.91 (0.84 – 0.98)
Use of insulin					
No use	3833 (93.8)	3512 (86.0)	696	1.00	1.00
Use within 1 year	252 (6.2)	573 (14.0)	75	0.99 (0.85 – 1.15)	1.13 (0.97 – 1.33)
Use of sulphonylureas					
No use	2196 (53.8)	1996 (73.3)	483	1.00	1.00
Use within 3 months	1889 (46.2)	1089 (26.7)	288	1.00 (0.78 – 1.28)	1.12 (0.87 – 1.47)

*Adjusted for all covariates

Table 2: Unadjusted and adjusted hazard ratios for secondary outcomes; with adjusted hazard ratios (with 95% CI) for incidence of cancer stratified by maximum prescribed dose and duration of follow-up (with comparators as the reference category).

	No (%) with outcome	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio[*] (95% CI)
Incidence of bowel cancer			
Comparators	76 (1.9)	1.00	1.00
Users	40 (1.0)	0.41 (0.28 – 0.61)	0.60 (0.38 – 0.94)
Incidence of lung cancer			
Comparators	58 (1.4)	1.00	1.00
Users	35 (0.9)	0.49 (0.32 – 0.74)	0.70 (0.43 – 1.15)
Incidence of female breast cancer			
Comparators	41 (2.2)	1.00	1.00
Users	24 (1.3)	0.44 (0.26 – 0.73)	0.60 (0.32 – 1.10)
Overall mortality			
Comparators	1422 (34.8)	1.00	1.00
Users	609 (14.9)	0.32 (0.29 – 0.35)	0.42 (0.38- 0.47)
Mortality from cancer			
Comparators	248 (6.1)	1.00	1.00
Users	123 (3.0)	0.48 (0.39 – 0.60)	0.63 (0.49 - 0.81)
Incidence of cancer			
Maximum prescribed dose during follow up (number of patients)	**Adjusted hazard ratios (95% CI)[*]**	**Adjusted hazard ratios (95% CI)[*]**	**Adjusted hazard ratios (95% CI)[*]**
	< 2 years follow-up	2-4 years follow-up	>4 years follow-up
Low (1,017)	3.15 (1.92 – 5.18)	0.99 (0.44 – 2.25)	0.16 (0.06 – 0.44)
Medium (2,090)	1.94 (1.20 – 3.13)	0.51 (0.31 – 0.82)	0.40 (0.27 – 0.60)
High (978)	2.76 (0.56 – 13.45)	0.28 (0.12 – 0.70)	0.15 (0.09 – 0.25)

FIGURE LEGENDS

Fig 1: Flow chart showing how metformin users and comparators were selected for the study.

Fig 2: Kaplan Meier plot with 95% confidence intervals showing time to cancer among metformin users and comparators.