Metformin use and the incidence of colorectal and lung cancer in patients with type 2 diabetes mellitus

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Abstract:

Background: Metformin is an oral hypoglycemic agent (OHA) commonly prescribed in patients with type 2 diabetes (T2DM). *In vivo* and *in vitro* studies have suggested that metformin has potential anti-tumour effects and may thus decrease cancer incidence. While observational studies have also suggested similar effects, they had important methodological limitation so that their results need to be confirmed using more rigorous methods.

Objective: To assess whether the anti-diabetic medication metformin is associated with a decreased incidence of lung and colorectal cancer in patients with T2DM.

Research Design and Methods: Using data from the United Kingdom General Practice Research Database, a large computerized database composed of primary care longitudinal patient records; two population-based cohort studies were conducted, one investigating colorectal cancer incidence in patients with T2DM and the other focused on lung cancer incidence in patients with T2DM. All colorectal or lung cancer cases occurring during follow-up of the cohort (depending on cancer being studied) of all patients with T2DM who had been prescribed at least one OHAs between 1988 and 2009 were identified. For each study, up to 10 randomly selected controls were selected from the cohort within the risk set and matched on age, sex, calendar year of cohort entry, and duration of follow-up. Primary exposure was defined as ever exposure to metformin prior to the risk set follow-up time, as well as in terms of number of metformin prescriptions received to examine the dose-response relationship. Conditional logistic regression was used to estimate adjusted rate ratios (RRs) and corresponding 95% confidence intervals (CIs).

Results: The colorectal cancer cohort was comprised of 115,578 users of OHAs, including 607 cases of colorectal cancer and 5837 matched controls. Metformin was not associated with a change in the incidence of colorectal cancer (RR: 0.94; 95% CI: 0.7-1.2). The lung cancer cohort consisted of 115,923 users of OHAs, where 808 cases of lung cancer were matched with 7764 controls. Metformin had no impact on the risk of lung cancer (RR: 0.94; 95% CI: 0.8-1.2). There were also no significant changes observed in incidence of either cancer when examining the number of prescriptions of metformin patients' received.

Conclusions: Metformin use is not associated with a decreased colorectal or lung cancer incidence in patients with T2DM. These findings contradict decreased cancer incidence associated with metformin use reported in previous observational studies that did not properly account for time.

Keywords: Type 2 Diabetes (T2DM), Oral Hypoglycemic Agents (OHAs), Metformin, Lung Cancer, Colorectal Cancer, United Kingdom General Practice Research Database (GPRD)

Résumé:

Contexte: Metformine est un agent hypoglycémique oral (AHO) couramment prescrit chez les patients avec diabète de type 2 (DNID). *In vivo* et *in vitro* ont suggéré que la metformine a un potentiel anti-tumoral effets et donc le potentiel pour diminuer l'incidence du cancer. Alors que les études observationnelles ont également suggéré revendications similaires, ils avaient d'importantes limites méthodologiques afin que leurs résultats doivent être confirmés en utilisant des méthodes plus rigoureuses.

Objective: Évaluez si la médication contre le diabète, metformine, est associé avec une diminution du taux d'incidence de cancer des poumons, et colorectal chez les patients avec DNID.

Méthodes d'analyse et de conception: En utilisant les données de la base de données du United Kingdom General Practice Research Database (GPRD), une grande base de données informatisée composée de dossiers de soins primaires aux patients longitudinale, deux basées sur la population des études de cohorte ont été menées, l'une d'incidence du cancer colorectal enquêter chez les patients atteints de DNID et l'autre axée sur l'incidence du cancer du poumon chez les patients atteints de DNID. Tous les cas de cancer colorectal ou du poumon survenant au cours du suivi de la cohorte (selon la cohorte du cancer) de tous les patients atteints de DIND qui avaient été prescrits au moins un AHO entre 1988 et 2009 ont été identifiés. Jusqu'à 10 contrôles choisis au hasard ont été choisis au sein de la cohorte des risques fixés et appariés selon l'âge, le sexe, l'année civile de l'entrée dans la cohorte, et la durée du suivi. L'exposition primaire a été définie comme l'exposition à la metformine jamais avant l'ensemble des risques de suivi du temps, ainsi qu'en termes de nombre d'ordonnances a reçu la metformine pour examiner la relation dose-

réponse. Une régression logistique conditionnelle a été utilisée pour estimer les rapports de taux ajustés (RRs) et les intervalles de confiance à 95% (IC).

Résultats: La cohorte du cancer colorectal été compris de 115 578 utilisateurs des AHO, incluant 607 cas de cancer colorectal et 5837 contrôles jumelées. Metformine n'étais pas associé avec la risque du cancer colorectal (RR: 0.94; 95% IC: 0.7-1.2). De plus, il n'y avait aucune changement significatif observer concernant l'incidence des deux cancers quand le nombre de prescription de metformine et pris en compte. La cohorte se composait de cancer du poumon 115 923 utilisateurs des AHOs, où 808 cas de cancer du poumon ont été appariés à 7764 contrôles. La metformine n'a eu aucune incidence sur le risque de cancer du poumon (RR: 0.94; 95% IC: 0.8-1.2). Il n'y avait aussi pas de changements significatifs observés dans l'incidence du cancer soit lors de l'examen pour le nombre de prescriptions de metformine reçue.

Conclusions: L'utilisation de metformine n'est pas associée à une diminution colorectal ou de l'incidence du cancer du poumon chez les patients atteints de DNID. Ces résultats contredisent l'incidence du cancer ont diminué associés à l'utilisation de metformine rapportés dans les études observationnelles précédentes qui n'ont pas correctement en compte le temps.

Mots Clés: Diabète de type 2 (DNID), Agent hypoglycémique oral (AHOs), Metformine, Cancer des poumons, Cancer colorectal, United Kingdom General Practice Research Database (GPRD)

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Abbreviations

ACF	rectal aberrant crypt foci
AMPK	5' adenosine monophosphate-activated protein kinase
BMI	body mass index
CI	confidence intervals
COPD	chronic obstructive pulmonary disease
CRC	colorectal cancer
DPP-4	dipeptidyl peptidase 4
GLP-1	glucagon-like peptide-1
GPRD	United Kingdom General Practice Research Database
HbA1c	glycosylated haemoglobin levels
HCC	hepatocellular cancer
HR	hazard ratio
LKB1	serine–threonine liver kinase B1
NHL	non-Hodgkin lymphoma
NSAIDs	non-steroidal anti-inflammatory drugs
OHAs	oral hypoglycaemic agents
OR	odds ratio
RR	rate ratio
SD	standard deviation
T2DM	type 2 diabetes mellitus
TZD	thiazolidinedione
UTS	up-to-standard
WHO	World Health Organization

Tables and Figures

Tables:

Figures:

1. Introduction

Multiple studies have shown that patients with type 2 diabetes mellitus (T2DM) are more likely to develop cancer and, once diagnosed, have a worse prognosis than the non-diabetic, general population (1-5). As a result, the possible influence of diabetes and anti-diabetic agents on cancer incidence has recently become a heavily debated and studied topic in diabetes and cancer research circles (6-13). According to some studies including the work of Currie *et al.* 2009 (14), diabetic patients using the oral hypoglycemic agent (OHA) sulfonylureas have a 36% increased risk in colorectal cancer risk compared to patients on other diabetes medications. While a different study conducted by Hemkens *et al.* in 2009 (15), showed that patients with T2DM who were prescribed insulin glargine were observed to have a 9-31% increased risk of cancer, depending on their dosage, compared to patients with T2DM not prescribed the insulin. In studies looking at patients with T2DM use of the OHA metformin and corollary changes in cancer incidence, patients with T2DM prescribed metformin exhibited a 23% reduction in overall cancer incidence (16) and a 31% reduction in overall cancer-related mortality compared to patients not prescribed metformin (8).

Despite the findings of these and other observational studies (6, 8, 12, 17-19), speculation and skepticism still remains as to what extent T2DM medication truly does influence cancer risk. In more recent research, a number of studies have observed that metformin, insulin and OHAs do not significantly affect cancer incidence and have no influence on overall mortality when prescribed to patients with T2DM (9, 20, 21). Such discrepancies in study findings are, in primarily due to faulty study designs and methodologies; the results of several studies have been skewed by small sample sizes, improperly used analysis techniques as well as selection and

information biases, while some studies additionally exhibit evidence of residual confounding, immortality bias, and allocation bias (8, 10, 14, 17, 18, 22-25).

Of the cancers investigated to date, lung cancer is one of the few cancers types that T2DM and obesity are not thought to cause an increase in overall incidence (26-29). Due to this, studying lung cancer incidence and metformin use in a T2DM cohort creates results that are less confounded than many of the studies focused on metformin and cancers significantly associated to obesity and T2DM. There is yet to be an observational study with appropriate methods, power and sample size that has been performed to look specifically at lung cancer, although a number of experimental and clinical studies have been conducted (30-32). Colorectal cancer is also one of the site-specific cancers for which the relationship between metformin use and cancer risk is still unclear (8, 11, 19, 33). Furthermore, recent *in vitro*, *in vivo*, and experimental research has suggested that metformin may also reduce the risk of colorectal cancer (19, 20, 33, 34), while observational studies focusing on the association between metformin and colorectal cancer have produced conflicting findings (11, 33).

2. Study Aim

The goal of this thesis project is evaluate whether metformin decreases either, or both, lung and colorectal cancer risk in patients with T2DM. Using data from the United Kingdom General Practice Research Database (GPRD), the two cohort studies in this project have been conducted with improved design and methodology, as well as longer patient follow-up time in hopes to avoid the errors of previous studies. It is hoped that these two studies focused on two very different cancer types, will help put an end to the number of poorly conducted studies in the research of metformin, cancer incidence and T2DM, and discourage researchers from creating unnecessary and misleading meta-analyses based on observational studies that are plagued with uncontrolled biases and poor methods. Metformin may possess the potential to influence the way we treat and prevent cancer, but many of the observational studies that have been conducted to date have produced alarmingly deceptive results and conclusions. Using a vigorous pharmacoepidemiologic approach, these two population-based studies will provide the medical community with strong evidence on the long-term effects of the most widely used anti-diabetic agent. If metformin is indeed associated with a decreased risk of either colorectal or lung cancer, it can provide additional rationale to conduct randomized controlled trials in high risk patients

3. Review of Literature

3.1 Introduction

Type 2 Diabetes Mellitus (T2DM) has become one of the fastest growing disease epidemics in the world. According to the World Health Organization (WHO), as of January 2011 over 200 million people worldwide live with diabetes and, of those patients, 90% of them have been diagnosed with T2DM. In 2004 alone, WHO estimated that 3.4 million patients died from complications related to high blood sugar and projected that by the year 2030, diabetes-related deaths will have doubled (35, 36). Given the dramatic increase of diagnosed patients, the complexity of the disease, the many associated diseases, disorders and comorbidity factors that may also affect a patient and their prognosis, gaining a greater understanding of the disease has become a priority in both the clinical and research sectors (28, 36-38).

3.2 Type 2 diabetes mellitus and cancer

Since the early 1900s, researchers have sought to understand the association between T2DM and cancer incidence (28, 29, 37-39). Researchers have determined that T2DM results from the body's increasing inability to use insulin effectively and is responsible for the chronic elevation of fasting and non-fasting blood levels of insulin and glucose, resulting in hyperinsulinemia and hyperglycaemia. Research has also shown that diabetes is associated with numerous comorbidities including obesity, cardiovascular disease, dyslipidemia, and renal disorders (27, 28, 35, 40).

Risk of Cancer

The increased occurrence of individuals with both T2DM and cancer has prompted researchers to focus on how these two diseases are related and to determine the temporality and strength of their relationship. Recent studies have determined that patients with T2DM are more likely to develop cancer and have a worse prognosis than non-diabetic individuals (1-4, 12, 26). Researchers have also verified that T2DM causes an increase in overall cancer risk for many site-specific cancers, including breast, colorectal, pancreatic, and endometrial, but a possible inverse relationship in prostate cancer (4, 9, 26, 39). Additional research has also been conducted looking at the relationship between T2DM and lung, lymphoma, and renal cancer incidence. Researchers have not been able to conclude if there are any increased risks for patients with T2DM because of methodological flaws, lack of necessary study power, sample size issues, and the inability to correct for substantial confounding variables such as obesity, smoking and other related comorbidities (9, 26, 28, 39, 41).

Studies have shown that the changes in blood glucose, insulin, and insulin-like growth factors (IGF) in patients caused by the progression of T2DM may be responsible for the observed increase in cancer incidence among patients with T2DM (4, 41). Hyperinsulinemia, hyperglycaemia, and insulin resistance – all conditions associated with T2DM – are thought to contribute to the metabolic and hormonal changes that may stimulate new cancer cell proliferation, decrease cellular apoptosis, and increase the survival and progression of early malignant cells via tumour growth (37, 42). Obesity is a comorbidity shown to be associated with the incidence of T2DM and specific cancers including kidney, colon and endometrial (28, 38, 41). Although obesity greatly confounds the possible causal relationship of T2DM and cancer, research has successfully shown that when obesity has been adjusted for, there is still a significant positive correlation between T2DM and cancer incidence (28, 37-39, 42).

Risk of Lung Cancer

General disagreement on the causal relationship between T2DM and lung cancer incidence still exists despite numerous studies exploring this association (26-29, 41). Research has successfully identified a link between a patient's smoking status and the incidence of lung cancer and the prognosis of T2DM; however conclusions regarding possible differences in lung cancer incidence between T2DM and non-diabetic patients have yet to be drawn (28). One of the main reasons for inconclusive results in the studies looking at T2DM and lung cancer is researchers' inability to correctly adjust for smoking in the model. Despite researchers' attempts to adjust for smoking in hopes of eliminating the effects of this confounder on the study model, their success has been limited, and residual confounding continues to obscure other causal relationships that might exist (27, 29).

Researchers' failure to account for the overall shorter life span of patients with T2DM compared to their non-diseased counterparts has also introduced study flaws leading to questionable results. Survivor bias caused by the reduced life expectancy observed in diabetes patients has led some researchers to conclude that T2DM may decrease the risk of, and be protective against, lung cancer (35). What has not been taken into account in these studies is that many patients with T2DM do not live long enough for cigarette smoke to exert its damage on the lungs; most patients die from another health-related complication before lung cancer has had the opportunity to proliferate and metastasize (29, 41). The lower incidence of lung cancer in patients with T2DM is therefore an artificial decrease resulting from the omission of life expectancy as a significant confounding variable. The influence of body mass index (BMI), waist circumference, and obesity on the relationship between T2DM and lung cancer is similarly unclear, despite extensive research on the subject (28).

Risk of Colorectal Cancer

Although some speculation remains, the majority of studies on the relationship between T2DM and the incidence of colorectal cancer have shown that there is at least a modest increase of cancer incidence in patients diagnosed with T2DM compared to those without T2DM. This association was also observed in most studies in which the models had been adjusted for possible confounding variables such as obesity and physical inactivity. This detected increase in cancer incidence has been show to exist in both genders, although some studies suggest that the increase in colorectal cancer incidence is higher among male patients with T2DM than females (3, 11, 26, 43, 44).

Problems with study design and methodology have afflicted the vast majority of studies dedicated to looking at the relationship between T2DM and colorectal cancer. Failure to take into account the possibility of reverse causality, small sample size, low power, selection biases, information biases, misclassification of the exposures and outcomes, and incorrectly adjusting for potentially confounding variables are among the major methodological issues that have led researchers to draw misleading conclusions from study results. (17, 44)

3.3 Type 2 diabetes medications, type 2 diabetes and cancer

In addition to the increasingly complex relationship between T2DM and cancer, researchers' have hypothesized that the type of medications a patient has been prescribed may also affect the risk of cancer incidence and cancer-related mortality (6-8, 10, 14, 18). Despite claims that many diagnosed patients with T2DM are able to control their disease and lower their blood glucose levels by changing their lifestyle habits and losing weight, this is, in reality, rarely the case (36, 45). Most newly diagnosed patients with T2DM are prescribed an OHA soon after

their initial diagnosis. Depending on disease progression and a patient's individual characteristics, a doctor may prescribe a patient an OHA that increases insulin production, such as sulfonylureas, or an OHA that improves the effectiveness of insulin, such as metformin. As a patient`s diabetes progressively worsens and insulin resistance begins to occur, he or she may be prescribed other OHAs or insulin to replace or supplement their current treatment. These additional prescriptions include the insulin sensitizer thiazolidinedione (TZD) or a combination OHA drugs such as amylin or incretin (24, 25). To combat increasing insulin resistance, a patient may also be required to start treatment on a rapid short-action, or long acting, insulin or insulin analog such as NovoRapid, Humulin-R, or Lantus (7, 45). Many researchers have hypothesized that many of these drugs may greatly influence the risk and incidence of cancers. However, it is still unclear if this is the case or if, instead, the inverse is true, and the progression of diabetes and undetected cancer changes are responsible for the need for changes in medications (18, 20, 21, 46).

OHAs and Cancer

Two groups of OHAs – insulin producers (sulfonylureas) and insulin sensitizers (biaguanides and thiazolidinediones) – have garnered significant attention for their potential influence on overall cancer risk in both patients with T2DM and without T2DM. Extensive laboratory and clinical research has shown that insulin producers and sensitizers may have the capability to decelerate cancer progression in already-diagnosed patients and decrease overall cancer mortality (6, 8, 12, 14, 17, 18, 20, 22-24, 37, 39, 42, 47-49). Hypotheses suggest that sulfonylureas (glyburide, gliclazide, glipizide, glimepiride, glibornuride, gliquidone, tolbutamide, chlorpropamide, tolazamide, or acetohexamide) may not only encourage new insulin production, but also to contribute to the body's increase in insulin resistance thus leading

to a possible influence on the increase of cancer incidence in patients with T2DM versus non-diabetic patients not receiving this type of treatment (11, 14, 18). Biguanides (metformin), and thiazolidinediones (pioglitazone, rosiglitazone, ciglitazone, and troglitazone), both types of insulin sensitizers, are used to lower glucose, insulin and fatty acid levels, and may possess unique anti-carcinogenic properties. Researchers believe that because of their ability to decrease the levels of circulating insulin as well as insulin resistance in patients with T2DM, they may serve a protective function and decrease both overall cancer risk and the incidence of several site-specific cancers (4, 8, 12, 14, 20, 23, 26, 41). However, not all observational studies agree on either of those claims (6, 7, 9, 20).

Insulin and Cancer

Insulin, much like OHAs, has been hypothesized to have an effect on the risk of cancer and cancer mortality in patients with T2DM. Insulin analogs (insulin aspart, insulin lispro, insulin glargine, insulin detemir, and insulin analog B10Asp) are usually introduced as treatment in combination with OHA therapy when OHA therapy alone has been deemed ineffective against the progression of T2DM and further assistance in insulin control is necessary, due to a patient's continuing decline in endogenous insulin production (11, 26). Insulin analogs have the ability to replicate the true physiological function of insulin (37) but do so differently than human insulin. Compared to human insulin, insulin analogs have an altered amino acid sequence, a different metabolic profile, an enhanced cell-growth and exhibit mitogenic potencies that may enhance the risk of malignancy – properties that have led some researchers to investigate whether these drugs have carcinogenic potentials (7, 21, 37).

Many researchers previously postulated that the recombinant insulin analogue insulin glargine, as well as other exogenous insulin types, increased the risk of cancer in patients using it as a T2DM treatment compared to those individuals who relied on other medications (11, 15, 18, 50). However, further analyses concluded that this might not actually be the case, as these studies may have been affected by methodological errors, such as selection biases, immortal time bias, incorrectly defined time-to-event and patient follow-up time as well as statistical deficiencies in the method sections (18, 21, 22, 41). *In vivo and in vitro* studies have shown that there may be a trend of cancer increase with the continuous use of exogenous insulin, but the dosage used in many of these studies is unknown or at a level that would not be appropriate for human use (15, 21, 50). Recent research has shown that the epidemiological link between insulin treatments and increase of cancer may be overestimated and that the influence of many other covariates, including disease severity and family medical history may play a role in cancer incidence among patients with T2DM. Despite numerous studies investigating how diabetes, insulin use, and cancer interact in patients with T2DM, research has thus far failed to clearly define the relationship between these factors (21, 43).

3.4 Metformin, type 2 diabetes and cancer

The OHA metformin, a drug introduced in the 1950s, is a common medication prescribed to patients with T2DM. This OHA is a type of biguanide derivative; it works as an insulin sensitizer and is used to reduce insulin resistance and inhibit hepatic glucose production. Metformin reduces the levels of circulating glucose and decreases plasma insulin levels in patients, helping to reduce the incidence of diabetes-related complications and allowing patients to better control their glycemic levels in the long-term. Metformin is also a very desirable drug to prescribe based on its short list of serious side effects and its level of safety when compared to

many other OHAs. In addition, metformin is an inexpensive drug that has a low level of toxicity and no major influence on weight (as observed in animal models). Metformin, unlike the insulin sensitizers phenformin, buformin and troglitazone that have been removed from most markets, has not been linked to a significant increase in the incidence of lactic acidosis and other severe side effects in prescribed patients (24, 42, 46, 51-53) .

Besides being an effective first-line, initial therapy for T2DM, metformin is also used in the treatment of polycystic ovary syndrome, non-alcoholic fatty liver disease, and premature puberty; historically, it was also used as a treatment for polyuria and halitosis (42, 51). Metformin is now currently being explored by researchers as a type of potential preventative treatment for T2DM, as a possible treatment and prevention agent for cancer, and as a weight loss aid for patients using anti-depressants (16, 24, 46, 49, 51, 54). Based on the positive preliminary findings of studies involving metformin, it is highly likely researchers will continue to investigate other potential pharmaceutical applications of the drug beyond its current proven effectiveness in controlling blood glucose levels and improving an individual's response to insulin (42, 46, 55).

Metformin is thought to possess numerous abilities that may influence the suppression of cancer growth directly and indirectly (37, 49, 56). Some of the potential capabilities of metformin that have been studied in *in vitro and in vivo* models include the ability to indirectly decrease circulating insulin and glucose levels, decrease plasma insulin levels in patients and inhibit hepatic glucose production. Metformin has been hypothesized to have the direct ability to decrease general protein synthesis and inhibit the unfolded protein response leading to tumour gene suppression through its ability to impair the mitochondrial adenosine-5'-triphosphate (ATP) production leading to the activation of the liver kinase B1 (LKB1)-5' AMP-activated protein

kinase (AMPK) mediated signaling pathway. Metformin may also possess the ability to active the immune system, induce cell cycle arrest and initiate cell apoptosis (24, 32, 42, 49). Other mechanisms that researcher have hypothesized that metformin may possibly possess include the ability to eradicate cancer stem cells in breast cancer, the ability to activate the immune system, and induce cell cycle arrest and possibly apoptosis (8, 24, 26, 32, 37, 42, 49). These discoveries have been observed in laboratory studies, and *in vivo* research has shown that metformin may be a safe and promising candidate for chemoprevention of colorectal cancer in all at-risk patients. Similar findings have been observed in both *in vivo and in vitro* lung cancer studies. However, very few observational studies have provided evidence significant enough to justify the proposal of using metformin in clinical trials for both treatment and prevention of colorectal and lung cancer (11, 26, 46, 51). In clinical research, researchers have speculated that metformin may be associated with the decreased risk of developing certain cancers and may function as a potentially preventative anticancer agent (6, 8, 16). Metformin has additionally been investigated as an agent that may decrease the risk of cancer-related mortality (12, 18, 22, 33), as some studies have shown patients to respond more successfully to chemotherapy when metformin is paired with this treatment (5, 32, 41). Although these positive results are certainly a motive to conduct further research, these evidence alone are not significant enough to initiate further clinical trials in this area unless evidence can be derived from observational studies that are free of major flaws and confounding.

3.5 Metformin, type 2 diabetes and cancer studies

One of the first studies commenting on the possible influence of metformin on cancer incidence was the 2004 retrospective cohort study conducted by Yang *et al.* (11). Although the

study had been focused on colorectal cancer risk among patients with T2DM who were prescribed insulin therapy, their nested case-control analysis had also reported the side observation that metformin. The researchers found that when metformin was used for three or more years, was associated with an odds ratio (OR) (95% CI) of 1.0 (0.6-1.7) and the use of metformin did not have an influence on colorectal cancer incidence (11). The 2005 study conducted by Evans *et al.* (16) was the first study to specifically suggest metformin's potential ability to protect against the development of cancers, and accordingly reduce the risk of cancer incidence, in patients with T2DM using the drug compared to patients who had not been prescribed metformin or were using other OHAs and insulin. In this small, case-control pilot study using data from 1993-2001, the researchers obtained an adjusted OR (95% CI) of 0.77 (0.64-0.92) for patients exposed to metformin since January 1993, compared to those patients who had not been exposed during that same period. From these results, the researchers suggested that metformin may have the ability to reduce overall cancer risk in patients with T2DM, that there is biological plausibility to support this possible finding, and that further research should be conducted to confirm their hypothesis (16).

In the years following the Evans *et al.* study, numerous *in vivo and in vitro* studies reported that metformin had the ability to suppress tumor proliferation and showed evidence that this agent could inhibit cancer cell growth in animal and cell model and could therefore be used in cancer prevention and treatment regimens (51, 53, 57-61). The publication of the study by Evans *et al.* also prompted a slew of other studies building on their initial results and hypotheses. In 2008, Chung *et al.*(62) conducted a case-control study specifically looking at insulin therapy and colorectal adenoma risk (adenomatous polyps that are thought to be the precursor lesion of colorectal cancer) among patients with T2DM. The study produced similar results to those of the

study by Yang *et al.* in 2004 (11), where the adjusted OR (95% CI) for colorectal adenoma risk for patients who had undergone continuous metformin treatment for more than a year was 0.7 (0.3-1.4), compared to those who did not receive chronic metformin treatment (62). Although this study agreed with the results of the previous observational study by Yang *et al.* (11) there were still a number of laboratory studies that provided evidence suggesting otherwise (48). Also conducted in 2008, was a retrospective cohort study by Oliveria *et al*. (25) looking at the incidence of colorectal, bladder, liver, pancreatic and melanoma cancer risk in patients with T2DM using various anti-diabetic pharmacotherapies versus those patients not using such medications. They found that metformin monotherapy had no significant overall effect on the risk of bladder, liver, pancreatic, or melanoma cancers in metformin *ever*-users, compared to *never*-users, although it did significantly decrease colorectal cancer risk in metformin *ever*-users with a reported relative risk (RR) (95% CI) of 0.67 (0.52-0.85) compared to *never* users. A similarly significant decrease in the risk of colorectal cancer was also detected in sulfonylurea/metformin dual therapy *ever*-users with a RR (95% CI) of 0.68 (0.51-0.91) compared to *never*-users (25).

In 2009, three major observational studies in regards to cancer risk and OHA-use in patients with T2DM were published. The studies by Currie *et al.* (14), Libby *et al.* (6), and Monami *et al.* (63) all focused on the changes in general cancer incidence or risk based on OHAs prescribed. These studies all reported findings in regards to the influence of being prescribed metformin versus other OHAs. The retrospective cohort study by Currie *et al.* (14) focused on the influence of all glucose-lowering therapies on the general risk of cancer and, specifically, on breast (in women), colorectal, pancreatic and prostate (in men) cancer in patients with T2DM. In their study, they reported that metformin monotherapy was responsible for a lower risk of cancer,

compared to the hazard ratios of metformin/sulfonylureas combination treatment, sulfonylureas monotherapy, and insulin based therapies. However, only the results of the insulin based therapy was statistically significantly higher. For site-specific cancers, metformin monotherapy was found to be responsible for a significantly lower risk of colorectal cancer, compared to the hazard ratios of other treatments. Metformin was also found to cause a lower risk of pancreatic cancer compared to sulfonylureas and insulin based therapies, but an increased risk compared to the hazard ratio of metformin plus sulfonylureas treatment. No significant differences were found in prostate or breast cancer risk when metformin use was compared to the hazard ratio of the other T2DM treatment options. The researchers consequently concluded that metformin, when used in combination with insulin treatment, may produce a lower risk of cancer compared to insulin treatment alone (14).

The new users of metformin and incidence of cancer cohort study conducted by Libby *et al*. (6), focused on the study of the primary outcome of use of metformin and its association with cancer risk in patients with T2DM. The secondary outcomes of the Libby *et al.* study also included the assessment of bowel, lung and breast cancer (in women), and all-cause mortality and mortality from cancer in patients with T2DM. When compared to patients with T2DM who had no record of metformin use, the adjusted hazard ratio (95% CI) for incidence of cancer among metformin users was significantly lower: 0.6 (0.53-0.75). There was a significantly lower incidence of bowel cancer among metformin users when compared to non-users, whereas there were no significant differences between the two groups for lung and breast cancer. When looking at dose during follow-up, the metformin users that had been followed for 2-4 years showed significantly lower HRs in cancer incidence when prescribed medium or high doses whereas patients with T2DM who were followed for 4 years or more showed significantly lower HRs for

incidence of cancer in all dose groups (6). Monami *et al.* (63) conducted a related matched case-control study in 2009 focused mainly on sulfonylureas influence on cancer incidence in patients with T2DM. However, this study also reported an additional alternative multivariate analysis, which concluded that prolonged exposure to metformin for more than 12 or 36 months was associated with a significant reduction of cancer incidence and produced a protective effect when paired with other OHAs that were suspected of increasing cancer incidence (61).

The cohort study conducted by Ferrara *et al.* (20) looked primarily at the influence of pioglitazone treatment on the risk of cancer incidence. Ferrara and their colleagues were focused on the 10 most common cancer types (prostate, female breast, lung/bronchus, endometrial, colon, non-Hodgkin lymphoma (NHL), pancreatic, kidney/renal pelvis, rectal, and melanoma), but also reported results on metformin use and its influence on the risk of cancer incidence. From their study Ferrara *et al.* reported that *ever*-use of metformin by patients with T2DM was not associated with any significant changes in cancer risk in the cancer types studied, except for a small-observed increase in the risk of pancreatic cancer when compared to patients with *never*-use of metformin.

Unlike the study findings of Ferrara *et* al, another nested case-control study focusing on metformin and cancer incidence in insulin-treated patients with T2DM finding different results was published by Monami *et al.* in 2011 (64)**.** In this study the cohort of patients with T2DM were all treated with different types of insulin. After adjusting for insulin type, metformin use by insulin-receiving patients with T2DM was associated with a reduced incidence of cancer with an OR (95% CI) of 0.46 (0.25-0.85) compared to those patients who did not receive metformin (64).

While most studies focused on metformin use among the general T2DM population, a 2011 study focused on its effects in the Oriental-specific population. This 800,000 patient

prospective Taiwanese cohort study was conducted in 2011 by Lee *et al.* (17). This study was focused on metformin's possible protective effect against cancer risk and focused on esophageal, gastric, colorectal, hepatocellular and pancreatic cancer incidence in a cohort of Oriental patients with T2DM. Taking varying diabetes statuses and medications into account, this study hoped to focus on further investigating changes in site-specific cancer incidence in relation to metformin dosage and duration. Lee *et al.* compared cancer incidence rates in patients with T2DM by categorized their cohort into four groups. These four groups were patients with T2DM who used metformin with at least two different prescriptions during the study periods, patients with T2DM who did not use metformin but had at least two prescriptions of OHAs, patients with T2DM who did not use any OHAs, and a group of subjects without any diagnosis of T2DM or any use of T2DM medications during the study period. The authors concluded that patients on low doses of metformin had a significant decrease in total cancer incidence, colorectal cancer incidence in women, in hepatocellular cancer incidence for men, and in pancreatic cancer incidence for all patients with T2DM who used metformin. It was also found that patients with T2DM that were on metformin had a decreased incidence of total cancer, colorectal cancer, and hepatocellular cancer that was comparable to levels of cancer incidence in the non-diabetic population (17).

Further studies during 2009-2011 were published focusing on more site-specific cancer incidence and metformin use in patients with T2DM. Two of these studies focused on breast cancer incidence and metformin were by Bodmer *et al.* (10) and by Bosco *et al.* (65), while the other by Azoulay *et al.* (9) focused on prostate cancer incidence and metformin use. The two breast cancer studies concluded that metformin use may be protective against breast cancer in peri- and postmenopausal female patients with T2DM and that long term use of metformin was responsible for a decreased risk of breast cancer in female patients with T2DM (10). Unlike the

breast cancer studies, Azoulay *et al.* (9) found that metformin use was not associated with a reduced risk of prostate cancer in patients with T2DM and that, based on their secondary analysis, there may in fact be a higher incidence in prostate cancer depending on dose. Despite these results, Azoulay's team did include a disclaimer stating that these finding should be interpreted with caution because of the complicated relationship between prostate cancer and T2DM. Metformin has been observed to improve the metabolic derangement associated with diabetes (leading to a decrease in prostate cancer risk in patients with T2DM). The use of metformin gives the illusion that it may increase prostate cancer risk among patients with T2DM although other mechanisms and factors are involved (9). Yet another site-specific cancer and mortality observational study that focused on the effects of metformin on clinical outcomes of colorectal cancer in patients with T2DM was published in 2011 by Lee *et al.* (33) In this study, the researchers assessed metformin use and its influence on overall mortality and colorectal cancer-specific mortality in patients with T2DM. Lee *et al.* concluded that T2DM, CRC patients with diabetes who received metformin had a lower overall and colorectal cancer -specific mortality compared to those who did not receive metformin and suggested that metformin should be further studied as a potential anti-tumor agent (33).

Various commentaries, clinical and additional laboratory research were also published during this time exploring the many capabilities metformin may hold in regards to cancer incidence and mortality as a well as focusing on the *in vitro* and *in vivo* study findings involving metformin and its chemopreventive abilities. Many of these publications encouraged and suggested that more observational and clinical research should be conducted on metformin's potential to prevent and suppress cancer tumor growth in patients with T2DM and without T2DM (49, 66). The discussion of utilizing metformin in the prevention of tobacco carcinogen-

induced lung tumorigenesis was introduced in the animal study conducted by Memmott *et al.* (31). In this study, researchers found metformin to be effective in preventing tobacco carcinogen-induced tumor growth in a non-diabetic mouse model of lung tumorigenesis and hypothesized that the drug may be a chemopreventive agent for lung cancer through the activation of AMPK, which inhibits tumor formation in lung cancer. Memmott *et al.* consequently concluded that it would be in the best interest of the research community to commence clinical testing of metformin as a chemopreventive agent for lung cancer to ascertain whether their hypotheses were correct. Following this study was a commentary by Antonoff and D'Cunha (30) further elaborating on the mechanisms responsible for metformin's chemopreventive properties/potential and seconding Memmott et al.'s call for additional clinical trials to examine metformin's ability to prevent tumor growth (68).

Around the same time as Memmott *et al* published their findings, Hosono *et al* produced two studies (48, 67), the first being an animal study focused on metformin's chemopreventive effects and its ability to influence the proteins, mechanisms and pathways responsible for influencing the suppression of colorectal carcinogenesis. In this study, the researchers concluded that metformin did show evidence that it could suppress colonic epithelial proliferation through the activation of the AMPK and the inhibition of certain mTOR pathways, suggesting that metformin may be a safe and promising chemopreventive agent for colorectal cancer (46). Shortly thereafter, Honsono *et al.*'s second study, published a 23-patient pilot clinical trial focused on observing metformin's chemopreventive effect on rectal aberrant crypt foci ([ACF] a surrogate marker for colorectal cancer) in patients without T2DM who had clinically, or historically, been diagnosed with the disease (69). Nine cases treated with metformin and fourteen untreated controls were examined via magnifying colonoscopies at the beginning of the

trial, followed for one month, and then re-examined. The study concluded that metformin clearly suppressed the formation of human colorectal ACF in patients without T2DM when taking a dose of 250 mg/d over 1 month and did not have any impact on blood glucose, insulin resistance, plasma cholesterol, or plasma triglyceride levels. Honsono *et al.* concluded that the ACF suppression by metformin was probably through a direct effect, rather than being mediated by insulin resistance or hyperlipidemia, and more in-depth studies on metformin's potential to be used in CRC chemoprevention in both T2DM and T2DM populations were warranted (69).

In 2010 and 2011, three systematic reviews with meta-analyses were published that focused on the research interest area of metformin and cancer incidence and mortality in patients with T2DM (8, 13, 19). The first, by DeCensi *et al*. (19), included five independent observational studies for an all-cancer site meta-analysis (6, 12, 14, 18, 63) and six independent observational studies for a single-cancer site meta-analysis. The studies chosen for this meta-analysis were selected based on the observational studies' interventions, the populations studied, and the researchers' ability to correctly report true cancer incidence or mortality. After showing that there was heterogeneity between the trials and no evidence of publication bias, the researchers determined that there was a 31% reduction in cancer incidence or mortality in patients with T2DM that had been prescribed metformin compared to those who were prescribed other anti-diabetic agents. DeCensi *et al.* continued their analysis by separately analyzing incidence of general cancer and general cancer mortality, and observed a significant reduction of both outcomes among patients with T2DM who were metformin users. The researchers additionally noted a dose-dependent trend, where metformin's effect on decreasing cancer incidence increased by each additional year of using the OHA. Looking specifically at metformin's association with the changes of colorectal cancer incidence (6, 11, 14)**.** In the final portion of

their study, DeCensi *et al.* reported another inverse relationship between metformin and cancer incidence, but unlike the general cancer estimate, this decrease in incidence was not significant (8).

A second meta-analysis that was published was by Johnson and Bowker (13) that examined previously conducted major clinical trials to identify the ways in which intensive glycaemic control influenced cancer risk in patients with T2DM. Using the systematic review of trials conducted by Turnbull *et al.* (68) which had been originally conducted to explore the relationship between intensive glucose control and cardiovascular outcomes in patients with T2DM, Johnson and Bowker selected four trials to include in their meta-analysis. The authors concluded that cancer risk was not reduced by improving glycaemic control in patients with type 2 diabetes. The authors also suggested that despite these results, metformin- an OHA used for glycaemic control might be the exception. Based on the results of only the metformin-specific study (the United Kingdom Prospective Diabetes Study on metformin), the authors concluded that metformin when used as a glycaemic control agent in overweight patients with T2DM compared to just dietary management for conventional glycaemic control for overweight patients with T2DM, had the ability to reduce cancer mortality (13). The authors also suggested that metformin has far more capabilities beyond being a treatment agent for diabetes and, unlike the other glucose control agents, may have a significant influence on reducing cancer incidence and risk (13).

The final systematic review conducted was a meta-analysis by Zhang *et al.*(19) focused on the conclusions of observational studies looking at metformin's effect on colorectal cancer risk in patients with T2DM. Using effect estimates from five different observational studies, the authors found that the pooled relative risk (95% CI) for patients with T2DM treated with

metformin was 0.63 (0.5-0.79), while those who did not receive metformin treatment were found to have a significantly lower risk of colorectal neoplasm (19).

Table 1: Observational studies focused on metformin use and changes in overall, lung or colorectal cancer incidence

Reference	Study Design	Sample Size	Exposure	Cancer	Results: OR/RR/HR (95%CI)
Yang et al 2004 (11)	retrospective cohort	52,872	any vs. no use metformin	colorectal	OR: 1.0 (0.6-1.7)
Evans et al 2005 (16)	case-control	11,876	any vs. no use metformin	overall	OR: 0.77 (0.64-0.92)
Chung et al 2008 (62)	case-control	100	any vs. no use metformin	colorectal	OR: 0.7 (0.3-1.4)
Oliveria et al 2008 (25)	retrospective cohort	191,223	ever vs. never metformin	colorectal	RR: 0.67 (0.52-0.85)
Oliveria et al 2008 (25)	retrospective cohort	191,223	ever vs. never metformin/sulfonylureas	colorectal	RR: 0.68 (0.51-0.91)
Monami et al 2009 (63)	case-control	390	any vs. no metformin use	overall	OR: 0.28 (0.13-0.57)
Libby et al 2009 (6)	retrospective cohort	8,170	any vs. no metformin use	overall	HR: 0.63 (0.49-0.81)
Libby et al 2009 (6)	retrospective cohort	8,170	any vs. no metformin use	lung	HR: 0.70 (0.43-1.15)
Currie et al 2009 (14)	retrospective cohort	62,809	sulfonylureas use vs. metformin use	colorectal	HR: 1.80 (1.29-2.53)
Currie et al 2009 (14)	retrospective cohort	62,809	sulfonylureas/metformin use vs. metformin use	colorectal	HR: 1.43 (1.05-1.94)
Currie et al 2009 (14)	retrospective cohort	62,809	insulin-based use vs. metformin use	colorectal	HR: 1.69 (1.23-2.33)
Lee et al 2011a (17)	prospective cohort	480,984	any vs. no use metformin	colorectal	HR: 0.36 (0.13-0.98)
Lee et al 2011b (33)	retrospective cohort	595	any vs. no metformin use	colorectal	HR: 1.45 (1.09-1.93)
Monami et al 2011 (64)	case-control	1,340	any vs. no use metformin	overall	OR: 0.46 (0.25-0.85)
Ferrara et al 2011 (20)	retrospective cohort	252,467	ever vs. never metformin	colon	HR: 1.0 (0.9-1.2)
Ferrara et al 2011 (20)	retrospective cohort	252,467	ever vs. never metformin	lung	HR: 1.0 (0.8-0.1.1)

3.6 Limitations of studies conducted

Observational Studies

A reoccurring and concerning trend has appeared in many of the studies exploring the relationship between OHAs and cancer incidence and mortality in patients with T2DM. Many of the studies that have found significant changes in cancer incidence and mortality due to metformin use in patients with T2DM have suffered from detrimental biases and confounding factors, and have lacked the necessary follow-up and dose-trend information necessary to provide sound results. Many other issues that have led to discrepancies in study findings include flaws in study design and methodology, and the use of small sample sizes. Unaccounted for residual confounding, the occurrence of immortal time and selection bias, miscalculated person-time of the study populations, the misclassified patients' exposure statuses, and possible reverse causation due to an insufficient length of follow up time may also be responsible for the inconclusive findings (6, 12, 14, 17, 18, 22, 69).

The initial study by Evans *et al.*(16) that sparked an interest in the possibility of metformin as being protective against cancer, provides disclaimers about the study; for instance, the researchers admitted to using a crudely defined case series of cancer patients and also acknowledged that much larger observational studies on site specific cancers as well as better defined, and verified, outcome measurements were necessary to obtain more conclusive results. In addition, the authors did not adjust for other diabetic medications used including insulin and OHAs – factors that could have greatly influenced the adjusted odds ratio.

Like the study by Evans *et al*, the 2011 study by Lee *et al* (17) assessing metformin use and its influence on cancer incident in Oriental populations is plagued with numerous methodological issues. These issues include the differential cohort entry depending on T2DM status and OHAs received and the criteria used to define group status. To form their T2DM cohort, the researchers selected subjects who were at least twenty years or older, were diabetes

and cancer free on January 1st 2000, and were non-users of diabetes medication from 1996-1999. They then stated that to be included in the cohort, patients were determined to be diagnosed with T2DM if they had a record of T2DM within one year during 2000-2007. Patients were categorized into four groups: the first group was classified as metformin users only if they had had at least two prescriptions of metformin, with the same criteria used to develop a second group of patients with T2DM who were users of other OHAs but not metformin. Lee's team then determined a third group of patients with T2DM based on having a lack of two prescriptions and categorized them as untreated patients with T2DM. The fourth group was then selected from an entirely different cohort of unspecified origin and different exclusion/inclusion criteria. These patients had no diagnosis of T2DM, nor had they used T2DM medication during the study period and had index date randomly selected to them so that they would correspond with the same gender and age as a metformin user. Another major issue that can be identified in this study is immortal time bias and exposure misclassification based on the author's definition of their index date and their previously described methods of deciding exposure classification. The authors determined that the index date was to be defined by three attributes: the date of first metformin prescription (for metformin users), the first diagnosis of T2DM (for T2DM patients without record of medication use), and the author assigned an index date for the non-T2DM group. The authors also decided that, because of limitations regarding the index date assignment for T2DM patients without medication, they would limit their consideration of any effect of metformin use to only those who had had OHAs (17).

The two studies conducted by Bowker *et al* in 2006 and 2010 (18, 22), both suffer from differences in the comparator groups, show immortal time bias (71), and do not properly adjust and assess person-time and time-dependent covariates. In the study conducted by Libby *et al.*(6),

the authors concluded that users of metformin are at a lower risk of cancer compared with patients with T2DM on other treatment regiments, but these results seem to have been distorted by a number of potential biases including selection bias, misclassification bias, and immortal time biases. In addition, the study did not take into account confounding by severity, and was unadjusted for confounders such as insulin use and obesity. Further methodological issues are evident in the matching of cases and control in this study. For instance, after the authors created their cohort, comparators were identified but subsequently discarded for that case (although recycled and made available for other cases) if there had been a record of cancer or death prior to the index date. This process was repeated until a suitable comparator was identified. Libby *et al.* justify this selection of comparators as a way to eliminate survival bias; however it more likely resulted in an incorrect estimate of the number of cases and controls in both the exposed and un-exposed groups, leading to misleading incidence rates.

Meta-analyses

As discussed by Ioannou and Boyko (70) in regards to the meta-analysis conducted by Zhang *et al.*(19) it must be reaffirmed that meta-analyses of observational studies are prone to a number of limitations in their conclusions and need to be interpreted differently than meta-analyses of randomized controlled trials. The meta-analysis by Decensi *et al.* (8) which is based on observational trials, showcases the problems frequently associated with these types of studies. In general, the limitations experienced in observational studies, such as incomplete adjustment of confounding, are still evident when such studies are combined together into a meta-analysis. Major issues that are evident in the studies used in both Zhang et al. and DeCensi et al.'s meta-analyses include confounding by indication and disease severity (6, 11), immortality bias (6, 17, 18**)**, misclassification of person-time (17, 18, 22), misclassification of the exposure (6), different

study entry criteria depending on drug prescribed (17) and an inherent lack of sufficient follow-up time to detect a true effect of metformin on colorectal cancer incidence (14). Also, by combining a group of studies using differently defined exposure comparators, it becomes unclear whether metformin actually has a protective effect or if all the other diabetes treatments grouped together create a much higher hazard rate (70). Instead of conducting meta-analyses filled with inherited issues and biases, it would have been more beneficial for these authors to have conducted full systematic reviews, pointing out strengths and trends of current research regarding cancer outcomes and metformin use in patients with T2DM and thereby providing the research community new hypotheses to test instead of producing results that are unclear, misleading and extrapolated.

The results of the meta-analysis by Johnson and Bowker (13) must also be considered with a degree of scepticism. Although the authors used randomized control trials in their meta-analysis, which help decrease the inherited biases and confounders that are introduced when observational trials are used, they explicatively stated that they did not conduct their own systematic review. Instead, they used previously assembled collection of literature used from a systematic review of large trials conducted by another research group looking at different outcome. The decision to use such trials is understandable, given that their long follow-up time would allow for a meaningful comparison of cancer incidence and outcome in patients with T2DM as well as the effects of dose on cancer incidence and mortality. However, by depending on the search outcomes of this macrovascular study instead of developing their own search criteria, the authors risked overlooking large randomized control trials involving outcomes of cancer incidence or mortality. Furthermore, because the majority of the randomized control trials used in this meta-analysis had been primarily focused on macrovascular outcomes and not cancer

incidence and mortality, the level of accuracy used to measure and determine cancer outcomes is unclear. The validity of classified cases is therefore unknown in these trials. There is also some uncertainty regarding the types of methods used for the analysis of these trials and the selection criteria for the trials included. The suggestions made in regards to metformin's potential influence on cancer mortality based on the findings of only one of the trials used in the meta-analysis is suspect and it is unclear why the author chose to elaborate on this single study's finding. Although the authors specify that their study should be taken as hypothesis-generating and not as conclusive evidence, the number of assumptions made on the trials used indicates that the study's results are questionable, if not misleading (13). As in the meta-analyses conducted by Decensi et al. and Zhang et al., it may have been more beneficial to the research community if Johnson and Bowker had conducted a well-constructed systematic review instead.

3.7 Studies that exemplify proper methods

Despite a trend of highly confounded observational and meta-analyses studies, some observational studies have employed straightforward methods and have correctly classified person-time, introduced a longer and more appropriate follow-up time, avoided issues pertaining to immortal time bias, and have better accounted for confounding by indication and disease severity (9, 20). In the study conducted by Azoulay *et al.* (9), the authors used the GPRD for their study population, allowing for the assessment of the long-term effects of metformin use in a large population. Other techniques and methods employed in this study that enhance the robustness of the results include the use of new users of diabetic OHAs who had not had a prior diagnosis of prostate cancer, a sampling scheme that allowed for time-dependent exposure definition and covariate information, and a focus on methods to decrease the misclassification of cases and exposure. By using sensitivity analyses, including the analysis of cases and matched

controls with at least five years of follow up, the authors were able to show long-term effects of metformin and to remove any issues pertaining to reverse causality.

The 2011 study by Ferrara *et al.* (20) is another example of a well-executed observational study. In this large cohort study the authors properly assesses the data using a Cox Proportional Hazards Regression Model correctly adjusting for time-dependent covariates. Although the study is focused on pioglitazone use and cancer incidence, it correctly displays and discusses the effects of different follow-up periods, dose, and prescriptions used and how they affect the hazard ratios obtained (20).

4. Methods

4.1 Data source:

The data source used for these two cancer incidence studies comes from the United Kingdom General Practice Research Database (GPRD). The GPRD is currently one of the world's largest computerized databases; it contains the primary care longitudinal records of over 11 million patients and provides over 67 million person-years of data. The GPRD currently contains information representing over 8% of the United Kingdom's estimated population and has over 600 general practices presently enrolled (71). The GPRD provides universal and uniform representation of the entire UK general population with only minor variations observed between geographic regions and contains patient age and sex distributions comparable to those reported by the National Population Census (72). The GPRD is a reliable database that allows for the study of rare outcomes because of its size and its ability to grant researchers access to anonymous, original medical records. All information collected by the database has been subjected to validation studies and is of consistent and proven high quality (73).

The electronically stored information found in the GPRD includes data on age, sex, and registration of all patients as entered into the GPRD computer software by trained participating general practitioners. Using the Read classification system (a universal classification system developed in the UK, as funded by the National Health Service), all medical diagnoses, laboratory results, and diagnostic procedures from routine care and hospitalization to consultations and emergency care are all recorded with clearly stated dates and location of events. In-depth prescription files with date, potency, amount, and doses are recorded automatically and are electronically transcribed as a computer record in the database using prescription coding established by the UK Prescription Pricing Authority. Indications for all new prescriptions (which can be referenced with the medical events recorded on the same dates) are also available and include information on any possible events that could have influenced

treatment changes or withdrawals. Other patient information that is available in the database include vaccinations and immunizations received, contraceptive use, pregnancy, dates of births, death, the entry and exit of a patient from a practice, as well as many important lifestyle variables such as smoking, height, weight, and glycosylated haemoglobin levels (HbA1c) (74). In creating these two studies, protocol and ethics approval was granted by GPRD Ethical and Scientific Advisory Committee.

4.2 Study design:

Using virtually identical methods, two population-based retrospective cohort studies were conducted with nested case-control analyses to assess whether metformin was associated with a decreased risk of lung or colorectal cancer in patients with type 2 diabetes. Variation in the two study designs did occur in cohort selection and in covariate inclusion during the analyses based on the cancer of interest studied.

4.2.1 Creation of the lung and colorectal study cohorts:

The cohorts created in our two studies included all patients (male and female) who were at least 40 years of age at cohort entry and had been prescribed at least one oral anti-diabetes agent between January 1, 1988 and December 31, 2009. Cohort entry for both studies was defined as the date of the first prescription for an oral anti-diabetic agent during this period. All patients included in the two studies were from up-to-standard (UTS) general medical practices verifying that their data met GPRD research quality standards, and were required to have at least one year of data available in the GPRD prior to their cohort entry. For both study cohorts any patient who received insulin as their first anti-diabetic treatment was excluded to ensure patients were diagnosed with type 2 diabetes mellitus, not type 1, thereby decreasing misclassification.

For the lung cancer cohort any patients previously diagnosed with lung cancer at any time prior to cohort entry was excluded, with the same exclusion criteria used for the colorectal cancer study cohort, where in this cohort any patients previously diagnosed with colorectal cancer at any time prior to cohort entry were excluded. All patients were followed until a first-ever diagnosis of lung cancer or colorectal cancer (depending on the outcome being studied), death from any cause as derived using a previously validated GPRD algorithm, end of registration with the general practice, or end of the study period (December 31, 2009), whichever had occurred first.

4.2.2 Case and control selection for the nested case-control analysis

In each cohort, a nested case-control analysis was conducted to allow for time matching of the cases and controls and to permit a multivariate analysis assessment. Each nested case-control analyses corresponded to a specific cancer type, colorectal or lung. All incident cases were identified by using strategically determined computerized algorithms based on medical codes, procedures, and treatments related to these two cancer outcomes (11, 29). The index date for each analysis was determined as the calendar date of each case's first lung or colorectal cancer diagnosis (depending on which cancer cohort). The dates of the risk-set were defined as the index date for the controls with equal duration of follow-up to that of the cases they matched.

For both analyses, up to ten controls were randomly selected from the defined risk set within the cohort and were matched to each case based on age (year of birth), sex, calendar year of cohort entry, and duration of follow-up. In order to avoid excluding cases, the matching criteria was relaxed for two colorectal cancer cases and for four lung cancer cases. Based on

these criteria, all controls were alive, not previously diagnosed with the specific cancer being study, and registered with the general practice when matched to a given case.

4.3 Exposure assessment:

For both cases and matched controls in each study, information on all anti-diabetic agents prescribed between cohort entry and index date was obtained. Exposures were excluded in the year immediately before the index date from our analysis in order to account for a suspected latent effect period. In both studies, subjects were grouped according to their anti-diabetic drug use: metformin, sulfonylureas, combinations of any two or more different oral hypoglycaemic agents (OHAs), and any other OHAs (thiazolidinediones, meglitinides, glucosidases, DPP-4 inhibitors, alpha-glucosidase inhibitors, GLP-1 analogs, or guar gum).

Primary Exposure Definition: patient's ever-exposure to metformin; where a patient was defined as ever exposed when they had had at least one prescription of metformin anytime between cohort entry and the year prior to index date. The non-metformin prescription categories were based on the distribution of drug use in the non-metformin users.

Secondary Exposure Definition: total number of metformin prescriptions; where a patient determined as ever exposed to metformin was further categorized according to number of prescriptions received between an individual's cohort entry until one year prior to index date. This secondary exposure was established to determine whether there was a dose-response relationship between metformin and cancer incidence. To evaluate this relationship, the use of a linear trend was employed to display the number of prescriptions as a continuous variable. The number of prescriptions received by patients was then categorized into five quartiles based on the overall distribution of prescriptions received. To increase the accuracy in determining a patient's

number of prescriptions, it was explicatively distinguished that receiving two or more of the same prescription concurrently counted independently towards a patient's total number of prescriptions.

4.4 Covariates and potential cofounders:

A number of covariates were considered and included in both study models; specifically considered were influential co-morbid conditions and variables measured at index date associated to the risk of the cancers under study and with the use of metformin as found documented in other studies and current literature. Conditions and variables considered as potential cofounders for both models were patient's weight, body mass index (BMI), excess alcohol use, smoking status, ever use of statins, aspirin and other NSAIDs, and use of other anti-diabetic agents. Also included was a patient`s last recorded Hb1Ac level at least one year prior to index date. For each of the specific cancer models additional covariates were considered. In the lung cancer study, record of chronic obstructive pulmonary disease (COPD) and asthma were collected at index date as well. Covariates specifically included for colorectal cancer were records of a cholecystectomy or inflammatory bowel diseases and any history of colonoscopy procedures or polyps as taken at index date (11, 29).

4.5 Statistical methods:

4.5.1 Statistical analysis:

For both lung cancer and colorectal cancer analyses, characteristics of the cases and controls were summarized using descriptive statistics. To estimate the overall incidence rate of cancer overtime the person-years of follow-up for each cohort was cumulated using 95% confidence intervals (CI) based on a Poisson distribution. Based on our criteria to match our

controls to cases, a conditional logistic regression was performed to estimate the rate ratio (RR) of each cancer incident associated with metformin use along with 95% CI. Each regression model was conditioned on four matching factors- age, sex, calendar year of cohort entry, and duration of follow-up and adjusted as appropriate for the potential confounders listed in the above section. To assess how diabetes duration can influence the risk of cancer and the length of metformin exposure in a patient, both models were also adjusted in order to observe how this possible effect-modifier could influence the relationship between metformin use and cancer risk. Also in regards to diabetes duration, the mean patient follow-up was also calculated for the secondary exposure of total number of metformin prescriptions. The information for diabetes duration was collected retrospectively before cohort entry as available in the GPRD up to one year prior to index date; the first available diabetes record in the GPRD was used to represent a diagnosis or presence of diabetes as the onset of disease. All analyses were conducted with SAS version 9.2 (SAS Institute, Cary, NC).

4.5.2 Sensitivity and additional analyses:

To assess the strength of the results, two sensitivity analyses were conducted in both the lung and colorectal cancer studies. The first sensitivity analysis repeated all statistical analyses using a 2-year or 6-month lag period instead of just 1-year before the index date to assess the appropriateness of the selected latent effect period. To assess how a patient's baseline physical characteristics could influence and attribute to their cancer risk, a second sensitivity analysis was conducted including available patients' baseline measurements for BMI and HbA1c. An additional analysis was also conducted in each study comparing *ever* only use of metformin to *ever* use of metformin and other OHAs. By stratifying the different types of metformin users by other drugs used, it was possible to observe any significant differences in patient follow-up time

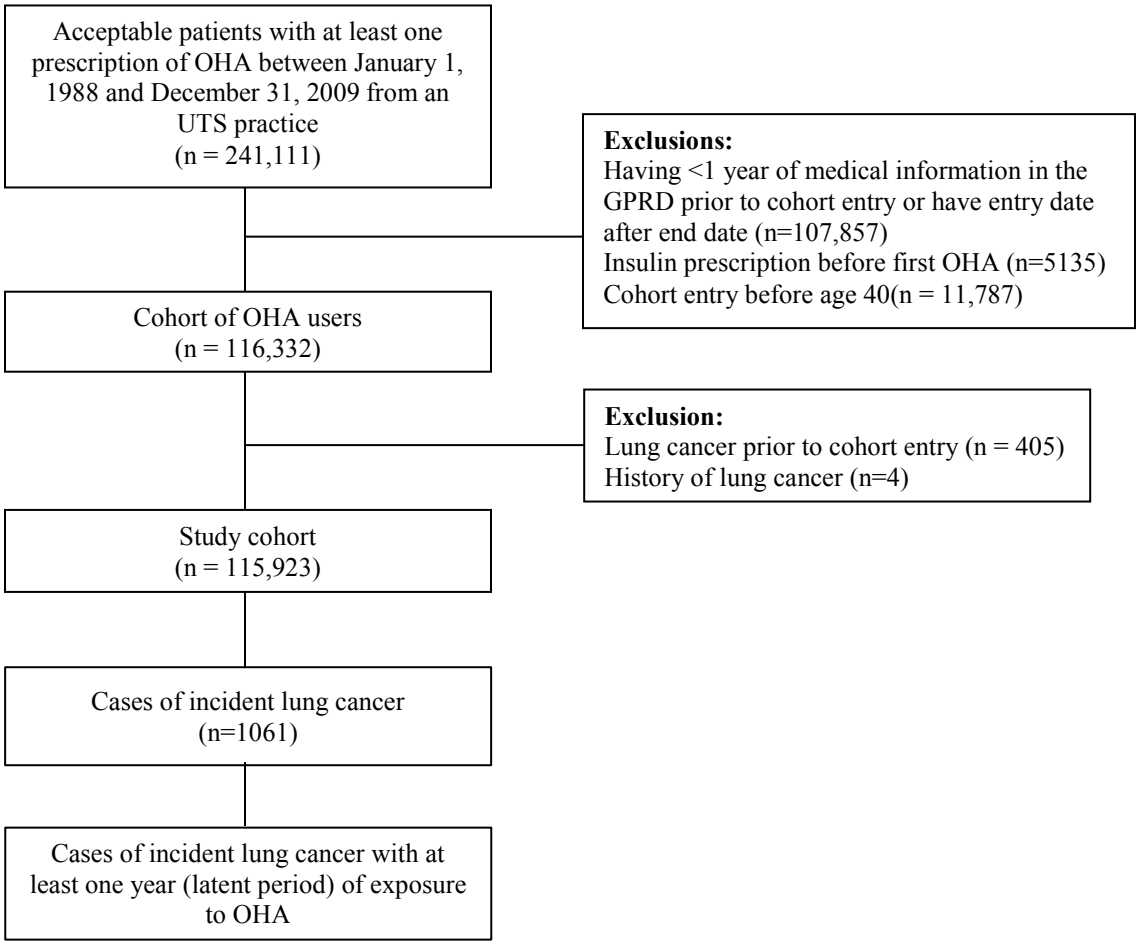
and changes in cancer risk due to the diabetes severity and progression. Crude relative risks of all unmatched covariates were also calculated in each study to assess the validity of our case ascertainment and to denote any significant differences between our cases and controls.

5. Results

5.1 Lung cancer

The initial lung cancer cohort extracted from the GPRD database consisted of 241,111 patients with at least one prescription of an OHA between January 1, 1988 and December 31, 2009 and were from an UTS practice. From this population, the final study cohort was narrowed down to 115,923 patients who had met the study inclusion criteria. The study cohort consisted of a population that was 55.23% male, had a mean (SD) age of 64.1 (12.0), and a median HbA1c level of 8.2%. Of the patients included in the cohort, 67.4% of them had received metformin as their first prescribed OHA while 29.6% had entered the cohort on sulfonylureas. 1.3% patients entered on other OHAs, and 1.7% were initially prescribed combination of any two OHAs. As stated in the method section no patients had received insulin prior to first OHA prescription in order to decrease the misclassification of type 1 diabetes patients as type 2. The mean (SD) duration of patient follow up for the cohort was 4.6 (3.6) years.

Figure 1: Lung cancer study flow chart



During 528,356 person-years of follow-up 808 patients with at least one year exposure to OHA were diagnosed with lung cancer producing a disease rate of 2.0 cases per 1000 persons per year (95% CI: 1.9-2.1). For the nested case control analysis, the cases were restricted to 808 subjects including only patients who had at least one year of exposure to OHAs. The cases were then matched appropriately to 7764 controls. In order to find an adequate number of controls for all cases in the lung cancer cohort, the matching criteria was relaxed for four cases. Three cases were relaxed to the year of cohort entry, ±1 year, and one case to the year of birth ±3 years and the year of cohort entry ±2 years.

The two groups of patients yielded many similar baseline characteristics including comparable rates of obesity with 38.5% of the case population and 39.5% of the control population exhibiting a BMI of 30 and over. NSAIDs use and HbA1c levels were also similarly distributed in the case and control groups. As expected, the number of *ever* smokers was higher in the cases at 85% compared to only 60% in the controls. The number of patients with a history of COPD and asthma were also higher for the cases at 31% for history of COPD and 13.4% for history of asthma compared to only 16.5% and 11.8% in the control groups. Aspirin, statins and excessive alcohol use were all observed as slightly higher in the cases compared to controls.

Table 2: Characteristics of lung cancer cases and controls at index date

	Cases	**Controls**	**Crude RR**
	(n= 808)	**(n= 7764)**	**95% CI**
Sex, males (%) *	526 (65.1)	5047 (65.1)	
Age at index date, (years), mean (SD) *	73.1 (8.5)	73.1 (8.3)	
Duration of follow-up (years), mean (SD) *	5.0 (3.4)	5.0 (3.2)	
Duration of disease prior to cohort entry (years), mean(SD)	2.0 (3.6)	2.1 (3.8)	
HbA1c (%), median (last result at year prior to index date)	7.1 (n= 656)	7.1 (n= 6417)	
< 6.5%, n (%)	157 (19.4)	1434 (18.5)	1.02 (0.8-1.3)
6.5-7.4%, n (%)	268 (33.2)	1434 (33.9)	0.93 (0.8-1.2)
7.5-8.9%, n (%)	145 (18.0)	1622 (20.9)	0.81 (0.6-1.0)
≥9%, n (%)	86 (10.6)	732 (9.4)	1.08 (0.8-1.4)
Unknown, n (%)	152 (18.8)	1347 (17.4)	
Body mass index			
< 30, n (%)	482 (59.7)	4557 (58.5)	1.00
≥ 30, n (%)	311 (38.5)	3067 (39.5)	0.97 (0.8-1.1)
Unknown, n (%)	15 (1.9)	140 (1.8)	
Smoking Status			
Never, n (%)	102 (12.6)	2946 (37.9)	1.00
Ever, n (%)	688 (85.2)	4639 (60.0)	5.21 (4.2-6.5)
Unknown, n (%)	18 (2.2)	179 (2.3)	
Excessive alcohol use, n (%)	110 (13.6)	820 (10.6)	1.35 (1.1-1.7)
COPD History, n (%)	247 (30.6)	1281 (16.5)	2.22 (1.9-2.6)
Asthma History, n (%)	108 (13.4)	918 (11.8)	1.18 (0.95-1.5)
Ever use of NSAIDs, n (%)	464 (57.4)	4481 (57.7)	0.99 (0.9-1.1)
Ever use of Aspirin, n (%)	522 (64.6)	4619 (59.5)	1.28 (1.1-1.5)
Ever use of Statins, n (%)	549 (68.0)	4949 (63.7)	1.41 (1.2-1.7)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Focusing on our primary exposure, we found that 76.3% of cases and 77.2% of control were classified as *ever* users of metformin. In both our crude and adjusted rate ratios (confounders adjusted for included- BMI at index date, *ever* status of smoking, COPD, asthma, statins, NSAIDS, aspirin, and alcohol, last HbA1c measurement prior to index date, diabetes duration and all other diabetes medications) we found that metformin *ever* use was not associated with a reduced risk of lung cancer.

Table 3: Metformin use among lung cancer cases and controls

Metformin exposure	Cases: n (%) (n=808)	Controls: n (%) (n=7764)	Crude RR	Adjusted RR (95% CI)**
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.94 (0.8-1.2)

****Adjusted for:** obesity, smoking, COPD, asthma, statins, NSAIDs, aspirin, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications

When *ever* metformin users were further categorized based on the number of metformin prescriptions received, no dose-response relationship were observed and there were no evidence that long term use of metformin had any significant influence on cancer incidence compared with never use of metformin.

Table 4: Number of metformin prescriptions among lung cancer cases and controls

# of prescriptions	Cases: n (%)	Follow-Up (years) mean, (SD)	Controls: n (%)	Follow-Up (years) mean, (SD)	Crude RR	Adjusted RR (95% CI)**
Never,	192 (23.8)	3.9 (2.6)	1769 (22.8)	4.2 (2.7)	1.00	1.00
1 to 7	173 (21.4)	3.3 (2.7)	1631 (21.0)	3.2 (2.6)	1.04 (0.8-1.3)	0.96 (0.7-1.3)
8 to 17	129 (16.0)	3.9 (2.5)	1373 (17.7)	3.6 (2.3)	0.89 (0.7-1.2)	0.84 (0.6-1.1)
18 to 37	150 (18.6)	5.4 (2.7)	1524 (19.6)	5.3 (2.4)	0.94 (0.7-1.2)	0.94 (0.7-1.3)
38 or more	164 (20.3)	8.5 (3.3)	1467 (18.9)	8.1 (3.0)	1.03 (0.8-1.4)	1.03 (0.8-1.4)

****Adjusted for:** obesity, smoking, COPD, asthma, statins, NSAIDs, aspirin, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications

Looking at all other *ever* versus *never* use of other individual OHA medications and adjusting for the same confounders, we found that sulfonylureas, TZD, alpha-glucosidase inhibitors, and all other OHA medication combined (meglitinides combinations, DPP-4 inhibitors, GLP-1analogs and guar gum) use was also not associated with a change in the risk of lung cancer.

Using identical procedures and adjusting for the same confounders as used in the primary analyses, the two sensitivity analyses presented similar results where *ever* exposure to metformin and the number of metformin prescriptions received had no significant influence on the incidence of lung cancer cases when compared to never users of metformin.

Table 5: Metformin use among lung cancer cases and controls (6 month lag period)

Metformin exposure	Cases n (%) (n=910)	Controls n (%) (n=8737)	Crude RR	Adjusted RR (95% CI)**
Never Metformin	214 (23.5)	1993 (22.8)	1.00	1.00
Ever Metformin	696 (76.5)	6744 (77.2)	1.01 (0.8-1.2)	1.04 (0.9-1.3)

****Adjusted for:** obesity, smoking, COPD, asthma, statins, NSAIDs, aspirin, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications

Table 6: Metformin use among lung cancer cases and controls (2 year lag period)

Metformin exposure	Cases n (%) (n=646)	Controls n (%) (n=6164)	Crude RR	Adjusted RR (95% CI)**
Never Metformin	165 (25.5)	1633 (26.5)	1.00	1.00
Ever Metformin	481 (74.5)	4531 (73.5)	1.1 (0.9-1.4)	1.03 (0.8-1.3)

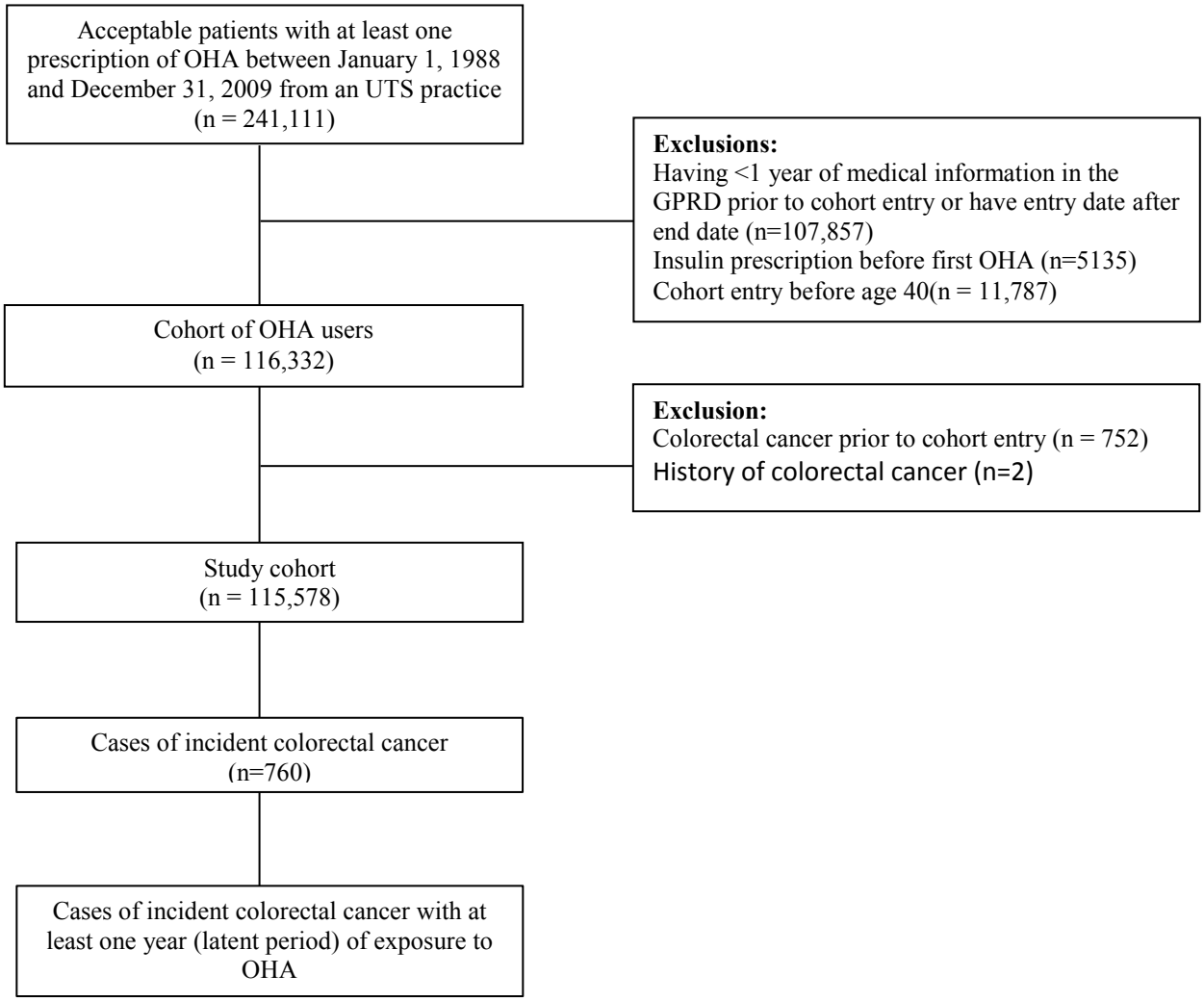
****Adjusted for:** obesity, smoking, COPD, asthma, statins, NSAIDs, aspirin, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications

Looking specifically if baseline BMI and HbA1c levels had an influence on lung cancer incidence, it was again observed that *ever* use of metformin was not associated with a decreased risk of lung cancer. When classifying metformin *ever* use based on other OHAs or insulin prescription ever used, there were also no changes in lung cancer incidence

5.2 Colorectal Cancer

The colorectal cancer cohort was extracted and created in an almost identical manner to that of the lung cancer cohort. All patients selected were from the GPRD database creating a preliminary cohort of 241,111 patients with at least one prescription of an OHA between January 1, 1988 and December 31, 2009 and were from UTS practices. With the same criteria as the lung cancer cohort, 107,857 patients were excluded for having less than 1 year of medical information in the GPRD prior to cohort entry. An additional 5135 patients were excluded for having insulin prescriptions before first OHA and another 11,787 were removed from the cohort for being under age 40 at cohort entry. After excluding 757 patients who were diagnosed or had a history of colorectal cancer, the population the final study cohort was narrowed down to 115,578 patients. The colorectal study cohort consisted of a population where 67.3% of the patients had received metformin as their first prescribed OHA while 29.7% had entered the cohort on sulfonylureas, 3% had a first prescriptions of another OHAs or combination of any two OHAs. The mean (SD) duration of patient follow up for the cohort was 4.5 (3.6) years with a colorectal cancer incident rate of 1.4 cases per 1000 persons per year (95% CI: 1.3, 1.6).

Figure 2: Colorectal cancer study flow chart



For the nested case control analysis, the cases were restricted to 607 patients who had at least one year of exposure to OHAs. The cases were matched appropriately to 5837 controls. For colorectal cancer, the match criteria were relaxed for two cases to the year of cohort entry, ±1 year in order to find appropriate controls. The two groups of patients yielded many similar baseline characteristics including comparable rates of obesity with 38.5% of the case population

and 39.5% of the control population exhibiting a BMI of 30 and over. NSAIDs use and HbA1c level distributions were also similar in the case and control groups.

Table 7: Characteristics of colorectal cancer cases and controls at index date			
	Cases	**Control**	**Crude RR**
	(n=607)	**(n=5837)**	**(95% CI)**
Sex, n=males (%) *	384 (63.3)	3712 (63.6)	
Age at index date, (years), mean (SD) *	72.8 (8.7)	72.5 (8.5)	
Duration of follow-up (years), mean (SD) *	4.8 (3.1)	4.8 (2.9)	
Duration of disease prior to cohort entry (years), mean(SD)	2.0 (3.4)	2.1 (3.9)	
HbA1c (%), median (last result at year prior to index date)	7.3 (n=492)	7.1 (n=4696)	
< 6.5%, n (%)	99 (16.3)	1067 (18.3)	1.00
6.5-7.4%, n (%)	185 (30.5)	1860 (31.9)	1.07 (0.8-1.4)
7.5-8.9%, n (%)	144 (23.7)	1224 (21.0)	1.28 (0.8-1.4)
≥9%, n (%)	64 (10.5)	545 (9.3)	1.27 (0.9-1.8)
Unknown, n (%)	115 (19.0)	1141 (19.6)	
Body mass index			
< 30, n (%)	358 (59.0)	3451 (59.1)	1.00
≥ 30, n (%)	252 (38.2)	2278 (39.0)	1.00 (0.8-1.2)
Unknown, n (%)	17 (2.8)	108 (1.9)	
Smoking Status			
Never, n (%)	235 (38.7)	2243 (38.4)	1.00
Ever, n (%)	356 (58.7)	3447 (59.0)	1.01 (0.8-1.2)
Unknown, n (%)	16 (2.6)	147 (2.5)	
Excessive alcohol use, n (%)	74 (12.2)	623 (10.7)	1.18 (0.9-1.5)
History of Colonoscopy, n (%)	24 (4.0	204 (3.5)	1.11 (0.7-1.7)
History of Polyps, n (%)	18 (3.0)	105 (1.8)	1.71 (1.0-2.8)
History of Inflammatory Bowel Diseases, n (%)	8 (1.3)	90 (1.5)	0.85 (0.4-1.8)
History of Cholecystectomy, n (%)	39 (6.4)	317 (5.4)	1.19 (0.8-1.7)
Ever use of NSAIDs, n (%)	358 (58.8)	3424 (58.7)	1.02 (0.9-1.2)
Ever use of Aspirin, n (%)	347 (57.2)	3412 (58.5)	0.95 (0.8-1.1)
Ever use of Statins, n (%)	357 (58.8)	3643 (62.4)	0.86 (0.7-1.1)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Focusing on our primary exposure, we found that 76.3% of cases and 77.2% of control were classified as *ever* users of metformin. In both our crude and adjusted rate ratios (confounders adjusted for included- BMI at index date, *ever* status of smoking, polyps, cholecystectomy, colonoscopy, statins, aspirin, NSAIDs and alcohol, last HbA1c measurement

prior to index date, diabetes duration, and all other diabetes medications) we found that metformin ever use was not associated with a reduced risk of colorectal cancer.

Table 8: Metformin use among colorectal cancer cases and controls				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.94 (0.7-1.2)
****Adjusted for:** obesity, smoking, polyps, cholecystectomy, colonoscopy, statins, aspirin, NSAIDS, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications				

Looking at all other *ever* versus *never* use of other individual OHA medications and adjusting for the same confounders, we found that sulfonylureas, TZD, alpha-glucosidase inhibitors, and all other OHA medications (meglitinide combinations, DPP-4 inhibitors, GLP-1analogs and guar gum) use was also not associated with a change in the risk of colorectal cancer. When *ever* metformin users were further categorized based on the number of metformin prescriptions received, no dose-response relationship were observed and there were no evidence that long term use of metformin had any significant influence on colorectal cancer incidence compared with *never* use of metformin.

Table 9: Number of metformin prescriptions among colorectal cancer cases and controls						
# of prescriptions	**Cases n (%)**	**Follow-Up (years) mean, (SD)**	**Controls n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never	163 (26.9)	4.1 (2.7)	1431 (24.5)	4.0 (2.5)	1.00	1.00
1 to 7	140 (23.0)	3.3 (2.7)	1176 (20.2)	3.0 (2.4)	1.13 (0.9-1.5)	1.14 (0.9-1.5)
8 to 17	94 (15.5)	3.3 (1.7)	1033 (17.7)	3.7 (2.3)	0.80 (0.6-1.1)	0.83 (0.6-1.1)
18 to 36	98 (16.1)	5.6 (2.4)	1104 (18.9)	5.2 (2.4)	0.75 (0.6-1.0)	0.77 (0.6-1.1)
37 or more	112 (18.5)	8.3 (2.8)	1093 (18.7)	7.7 (2.6)	0.83 (0.6-1.1)	0.86 (0.6-1.2)
****Adjusted for:** obesity, smoking, polyps, cholecystectomy, colonoscopy, statins, aspirin, NSAIDS, excessive alcohol use , hba1c, diabetes duration, and all other diabetes medications						

By conducting identical methods and adjusting for the same confounders as used in the primary statistical analysis, the two sensitivity analyses presented similar results where *ever* exposure to metformin and the number of metformin prescriptions received had no significant

influence on the incidence of colorectal cancer cases when compared to never users of metformin. Looking specifically if baseline BMI and HbA1c levels had an influence on colorectal cancer incidence, it was again observed that *ever* use of metformin was not associated with a decreased risk of colorectal cancer. When classifying metformin *ever* use based on other OHAs or insulin prescription ever used as well, there were also no changes in colorectal cancer incidence.

Table 10: Ever other drug use with metformin among colorectal cancer cases and controls

Ever Drug	Cases (n=444)	Follow-Up (years) mean (SD)	Controls (n=4406)	Follow-Up (years) mean (SD)	Crude RR	Adjusted RR (95% CI)**
Metformin	180 (40.5)	3.1 (1.9)	1885 (42.8)	4.8 (2.0)	1.00	1.00
Metformin+Sulfonylureas	156 (35.1)	5.7 (2.8)	1522 (34.5)	5.6 (2.8)	1.08 (0.8-1.4)	1.05 (0.8-1.4)
Metformin + TZD	16 (3.6)	5.1 (2.9)	236 (5.4)	4.6 (2.4)	0.72 (0.4-1.2)	0.70 (0.4-1.2)
Metformin + Insulin	2 (0.5)	4.1 (1.3)	22 (0.5)	4.8 (2.5)	1.01 (0.2-4.3)	1.03 (0.2-4.4)
Met + TZD + Sulf	51 (11.4)	6.6 (3.0)	352 (7.9)	7.3 (2.7)	1.55 (1.1-2.3)	1.48 (1.0-2.1)
Met + TZD + Insulin	1 (0.2)	6.8	9 (0.2)	8.1 (3.3)	1.23 (0.2-9.8)	1.03 (0.1-8.3)
Met + Sulf + Insulin	27 (6.1)	10.0 (3.2)	295 (6.7)	4.8 (1.9)	0.88 (0.6-1.4)	0.80 (0.5-1.3)
Met+Sulf+Insulin+TZD	11 (2.4)	9.5 (3.1)	85 (1.9)	8.7 (2.9)	1.37 (0.7-2.7)	1.26 (0.6-2.5)

****Adjusted for:** obesity, smoking, polyps, cholecystectomy, colonoscopy, statins, aspirin, NSAIDS, excessive alcohol use, hba1c, diabetes duration, and all other diabetes medications

6. Discussion

The results from these two observational studies using large populations obtained from the GPRD and nested case-control analyses provides evidence that the use of metformin does not decrease lung or colorectal cancer incidence in patient with T2DM when compared to those individuals who did not use metformin. The same null effect was observed in both cancer studies regardless of the number of prescriptions a patient had received. Similar results were also obtained from all sensitivity analyses conducted in both studies. From our results, we also observed that *ever* use of other OHAs and insulin versus *never* use of these substances in T2DM patients did not significantly change lung or colorectal cancer risk as well. The results obtained from our study counter the findings of most other studies conducted in this field. The use of more rigorous methods in this study may explain why there is such a contrast in this and previous studies results (6, 12, 14, 17, 18, 22, 33).

The findings of the lung cancer cohort study are comparable to those found in the secondary analyses by Ferrara *et al* where ever users of metformin compared to those with no use of metformin did not have a significant decrease in lung cancer incidence (HR: 1.0, 95%CI: 0.8-1.1) (20). The results of the colorectal cancer study provide further evidence supporting the claim that metformin is not associated with a decrease in colorectal cancer showing comparable findings to that of the research by Yang *et al* (OR: 1.0, 95%CI: 0.6-1.7) (11). This study's results greatly differ from those obtained from the recent meta-analysis by Zhang *et al* where the researchers associated metformin with an overall 37% decreased risk of colorectal cancer (RR: 0.63, 95%CI: 0.47-0.84) (19). However, most of the studies included in this meta-analysis, as discussed in the literature review section, had important methodological shortcomings that greatly limit the interpretation of their results.

Both study results are different from those found in experimental studies focused on the direct and indirect effect of metformin on cancer and tumour proliferation (31, 51, 59, 67). Many of the discrepancies between studies can be explained by the use of simple models in the experimental studies. Other differences in findings between the two study types are due to the use of high dosages and short-term treatment regiments in experimental studies that are not comparable to what would actually be prescribed to patient with type T2DM. The experimental studies executed are unable to replicate the conditions and complexities experienced in an observational and clinical setting and lack many key factors experienced in complex human models. Many of the indirect mechanisms that influence cancer incidence that are not captured in a simple, experimental model include circulating insulin and glucose levels, changes in disease severity and the types of medications, obesity, and a patient's lifestyle choices (75).

The two studies conducted exhibit a number of strengths; many of them arising from the use of a nested case control analysis and conditional logistic regression. By employing these strategies, it was possible to decrease exposure misclassification and allow for the sampling of cases and controls from the same well-defined, large source population (76). By using a nested case control analysis, time-dependent exposure definitions and covariate information were used in the sampling scheme to select controls. The nested case control strategy allowed for an appropriate number of controls within the risk-set to be matched to cases by age (year of birth), sex, calendar year of cohort entry, and duration of follow-up. By matching these two groups, it was possible to increase the comparability between our cases and controls' disease severity, better control for potential confounding, and create less opportunity for the occurrence of selection bias in the studies (77, 78). By using a conditional logistic regression instead of a cox

regression model, the two studies conducted were able to produce results using a less complex and much more straightforward analysis that helped to decrease error-plagued results and minimize misclassification of time and exposure (78). The introduction of bias (specifically immortal time bias and selection bias) due to the incorrect use of the cox regression model in previous studies may explain their result showing significant changes in cancer incidence due to metformin use (6, 14, 17).

Further study strengths were gain by using the GPRD. As a well-established and validated longitudinal primary care database (71, 74), the GPRD made it possible to assemble a large cohort of patients with T2DM for each study and obtain a large number of cases for both cancers types. The GPRD also allowed for the ascertainment of patient follow-up time, diabetes duration prior to cohort entry, a patient's number of prescriptions, and removed recall bias due to all information in the database being entered prospectively. The GPRD provided information on a number of confounder for such as smoking, BMI, and other OHAs prescribed. Unlike many administrative databases, it was also possible to obtain information on HbA1c levels, excessive alcohol use, and other non-diabetic medications. The availability of lung and colorectal cancer specific covariates made available by the GPRD also strengthened both of our studies, where it was possible to adjust our model for confounding variables such as history of COPD, asthma, polyps, cholecystectomies, and colonoscopies.

Two other strengths in our studies were gained by using individuals that were newly treated for diabetes and by conducting a number of sensitivity analyses. The use of a homogenous cohort for each study, made up of new users of OHAs allowed for a decrease in the number of biases related to prevalent user designs from our studies and allowed for the isolation of the effects of metformin on cancer with only minimal confounding from diabetes severity. By

running additional analyses, it was possible to confirm and strengthen the results of our primary analyses.

Despite the numerous strengths in both of these studies, there were some limitations encountered as well. Although the GPRD contains information on a large number of patients, it unfortunately lacks records on certain covariates that could be considered influential on the association between metformin and cancer. Some of the patient variables that were not available in the GPRD included family history of cancers, race, level of physical activity, diet, and information on past biopsies, bronchoscopy, CT scans and other hospital procedures related to both cancer types (76). Some residual confounding may existed due to the lack of information on these variables, but it can be reasonably assumed that this lack of information would not affect the validity of our results. In regards to race, because 92% of the population of the UK is white, it can be assumed that not having this information would not change our observed results (9). It can also be inferred that these unknown covariates would be non-differentially distributed between our exposed and unexposed users in our cohort. Therefore, these unknown covariates would not influence the prescription of metformin over other anti-diabetic treatments.

Another limitation found in the two studies was experienced due to an absence of information being available in regards to patient compliances to prescribed medications and the filling of given prescriptions; the GPRD only containing record of if a prescription had been written by a GP (76). Despite a lack of knowledge in regards to if prescriptions were actually taken by patients, this issue most likely would not have greatly differed between exposure groups or influenced the results observed. Due to a lack of patient data on cancer progression at diagnosis and on HbA1c baseline levels in the GPRD, two additional analyses were not executed. Further research should focus on how metformin may differentially increases cancer

risk among subgroups of patients depending on their level of detected cancer progression, and as well on how changes in a patient's HbA1c levels from baseline to index date may influence their cancer risk.

To date, this is one of largest observational study to investigate the effects of metformin on colorectal cancer incidence in patients with T2DM and the first observational study with a large enough sample size to look specifically at the effects of metformin on lung cancer incidence in patients with T2DM. Although some laboratory and clinical research still points towards metformin having tumour suppressive capabilities and other therapeutic effects on cancer in *in vitro and in vivo* research settings (49, 51, 57, 59, 60, 66), much more research is needed before clinical equipoise exists. There is yet the evidence necessary to justify the spending of substantial amounts of funding on large randomized controlled trials employing metformin as a cancer preventative agent or as an add-on cancer treatment (79). The laboratory research conducted and results obtained from these studies do give some merit towards researchers preforming further observational studies or pilot clinical trials. Future studies should focus on observing metformin's impact on different cancer stages and disease progression in already diagnosed patients with T2DM or patients with cancer precursors rather than on risk and changes in the incidence of cancer in patients with no history of cancer.

It is my hope that future research will concentrate on increasing the understanding of metformin's capabilities in human patients with and without T2DM and that through collaborative efforts across the various fields of research a consistency of similar conclusions will be determined between the findings of observational studies and in laboratory research. Until there is a uniform consensus across disciplines on what capabilities metformin actually possesses, the use of metformin in large experimental studies should be approached with high

caution and scepticism, where the safety and consequences of producing false hope for effected patients and the misuse of funding should be greatly considered before executing such trials.

7. Conclusion

Metformin use is not associated with a decreased incidence of colorectal or lung cancer in patients with T2DM. This null effect counters the significant decrease in cancer incidence associated with metformin use reported in other studies (6, 14, 17, 25, 65). Previously conducted observational studies should be interpreted with caution due to methodological and analytical issues. A Major focus of these two studies was to eliminate major bias and confounding by correctly accounting for study aspects such as time-dependent variables. Other major focuses of this thesis project were to correctly adjust for confounding covariates, accurately ascertain exposures and outcomes, and produce a model that allowed for a high level of comparability between cases and controls. By developing these two studies with an emphasis on avoiding previous method issues, the results produced in this paper add substantial evidence that further weakens the hypothesis made that metformin use decreases cancer risk in patients with T2DM.

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9. Appendix

List of Appendix Tables and Figures

1. Lung Cancer Tables and Figures

2. Colorectal Cancer Tables and Figures

Figure 1.1: Lung cancer Study flow chart

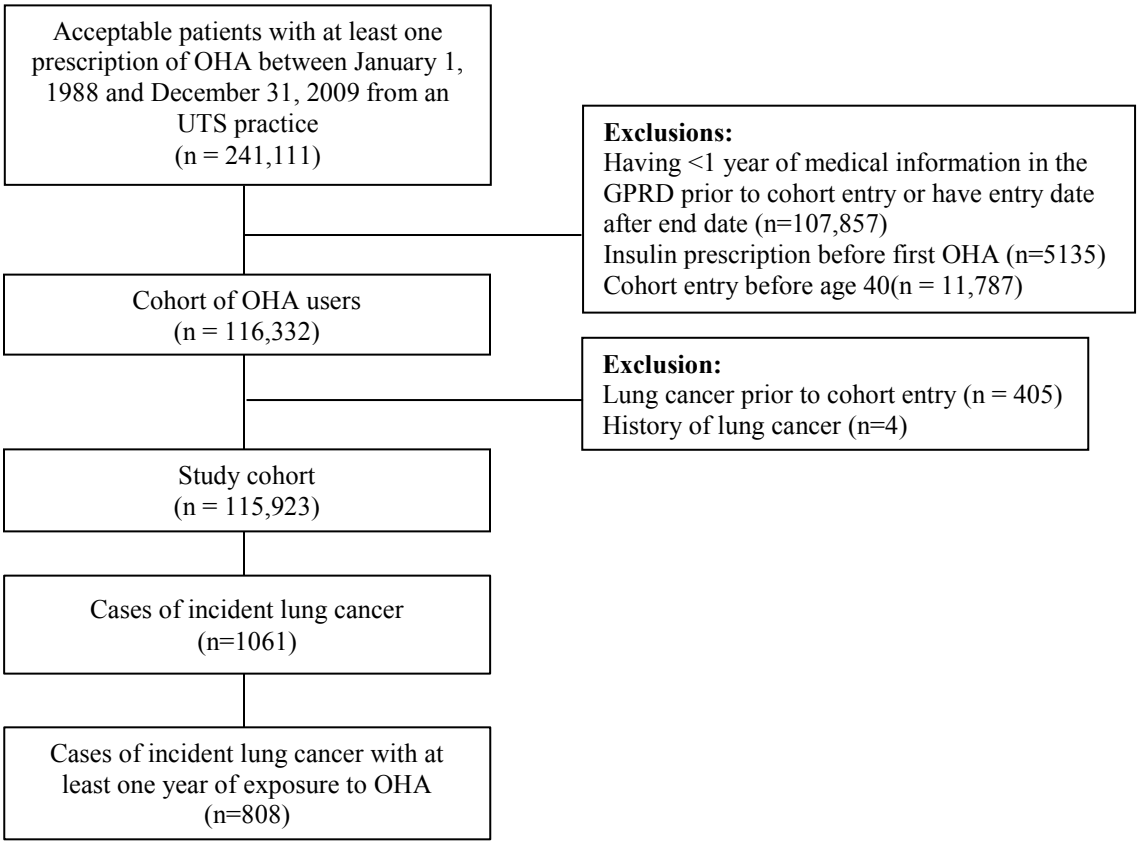

Table 1.1 Characteristics of the cohort (lung cancer)	
Cohort (n = 115,923)	
Age (years), mean (SD)	64.1 (12.0)
Duration of follow-up (years), mean (SD)	4.6 (3.6)
Total person-years of follow-up	528,356
Duration of disease, mean(SD)	3.7 (4.3)
HbA1c (%), median	8.2
Receipt of OHA at cohort entry, n (%)	
Metformin	78,092 (67.4)
Sulfonylureas	34,330 (29.6)
Other OHA	1489 (1.3)
Combinations*	2012 (1.7)
Rate of lung cancer, per 1000/year (95% CI)	2.0 (1.9, 2.1)
*Combinations of any two different OHAs	

Table 1.2: Characteristics of lung cancer cases and controls at index date			
	Cases	**Controls**	**Crude RR**
	(n= 808)	**(n= 7764)**	**95% CI**
Sex, males (%) *	526 (65.1)	5047 (65.1)	
Age at index date, (years), mean (SD) *	73.1 (8.5)	73.1 (8.3)	
Duration of follow-up (years), mean (SD) *	5.0 (3.4)	5.0 (3.2)	
Duration of disease prior to cohort entry (years), mean(SD)	2.0 (3.6)	2.1 (3.8)	
HbA1c (%), median (last result at year prior to index date)	7.1 (n= 656)	7.1 (n= 6417)	
< 6.5%, n (%)	157 (19.4)	1434 (18.5)	1.02 (0.8-1.3)
6.5-7.4%, n (%)	268 (33.2)	1434 (33.9)	0.93 (0.8-1.2)
7.5-8.9%, n (%)	145 (18.0)	1622 (20.9)	0.81 (0.6-1.0)
≥9%, n (%)	86 (10.6)	732 (9.4)	1.08 (0.8-1.4)
Unknown, n (%)	152 (18.8)	1347 (17.4)	
Body mass index			
< 30, n (%)	482 (59.7)	4557 (58.5)	1.00
≥ 30, n (%)	311 (38.5)	3067 (39.5)	0.97 (0.8-1.1)
Unknown, n (%)	15 (1.9)	140 (1.8)	
Smoking Status			
Never, n (%)	102 (12.6)	2946 (37.9)	1.00
Ever, n (%)	688 (85.2)	4639 (60.0)	5.21 (4.2-6.5)
Unknown, n (%)	18 (2.2)	179 (2.3)	
Excessive alcohol use, n (%)	110 (13.6)	820 (10.6)	1.35 (1.1-1.7)
COPD History, n (%)	247 (30.6)	1281 (16.5)	2.22 (1.9-2.6)
Asthma History, n (%)	108 (13.4)	918 (11.8)	1.18 (0.95-1.5)
Ever use of NSAIDs, n (%)	464 (57.4)	4481 (57.7)	0.99 (0.9-1.1)
Ever use of Aspirin, n (%)	522 (64.6)	4619 (59.5)	1.28 (1.1-1.5)
Ever use of Statins, n (%)	549 (68.0)	4949 (63.7)	1.41 (1.2-1.7)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 1.3: Metformin use among lung cancer cases and controls				
Metformin exposure	**Cases: n (%) (n=808)**	**Controls: n (%) (n=7764)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.94 (0.8-1.2)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.4: Number of metformin prescriptions among lung cancer cases and controls						
# of prescriptions	**Cases: n (%)**	**Follow-Up (years) mean, (SD)**	**Controls: n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never,	192 (23.8)	3.9 (2.6)	1769 (22.8)	4.2 (2.7)	1.00	1.00
1 to 7	173 (21.4)	3.3 (2.7)	1631 (21.0)	3.2 (2.6)	1.04 (0.8-1.3)	0.96 (0.7-1.3)
8 to 17	129 (16.0)	3.9 (2.5)	1373 (17.7)	3.6 (2.3)	0.89 (0.7-1.2)	0.84 (0.6-1.1)
18 to 37	150 (18.6)	5.4 (2.7)	1524 (19.6)	5.3 (2.4)	0.94 (0.7-1.2)	0.94 (0.7-1.3)
38 or more	164 (20.3)	8.5 (3.3)	1467 (18.9)	8.1 (3.0)	1.03 (0.8-1.4)	1.03 (0.8-1.4)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications						

Table 1.5: Other anti-diabetes medication use among lung cancer cases and controls				
Sulfonylureas use among lung cancer cases and controls				
Sulfonylureas exposure	**Cases: n (%)**	**Controls n: (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	289 (35.8)	2942 (37.9)	1.00	1.00
Ever Sulfonylureas	519 (64.2)	4822 (62.1)	1.06 (0.9-1.3)	0.99 (0.8-1.2)
TZD use among lung cancer cases and controls				
TZD exposure	**Cases: n (%)**	**Controls n: (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	697 (86.3)	6744 (86.9)	1.00	1.00
Ever TZD	111 (13.7)	1020 (13.1)	1.07 (0.9-1.3)	1.07 (0.9-1.4)
Insulin use among lung cancer cases and controls				
Insulin exposure	**Cases: n (%)**	**Controls n: (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	715 (88.5)	7093 (91.4)	1.00	1.00
Ever Insulin	93 (11.5)	671 (8.6)	1.36 (1.1-1.8)	1.23 (0.9-1.6)
Alpha-Glucosidase Inhibitors use among lung cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases: n (%)**	**Controls n: (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	778 (96.3)	7545 (97.2)	1.00	1.00
Ever Alpha-Glucosidase	30 (3.7)	219 (2.8)	1.31 (0.9-2.0)	1.20 (0.8-1.8)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
OTHER exposure	**Cases: n (%)**	**Controls n: (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never OTHER	793 (98.1)	7638 (98.4)	1.00	1.00
Ever OTHER	15 (1.9)	126 (1.6)	1.08 (0.6-1.9)	1.12 (0.6-2.0)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.6: Ever other drug use with metformin among lung cancer cases and controls						
Ever Drug	**Cases: n (%) (n=616)**	**Follow-Up (years) mean, (SD)**	**Controls: n (%) (n=5995)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Metformin	251 (40.1)	3.2 (1.9)	2573 (42.9)	3.2 (2.0)	1.00	1.00
Met + Sulfonylureas	204 (33.1)	6.0 (3.1)	2062 (34.4)	5.7 (3.0)	1.02 (0.8-1.3)	0.98 (0.8-1.2)
Met + TZD	28 (4.5)	4.6 (2.4)	275 (4.6)	4.7 (2.4)	1.07 (0.7-1.6)	1.10 (0.7-1.7)
Met + Insulin	3 (0.5)	4.8 (0.4)	27 (0.5)	5.8 (3.0)	1.17 (0.4-3.9)	1.10 (0.3-3.7)
Met + TZD + Sulf	51 (8.3)	7.2 (2.8)	502 (8.4)	7.2 (3.2)	1.07 (0.8-1.5)	1.02 (0.7-1.5)
Met + TZD + Insulin	1 (0.2)	2.8	10 (0.2)	6.5 (2.6)	1.05 (0.1-8.3)	1.12 (0.1-9.0)
Met + Sulf + Insulin	56 (9.1)	9.8 (4.1)	411 (6.8)	8.6 (3.3)	1.38 (1.0-2.0)	1.17 (0.8-1.7)
Met+Sulf+Insulin+TZD	22 (2.7)	9.0 (3.1)	135 (2.3)	9.3 (2.9)	1.72 (1.0-2.8)	1.48 (0.9-2.5)

****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications

Figure 1.2: Lung cancer study flow chart (6 month lag period)

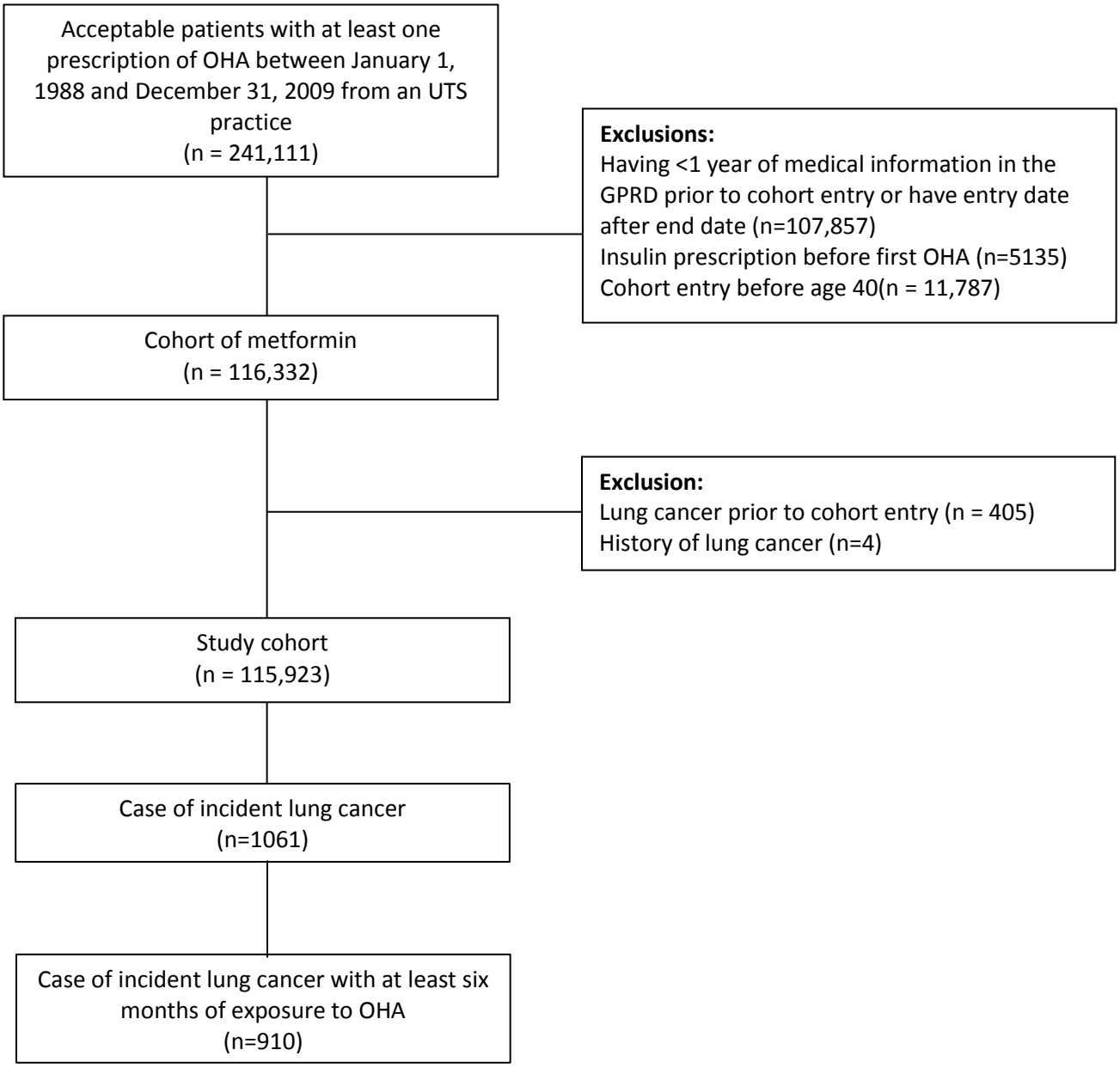

Table 1.7: Characteristics of lung cancer cases and controls at index date (6 month lag period)			
	Cases	**Controls**	**Crude RR**
	(n= 910)	**(n= 8737)**	
Sex, males (%) *	594 (65.3)	5691 (65.1)	
Age at index date, (years), mean (SD) *	73.1 (8.5)	73.1 (8.4)	
Duration of follow-up (years), mean (SD) *	4.5 (3.4)	4.5 (3.2)	
Duration of disease prior to cohort entry (years), mean(SD)	2.0 (3.6)	2.1 (3.6)	
HbA1c (%), median (last result at year prior to index date)	(n= 7.1)	(n= 7.1)	
< 6.5%, n (%)	162 (17.8)	1704 (19.5)	1.00
6.5-7.4%, n (%)	163 (17.9)	2832 (32.4)	1.09 (0.9-1.3)
7.5-8.9%, n (%)	295 (32.42)	1877 (21.5)	1.10 (0.9-1.4)
≥9%, n (%)	196 (21.5)	836 (9.6)	1.17 (0.9-1.5)
Unknown, n (%)	94 (10.3)	1488 (17.0)	
Body mass index			
< 30, n (%)	609 (66.9)	5245 (60.0)	1.00
≥ 30, n (%)	280 (30.8)	3324 (38.1)	0.72 (0.6-0.8)
Unknown, n (%)	21 (2.3)	168 (1.9)	
Smoking Status			
Never, n (%)	21 (2.3)	3252 (37.22)	1.00
Ever, n (%)	113 (12.4)	5286 (60.50)	5.08 (4.1-6.3)
Unknown, n (%)	776 (85.3)	199 (2.3)	
Excessive alcohol use, n (%)	143 (15.7)	952 (10.9)	1.56 (1.3-1.9)
COPD History, n (%)	294 (32.3)	1448 (16.6)	2.38 (2.1-2.8)
Asthma History, n (%)	124 (13.63)	1005 (11.5)	1.25 (1.0-1.5)
Ever use of NSAIDs, n (%)	533 (58.6)	5083 (58.2)	1.02 (0.9-1.2)
Ever use of Aspirin, n (%)	598 (65.7)	5299 (60.7)	1.30 (1.1-1.5)
Ever use of Statins, n (%)	617 (67.8)	5731 (65.6)	1.25 (1.0-1.5)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 1.8: Metformin use among lung cancer cases and controls (6 month lag period)				
Metformin exposure	**Cases n (%) (n=910)**	**Controls n (%) (n=8737)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	214 (23.5)	1993 (22.8)	1.00	1.00
Ever Metformin	696 (76.5)	6744 (77.2)	1.01 (0.8-1.2)	1.04 (0.9-1.3)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.9: Number of metformin prescriptions among lung cancer cases and controls (6 month lag period)						
# of prescriptions	**Cases n (%)**	**Follow-Up (years) mean, (SD)**	**Controls n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never,	214 (23.5)	3.5 (2.7)	1993 (22.8)	3.6 (2.8)	1.00	1.00
1 to 7	186 (20.4)	2.6 (2.4)	1828 (20.9)	2.7 (2.7)	1.01 (0.8-1.3)	1.00 (0.8-1.3)
8 to 19	166 (18.2)	3.5 (2.6)	1646 (18.8)	3.5 (2.5)	1.00 (0.8-1.3)	1.04 (0.8-1.4)
20 to 38	159 (17.5)	4.9 (2.7)	1585 (18.1)	5.0 (2.7)	0.99 (0.8-1.3)	1.06 (0.8-1.4)
39 or more	185 (20.3)	8.0 (3.3)	1685 (19.3)	8.1 (3.3)	1.06 (0.8-1.4)	1.17 (0.9-1.6)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications						

Table 1.10: Other anti-diabetes medication use among lung cancer cases and controls (6 month lag period)				
Sulfonylureas use among lung cancer cases and controls				
Sulfonylureas exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	330 (36.3)	3366 (38.5)	1.00	1.00
Ever Sulfonylureas	580 (63.7)	5371 (61.5)	1.07 (0.9-1.3)	1.02 (0.9-1.2)
TZD use among lung cancer cases and controls				
TZD exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	784 (86.2)	7436 (85.1)	1.00	1.00
Ever TZD	126 (13.9)	1301 (14.9)	0.92 (0.7-1.1)	0.94 (0.8-1.2)
Insulin use among lung cancer cases and controls				
Insulin exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	807 (88.7)	7972 (91.2)	1.00	1.00
Ever Insulin	103 (11.3)	765 (8.8)	1.32 (1.4-1.7)	1.24 (0.96-1.6)
Alpha-Glucosidase Inhibitors use among lung cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	878 (96.5)	8494 (97.2)	1.00	1.00
Ever Alpha-Glucosidase	32 (3.5)	243 (2.8)	1.26 (0.9-1.9)	1.20 (0.8-1.8)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
OTHER exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never OTHER	895 (98.4)	8591 (98.3)	1.00	1.00
Ever OTHER	15 (1.7)	146 (1.7)	0.93 (0.5-1.6)	0.95 (0.5-1.7)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.11: Ever other drug use with metformin among lung cancer cases and controls (6 month lag period)						
Ever Drug	**Cases (n=696)**	**Follow-Up (years) mean, (SD)**	**Controls (n=6744)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Metformin	285 (40.9)	2.7 (2.0)	2882 (42.7)	0.0 (2.1)	1.00	1.00
Metformin+Sulfonylureas	231 (33.2)	3.2 (2.2)	2180 (32.3)	5.7 (3.2)	1.07 (0.9-1.3)	1.02 (0.8-1.3)
Metformin + TZD	33 (4.7)	4.23 (2.5)	382 (5.6)	4.2 (2.4)	0.89 (0.6-1.3)	0.91 (0.6-1.4)
Metformin + Insulin	5 (0.7)	2.8	40 (0.1)	5.1 (3.7)	1.30 (0.5-3.4)	1.14 (0.4-3.00
Met + TZD + Sulf	58 (8.3)	5.4 (3.3)	651 (9.7)	5.3 (3.1)	0.9 (0.6-1.3)	0.90 (0.7-1.3)
Met + TZD + Insulin	1 (0.1)	9.7 (4.2)	7 (0.01)	7.0 (3.4)	1.39 (0.2-11.4)	0.93 (0.1-7.8)
Met + Sulf + Insulin	58 (8.3)	6.9 (2.6)	435 (6.5)	7.0 (3.2)	1.32 (0.9-1.9)	1.19 (0.8-1.7)
Met+Sulf+Insulin+TZD	25 (3.6)	9.1 (3.2)	167 (1.9)	9.0 (2.5)	1.5 (1.0-2.5)	1.51 (0.9-2.5)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications						

Figure 1.3: Lung cancer study flow chart (2 year lag period)

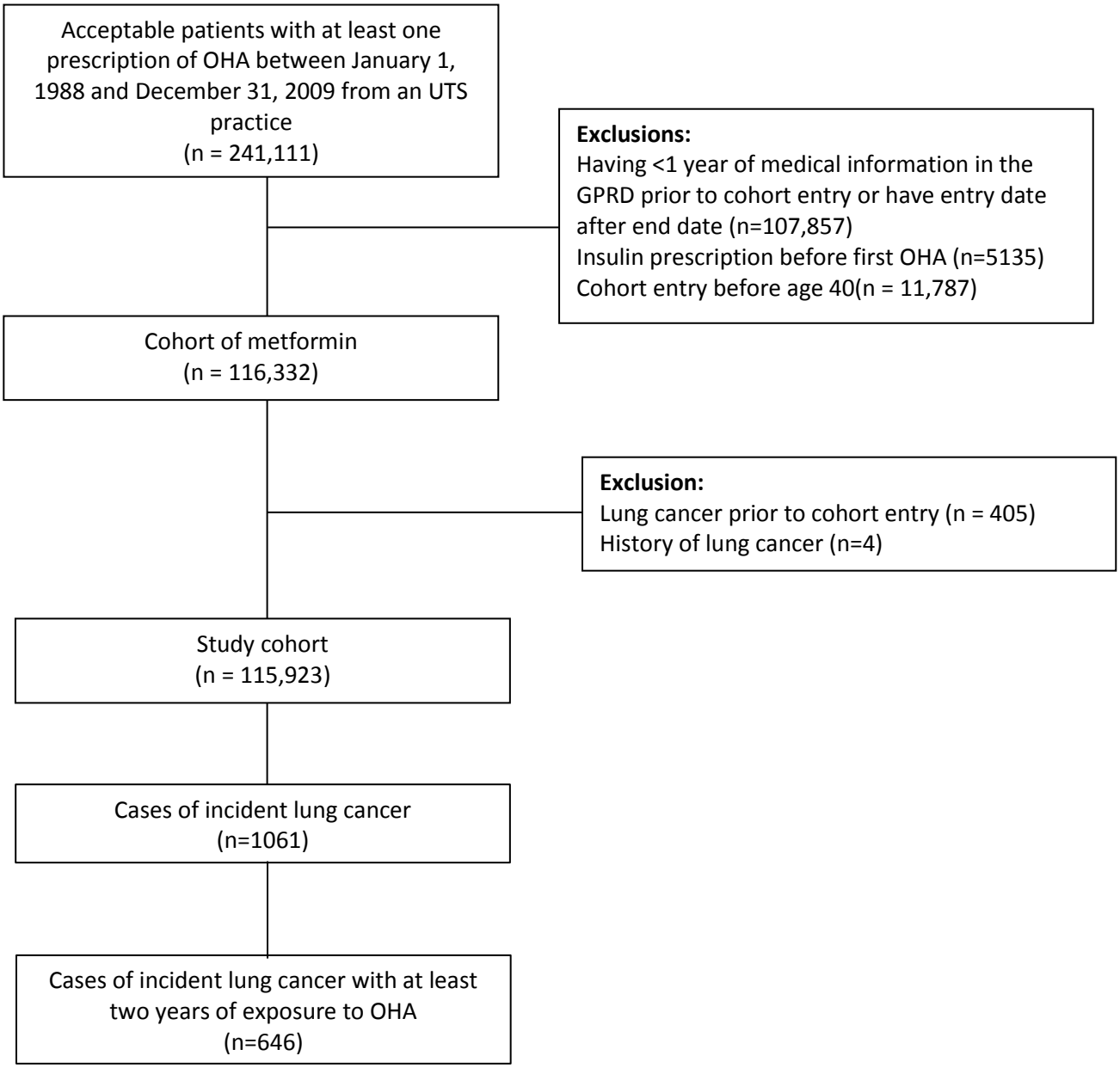

Table 1.12: Characteristics of lung cancer cases and controls at index date (2 year lag period)			
	Cases	**Controls**	**Crude RR**
	(n=646)	**(n= 6164)**	**95% CI**
Sex, males (%) *	426 (65.9)	4059 (65.9)	
Age at index date, (years), mean (SD) *	73.4 (8.3)	73.4 (8.4)	
Duration of follow-up (years), mean (SD) *	5.9 (3.2)	5.9 (3.0)	
Duration of disease prior to cohort entry (years), mean(SD)	2.2 (3.6)	2.0 (3.7)	
HbA1c (%), median (last result at year prior to index date)	(n=7.2)	(n=7.2)	
< 6.5%, n (%)	109 (16.9)	1115 (18.1)	1.00
6.5-7.4%, n (%)	198 (30.65)	1854 (30.1)	1.10 (0.9-1.4)
7.5-8.9%, n (%)	135 (20.9)	1352 (21.9)	1.02 (0.8-1.3)
≥9%, n (%)	72 (11.2)	604 (9.8)	1.2 (0.9-1.7)
Unknown, n (%)	132 (20.4)	1239 (20.1)	
Body mass index			
< 30, n (%)	382 (591)	3671 (69.6)	1.00
≥ 30, n (%)	254 (39.3)	2399 (39.0)	1.03 (0.9-1.2)
Unknown, n (%)	10 (1.6)	94 (1.5)	
Smoking Status			
Never, n (%)	85 (13.2)	2243 (36.4)	1.00
Ever, n (%)	550 (85.14)	3809 (61.8)	4.6 (3.6 -5.9)
Unknown, n (%)	11 (1.7)	112 (1.8)	
Excessive alcohol use, n (%)	88 (13.6)	659 (10.7)	1.33 (1.1-1.7)
COPD History, n (%)	206 (31.9)	1012 (16.4)	2.38 (2.0-2.9)
Asthma History, n (%)	87 (13.5)	696 (11.3)	1.26 (1.0-1.6)
Ever use of NSAIDs, n (%)	381 (59.0)	3633 (58.9)	1.00 (0.8-1.2)
Ever use of Aspirin, n (%)	431 (66.7)	3826 (62.1)	1.26 (1.1-1.5)
Ever use of Statins, n (%)	456 (70.6)	4065 (66.0)	1.48 (1.2-1.9)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 1.13: Metformin use among lung cancer cases and controls (2 year lag period)				
Metformin exposure	**Cases n (%) (n=646)**	**Controls n (%) (n=6164)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	165 (25.5)	1633 (26.5)	1.00	1.00
Ever Metformin	481 (74.5)	4531 (73.5)	1.1 (0.9-1.4)	1.03 (0.8-1.3)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.14: Number of metformin prescriptions among lung cancer cases and controls (2 year lag period)

# of prescriptions	Cases n (%)	Follow-Up (years) mean, (SD)	Controls n (%)	Follow-Up (years) mean, (SD)	Crude RR	Adjusted RR (95% CI)**
Never,	165 (25.5)	4.9 (2.5)	1633 (26.5)	4.9 (2.6)	1.00	1.00
1 to 7	138 (21.4)	4.3 (2.7)	1244 (20.2)	4.3 (2.6)	1.19 (0.9-1.5)	1.10 (0.8-1.5)
8 to 17	103 (15.9)	4.9 (2.4)	1033 (16.8)	4.7 (2.3)	1.03 (0.8-1.4)	0.94 (0.7-1.3)
18 to 35	111 (17.2)	6.2 (2.5)	1140 (18.5)	6.1 (2.4)	1.01 (0.8-1.3)	0.97 (0.7-1.3)
36 or more	129 (20.0)	9.2 (3.2)	1114 (16.6)	8.8 (2.8)	1.17 (0.9-1.6)	1.11 (0.8-1.6)

****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications

Table 1.15: Other anti-diabetes medication use among lung cancer cases and controls (2 year lag period)

Sulfonylureas use among lung cancer cases and controls				
Sulfonylureas exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	210 (32.5)	2097 (34.0)	1.00	1.00
Ever Sulfonylureas	436 (67.5)	4067 (66.0)	1.03 (0.9-1.3)	1.03 (0.8-1.3)
TZD use among lung cancer cases and controls				
TZD exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	564 (87.3)	5413 (87.8)	1.00	1.00
Ever TZD	82 (12.7)	751 (12.2)	1.06 (0.8-1.4)	1.02 (0.8-1.3)
Insulin use among lung cancer cases and controls				
Insulin exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	570 (88.2)	5620 (91.2)	1.00	1.00
Ever Insulin	76 (11.76)	544 (8.8)	1.36 (1.0-1.8)	1.25 (0.9-1.7)
Alpha-Glucosidase Inhibitors use among lung cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	620 (96.0)	5962 (96.7)	1.00	1.00
Ever Alpha-Glucosidase	26 (4.0)	202 (3.3)	1.22(0.8-1.9)	1.10 (0.7-1.7)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
OTHER exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never OTHER	634 (98.1)	6058 (98.3)	1.00	1.00
Ever OTHER	12 (1.9)	106 (1.7)	1.03 (0.6-1.9)	0.99 (0.5-1.9)

****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications

Table 1.16: Ever other drug use with metformin among lung cancer cases and controls (2 year lag period)						
Ever Drug	**Cases (n=481)**	**Follow-Up (years) mean, (SD)**	**Controls (n=4531)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Metformin	183 (38.1)	4.0 (1.8)	1832 (40.0)	4.1 (1.9)	1.00	1.00
Metformin+Sulfonylureas	174 (36.2)	6.7 (2.9)	1691 (37.3)	6.5 (2.9)	1.04 (0.8-1.3)	0.96 (0.8-1.2)
Metformin + TZD	20 (4.1)	5.5 (2.3)	188 (4.1)	5.4 (2.3)	1.10 (0.7-1.8)	1.03 (0.6-1.7)
Metformin + Insulin	3 (0.6)	4.8 (0.4)	28 (0.6)	6.9 (2.7)	1.32 (0.4-3.8)	0.96 (0.3-3.3)
Met + TZD + Sulf	39 (8.1)	8.2 (2.8)	372 (8.2)	8.2 (3.0)	1.09 (0.7-1.6)	1.00 (0.7-1.5)
Met + TZD + Insulin	1 (0.2)	2.8	8 (0.2)	6.5 (2.7)	1.29 (0.2-10.4)	1.34 (0.2-11.5)
Met + Sulf + Insulin	46 (9.5)	10.7 (3.7)	311 (6.8)	9.5 (3.0)	1.49 (1.0-2.2)	1.22 (0.8-1.9)
Met+Sulf+Insulin+TZD	15 (3.2)	9.7 (2.5)	101 (2.2)	9.9 (2.8)	1.57 (0.9-2.9)	1.26 (0.7-2.4)

****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications

Table 1.17 BMI and HbA1c characteristics of lung cancer cases and controls at baseline			
	Cases (n=808)	**Control (n=7764)**	**Crude RR (95% CI)**
HbA1c (%), median (last result prior to cohort entry)			
< 6.5%, n (%)	23 (2.9)	233 (3.0)	1.00
6.5-7.4%, n (%)	71 (8.8)	749 (9.7)	0.98 (0.6-1.6)
7.5-8.9%, n (%)	145 (18.0)	1599 (20.6)	0.93 (0.6-1.5)
≥9%, n (%)	155 (19.2)	1323 (17.0)	1.20 (0.8-1.9)
Unknown, n (%)	414 (51.2)	3860 (49.7)	
Body mass index at base line			
< 30, n (%)	491 (60.8)	4613 (59.4)	1.00
≥ 30, n (%)	302 (37.4)	3011 (38.8)	0.95 (0.8-1.1)
Unknown, n (%)	15 (1.9)	140 (1.8)	
Changes in Body Mass Index (%), (measured at baseline and index date)			
< 30 for baseline and index date, n (%)	428 (53.0)	4132 (53.2)	1.00
< 30 at baseline to ≥ 30 at index date, n (%)	54 (6.7)	425 (5.5)	1.23 (0.9-1.7)
≥ 30 at baseline to < 30 at index date, n (%)	63 (7.8)	481 (6.2)	1.28 (1.0-1.7)
≥ 30 for baseline and index date, n (%)	248 (30.7)	2586 (33.3)	0.94 (0.8-1.1)
Unknown, n (%)	15 (1.9)	140 (1.8)	

Table 1.18: Metformin use among lung cancer cases and controls (BMI at baseline)				
Metformin exposure	**Cases n (%) (n=808)**	**Controls n (%) (n=7764)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.94 (0.8-1.2)
****Adjusted for:** BMI at baseline, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.19: Metformin use among lung cancer cases and controls (change in BMI measured at baseline and index date)				
Metformin exposure	**Cases n (%) (n=808)**	**Controls n (%) (n=7764)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.92 (0.7-1.2)
****Adjusted for: (change in BMI from baseline to index date)**, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 1.20: Metformin use among lung cancer cases and controls (HbA1c at baseline)				
Metformin exposure	**Cases n (%) (n=808)**	**Controls n (%) (n=7764)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.92 (0.7-1.1)
****Adjusted for:** BMI, smoking, COPD, asthma, statins, NSAIDs, aspirin, **HbA1c at baseline**, diabetes duration, and all other diabetes medications				

Table 1.21: Metformin use among lung cancer cases and controls (BMI at baseline and HbA1c at baseline)				
Metformin exposure	**Cases n (%) (n=808)**	**Controls n (%) (n=7764)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	192 (23.8)	1769 (22.8)	1.00	1.00
Ever Metformin	616 (76.2)	5995 (77.2)	0.97 (0.8-1.2)	0.93 (0.8-1.2)
****Adjusted for: BMI at baseline**, smoking, COPD, asthma, statins, NSAIDs, aspirin, alcohol, **HbA1c at baseline**, diabetes duration, and all other diabetes medications				

Figure 2.1: Colorectal study flow chart

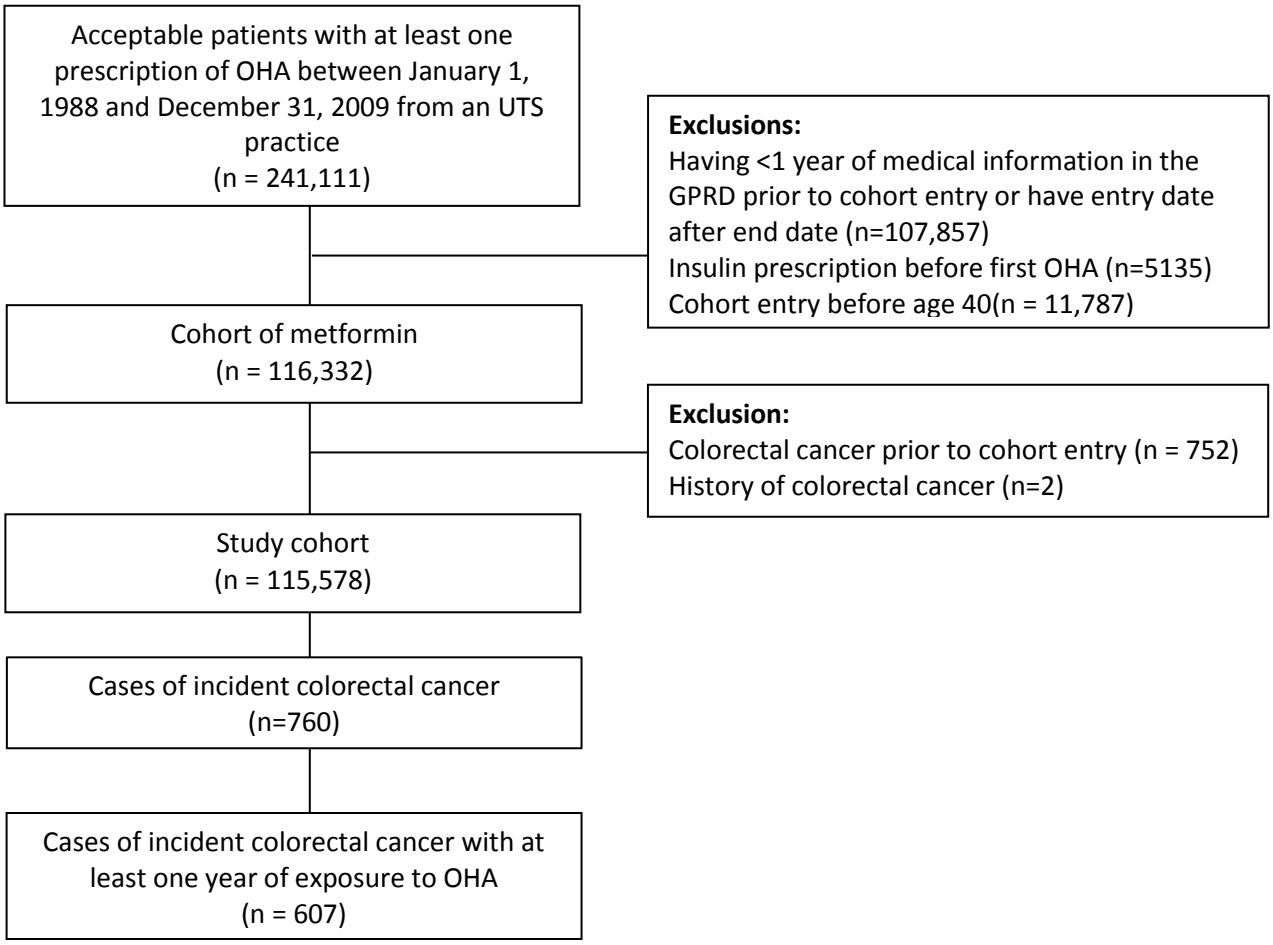

Table 2.1 Characteristics of the cohort (colorectal cancer)	
Cohort (n = 115,578)	
Age (years), mean (SD)	64.1 (12.0)
Duration of follow-up (years), mean (SD)	4.5 (3.6)
Total person-years of follow-up	525,587
Duration of disease, mean(SD)	3.7 (4.3)
HbA1c (%), median	8.2
Receipt of OHA at cohort entry, n (%)	
Metformin	77,793 (67.3)
Sulfonylureas	34,289 (29.7)
Other OHA	1483 (1.3)
Combinations*	2013 (1.7)
Rate of colorectal cancer, per 1000/year (95% CI)	1.4 (1.3, 1.6)
*Combinations of any two or more different OHAs.	

Table 2.2: Characteristics of colorectal cancer cases and controls at index date			
	Cases	**Control**	**Crude RR**
	(n=607)	**(n=5837)**	**(95% CI)**
Sex, n=males (%) *	384 (63.3)	3712 (63.6)	
Age at index date, (years), mean (SD) *	72.8 (8.7)	72.5 (8.5)	
Duration of follow-up (years), mean (SD) *	4.8 (3.1)	4.8 (2.9)	
Duration of disease prior to cohort entry (years), mean(SD)	2.0 (3.4)	2.1 (3.9)	
HbA1c (%), median (last result at year prior to index date)	7.3 (n=492)	7.1 (n=4696)	
< 6.5%, n (%)	99 (16.3)	1067 (18.3)	1.00
6.5-7.4%, n (%)	185 (30.5)	1860 (31.9)	1.07 (0.8-1.4)
7.5-8.9%, n (%)	144 (23.7)	1224 (21.0)	1.28 (0.8-1.4)
≥9%, n (%)	64 (10.5)	545 (9.3)	1.27 (0.9-1.8)
Unknown, n (%)	115 (19.0)	1141 (19.6)	
Body mass index			
< 30, n (%)	358 (59.0)	3451 (59.1)	1.00
≥ 30, n (%)	252 (38.2)	2278 (39.0)	1.00 (0.8-1.2)
Unknown, n (%)	17 (2.8)	108 (1.9)	
Smoking Status			
Never, n (%)	235 (38.7)	2243 (38.4)	1.00
Ever, n (%)	356 (58.7)	3447 (59.0)	1.01 (0.8-1.2)
Unknown, n (%)	16 (2.6)	147 (2.5)	
Excessive alcohol use, n (%)	74 (12.2)	623 (10.7)	1.18 (0.9-1.5)
History of Colonoscopy, n (%)	24 (4.0	204 (3.5)	1.11 (0.7-1.7)
History of Polyps, n (%)	18 (3.0)	105 (1.8)	1.71 (1.0-2.8)
History of Inflammatory Bowel Diseases, n (%)	8 (1.3)	90 (1.5)	0.85 (0.4-1.8)
History of Cholecystectomy, n (%)	39 (6.4)	317 (5.4)	1.19 (0.8-1.7)
Ever use of NSAIDs, n (%)	358 (58.8)	3424 (58.7)	1.02 (0.9-1.2)
Ever use of Aspirin, n (%)	347 (57.2)	3412 (58.5)	0.95 (0.8-1.1)
Ever use of Statins, n (%)	357 (58.8)	3643 (62.4)	0.86 (0.7-1.1)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 2.3: Metformin use among colorectal cancer cases and controls				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.94 (0.7-1.2)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.4: Number of metformin prescriptions among colorectal cancer cases and controls						
# of prescriptions	**Cases n (%)**	**Follow-Up (years) mean, (SD)**	**Controls n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never	163 (26.9)	4.1 (2.7)	1431 (24.5)	4.0 (2.5)	1.00	1.00
1 to 7	140 (23.0)	3.3 (2.7)	1176 (20.2)	3.0 (2.4)	1.13 (0.9-1.5)	1.14 (0.9-1.5)
8 to 17	94 (15.5)	3.3 (1.7)	1033 (17.7)	3.7 (2.3)	0.80 (0.6-1.1)	0.83 (0.6-1.1)
18 to 36	98 (16.1)	5.6 (2.4)	1104 (18.9)	5.2 (2.4)	0.75 (0.6-1.0)	0.77 (0.6-1.1)
37 or more	112 (18.5)	8.3 (2.8)	1093 (18.7)	7.7 (2.6)	0.83 (0.6-1.1)	0.86 (0.6-1.2)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications						

Table 2.5: Other anti-diabetic medication use among colorectal cancer cases and controls				
Sulfonylureas use among colorectal cancer cases and controls				
Sulfonylureas exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	204 (33.6)	2194 (37.6)	1.00	1.00
Ever Sulfonylureas	403 (66.4)	3643 (62.4)	1.18 (1.0-1.5)	1.14 (0.9-1.4)
TZD use among colorectal cancer cases and controls				
TDZ exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	521 (85.8)	5099 (87.4)	1.00	1.00
Ever TZD	86 (14.2)	739 (12.6)	1.18 (0.9-1.5)	1.15 (0.9-1.5)
Insulin use among colorectal cancer cases and controls				
Insulin exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	5351 (91.7)	557 (91.8)	1.00	1.00
Ever Insulin	486 (8.3)	50 (8.2)	0.90 (0.6-1.3)	1.21 (0.9-1.7)
Alpha-Glucosidase inhibitors use among colorectal cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	583 (96.1)	5674 (97.2)	1.00	1.00
Ever Alpha-Glucosidase	24 (4.0)	163 (2.8)	1.38 (0.9-2.2)	1.39 (0.9-2.2)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
Other exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Other	597 (98.4)	5753 (98.6)	1.00	1.00
Ever Other	10 (1.7)	84 (1.4)	1.17 (0.6-2.3)	1.16 (0.6-2.3)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.6: Ever other drug use with metformin among colorectal cancer cases and controls						
Ever Drug	**Cases (n=444)**	**Follow-Up (years) mean (SD)**	**Controls (n=4406)**	**Follow-Up (years) mean (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Metformin	180 (40.5)	3.1 (1.9)	1885 (42.8)	4.8 (2.0)	1.00	1.00
Metformin+Sulfonylureas	156 (35.1)	5.7 (2.8)	1522 (34.5)	5.6 (2.8)	1.08 (0.8-1.4)	1.05 (0.8-1.4)
Metformin + TZD	16 (3.6)	5.1 (2.9)	236 (5.4)	4.6 (2.4)	0.72 (0.4-1.2)	0.70 (0.4-1.2)
Metformin + Insulin	2 (0.5)	4.1 (1.3)	22 (0.5)	4.8 (2.5)	1.01 (0.2-4.3)	1.03 (0.2-4.4)
Met + TZD + Sulf	51 (11.4)	6.6 (3.0)	352 (7.9)	7.3 (2.7)	1.55 (1.1-2.3)	1.48 (1.0-2.1)
Met + TZD + Insulin	1 (0.2)	6.8	9 (0.2)	8.1 (3.3)	1.23 (0.2-9.8)	1.03 (0.1-8.3)
Met + Sulf + Insulin	27 (6.1)	10.0 (3.2)	295 (6.7)	4.8 (1.9)	0.88 (0.6-1.4)	0.80 (0.5-1.3)
Met+Sulf+Insulin+TZD	11 (2.4)	9.5 (3.1)	85 (1.9)	8.7 (2.9)	1.37 (0.7-2.7)	1.26 (0.6-2.5)

****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications

Figure 2.2: Colorectal study flow chart (6 month lag period)

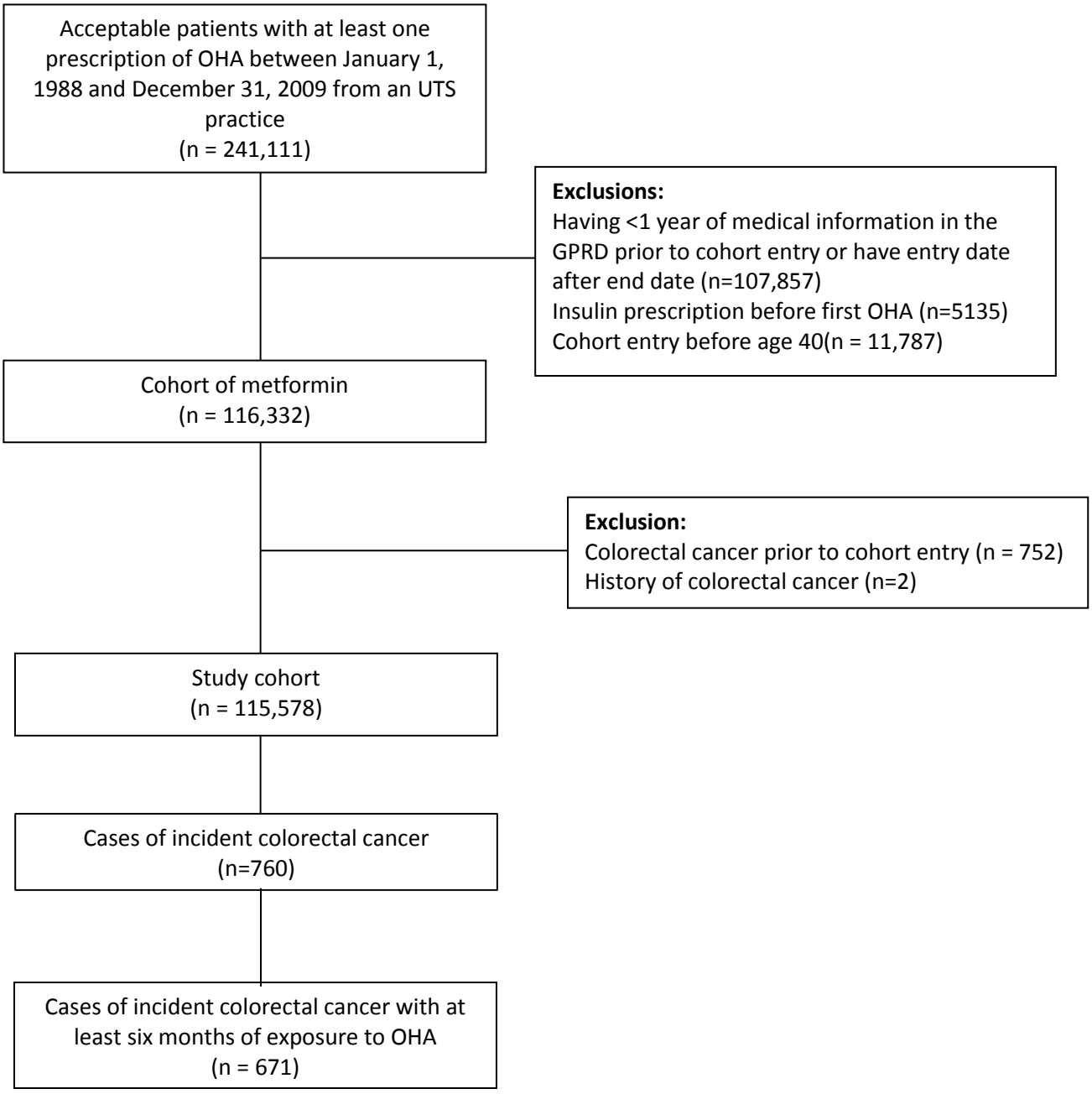

Table 2.7: Characteristics of colorectal cancer cases and controls at index date (6month lag period)			
	Cases (n=671)	**Control (n=6470)**	**Crude RR (95% CI)**
Sex, n=males (%) *	417 (62.2)	4035 (62.4)	
Age at index date, (years), mean (SD) *	72.9 (8.8)	72.9 (8.6)	
Duration of follow-up (years), mean (SD) *	4.4 (3.2)	4.4 (3.2)	
Duration of disease prior to cohort entry (years), mean (SD)	2.0 (3.4)	2.1 (3.6)	
HbA1c (%), median (last result at year prior to index date)	7.2	7.1	
< 6.5%, n (%)	112 (16.7)	1236 (19.1)	1.00
6.5-7.4%, n (%)	224 (33.4)	2038 (31.5)	1.21 (0.96-1.5)
7.5-8.9%, n (%)	134 (20.0)	1424 (22.0)	1.03 (0.8-1.3)
≥9%, n (%)	75 (11.2)	592 (9.2)	1.41 (1.0-2.0)
Unknown, n (%)	126 (18.8)	1180 (18.2)	
Body mass index			
< 30, n (%)	390 (58.1)	3815 (59.0)	1.00
≥ 30, n (%)	259 (38.6)	2536 (39.2)	1.72 (1.0-2.9)
Unknown, n (%)	22 (3.3)	119 (1.8)	
Smoking Status			
Never, n (%)	263 (39.2)	2469 (38.2)	1.00
Ever, n (%)	389 (58.0)	3796 (58.7)	0.98 (0.8-1.2)
Unknown, n (%)	19 (2.8)	205 (3.2)	
Excessive alcohol use, n (%)	79 (11.8)	615 (9.51)	1.3 (1.0-1.7)
History of Colonoscopy, n (%)	24 (3.6)	202 (3.1)	1.12 (0.7-1.7)
History of Polyps, n (%)	19 (2.8)	107 (1.7)	1.78 (1.1-2.9)
History of Inflammatory Bowel Diseases, n (%)	8 (1.2)	79 (1.2)	0.97 (0.5-2.0)
History of Cholecystectomy, n (%)	42 (6.3)	396 (6.1)	1.02 (0.7-1.4)
Ever use of NSAIDs, n (%)	389 (58.0)	3706 (57.3)	1.03 (0.88-1.2)
Ever use of Aspirin, n (%)	374 (55.7)	3709 (57.3)	0.93 (0.8-1.1)
Ever use of Statins, n (%)	383 (57.1)	3907 (60.4)	0.87 (0.7-1.1)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 2.8: Metformin use among colorectal cancer cases and controls (6month lag period)				
Metformin exposure	**Cases n (%) (n=671)**	**Controls n (%) (n=6470)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	167 (24.9)	1547 (23.9)	1.0	1.00
Ever Metformin	504 (75.11)	4923 (76.1)	0.97 (0.8-1.2)	1.03 (0.8-1.3)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.9: Number of metformin prescriptions among colorectal cancer cases and controls (6month lag period)						
# of prescriptions	**Cases n (%)**	**Follow-Up (years) mean, (SD)**	**Controls n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never	167 (24.9)	3.5 (2.6)	1547 (23.9)	3.6 (2.6)	1.00	1.00
1 to 7	162 (24.1)	3.1 (2.9)	1280 (19.8)	2.6 (2.6)	1.30 (1.0-1.7)	1.32 (1.0-1.7)
8 to 18	110 (16.4)	3.0 (1.9)	1186 (18.3)	3.3 (2.4)	0.87 (0.7-1.1)	0.91 (0.7-1.2)
19 to 38	109 (16.2)	5.1 (2.5)	1256 (19.4)	4.7 (2.4)	0.77 (0.6-1.0)	0.82 (0.6-1.1)
39 or more	123 (18.3)	8.0 (3.0)	1201 (18.6)	7.4 (2.6)	0.89 (0.7-1.2)	0.94 (0.7-1.3)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications						

Table 2.10: Other anti-diabetic medication use among colorectal cancer cases and controls (6month lag period)				
Sulfonylureas use among colorectal cancer cases and controls				
Sulfonylureas exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	233 (34.7)	2477 (38.3)	1.00	1.00
Ever Sulfonylureas	438 (65.3)	3993 (61.7)	1.15 (1.0-1.4)	1.17 (0.95-1.5)
TZD use among colorectal cancer cases and controls				
TDZ exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	572 (85.3)	5606 (86.7)	1.00	1.00
Ever TZD	99 (14.8)	864 (13.4)	1.16 (0.9-1.5)	1.13 (0.8-1.6)
Insulin use among colorectal cancer cases and controls				
Insulin exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	614 (91.5)	5934 (91.7)	1.00	1.00
Ever Insulin	57 (8.5)	536 (8.3)	0.94 (0.7-1.3)	1.14 (0.9-1.5)
Alpha-Glucosidase inhibitors use among colorectal cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	646 (96.3)	6303 (97.4)	1.00	1.00
Ever Alpha-Glucosidase	25 (3.7)	167 (2.6	1.40 (0.9-2.2)	1.39 (0.9-2.2)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
Other exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Other	359 (98.2)	6362 (98.3)	1.00	1.00
Ever Other	12 (1.8)	108 (1.7)	1.09 (0.6-2.0)	1.08 (0.6-2.0)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.11: Ever other drug use with metformin among colorectal cancer cases and controls (6 month lag period)

Ever Drug	Cases (n=504)	Follow-Up (years) mean (SD)	Controls (n=4923)	Follow-Up (years) mean (SD)	Crude RR	Adjusted RR (95% CI)**
Metformin	204 (40.5)	2.6 (2.0)	2160 (43.9)	2.8 (2.1)	1.00	1.00
Metformin+Sulfonylureas	174 (34.5)	5.4 (2.9)	1631 (33.1)	5.2 (2.9)	1.14 (0.9-1.4)	1.14 (0.9-1.4)
Metformin + TZD	22 (4.3)	4.4 (3.0)	251 (5.1)	4.3 (2.4)	0.95 (0.6-1.5)	0.95 (0.6-1.5)
Metformin + Insulin	2 (0.4)	4.1 (1.3)	21 (0.4)	5.1 (2.4)	1.07 (0.3-4.6)	1.03 (0.2-4.5)
Met + TZD + Sulf	57 (11.3)	6.5 (3.0)	415 (8.4)	6.8 (2.9)	1.50 (1.1-2.1)	1.47 (1.0-2.1)
Met + TZD + Insulin	2 (0.4)	4.8 (2.9)	9 (0.2)	5.8 (2.4)	2.53 (0.5-11)	2.5 (0.5-12)
Met + Sulf + Insulin	31 (6.2)	9.3 (3.6)	332 (6.7)	7.7 (3.4)	0.93 (0.6-1.4)	0.88 (0.6-1.4)
Met+Sulf+Insulin+TZD	12 (2.4)	9.7 (3.0)	104 (2.1)	8.3 (3.0)	1.25 (0.7-2.4)	1.20 (0.6-2.3)

****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications

Figure 2.3s: Colorectal cancer study flow chart (2 year lag period)

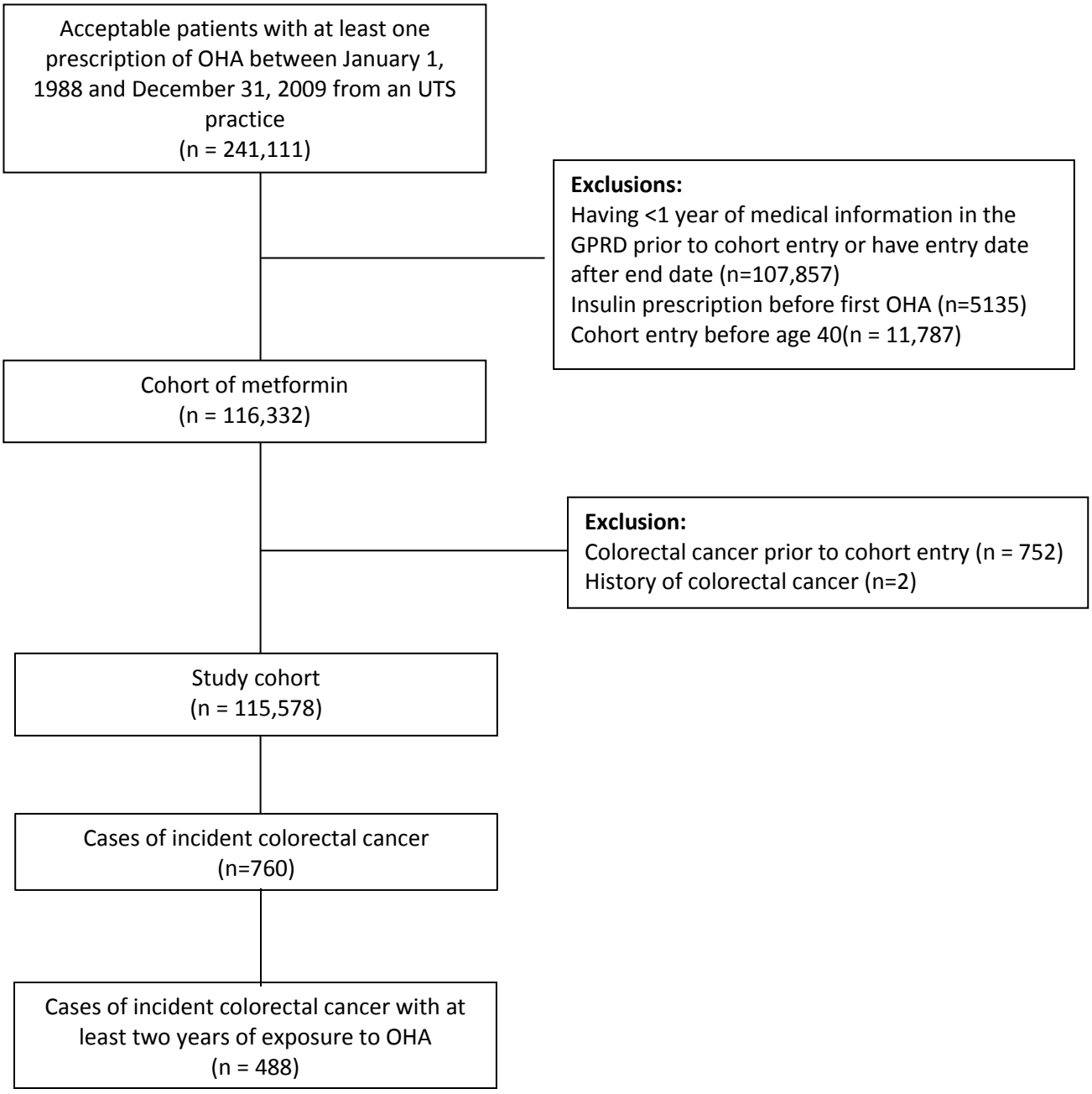

Table 2.12: Characteristics of colorectal cancer cases and controls at index date (2 year lag period)			
	Cases (n=488)	**Control (n=4655)**	**Crude RR (95% CI)**
Sex, n=males (%) *	313 (64.1)	3004 (64.5)	
Age at index date, (years), mean (SD) *	73.3 (8.7)	73.3 (8.4)	
Duration of follow-up (years), mean (SD) *	5.6 (2.9)	5.6 (2.7)	
Duration of disease prior to cohort entry (years), mean (SD)	1.8 (2.8)	2.1 (3.8)	
HbA1c (%), median (last result at year prior to index date)	7.2	7.2	
< 6.5%, n (%)	75 (15.4)	810 (17.4)	1.00
6.5-7.4%, n (%)	142 (29.1)	1386 (29.8)	1.12 (0.8-1.5)
7.5-8.9%, n (%)	103 (21.1)	1035 (22.2)	1.08 (0.8-1.5)
≥9%, n (%)	48 (9.8)	467 (10.0)	1.11 (0.8-1.7)
Unknown, n (%)	120 (24.6)	957 (20.6)	
Body mass index			
< 30, n (%)	292 (59.8)	2763 (59.4)	1.00
≥ 30, n (%)	184 (37.7)	1830 (39.3)	1.55 (0.8-3.1)
Unknown, n (%)	12 (2.5)	62 (1.3)	
Smoking Status			
Never, n (%)	191 (39.1)	1738 (37.3)	1.00
Ever, n (%)	287 (58.8)	2821 (60.6)	0.95 (0.8-1.2)
Unknown, n (%)	10 (2.1)	96 (2.1)	
Excessive alcohol use, n (%)	58 (11.89)	489 (10.5)	1.16 (0.9-1.6)
History of Colonoscopy, n (%)	19 (3.9)	172 (3.7)	1.03 (0.6-1.7)
History of Polyps, n (%)	14 (2.9)	84 (1.8)	1.7 (0.9-3.0)
History of Inflammatory Bowel Diseases, n (%)	6 (1.2)	75 (1.6)	0.77 (0.3-1.8)
History of Cholecystectomy, n (%)	33 (6.8)	293 (6.3)	1.08 (0.7-1.6)
Ever use of NSAIDs, n (%)	286 (58.6)	2745 (59.0)	0.99 (0.8-1.2)
Ever use of Aspirin, n (%)	284 (58.2)	2747 (59.0)	0.97 (0.8-1.2)
Ever use of Statins, n (%)	297 (60.9)	2972 (63.9)	0.91 (0.7-1.2)
*** Controls matched to cases on these variables as well as calendar year of cohort entry**			

Table 2.13: Metformin use among colorectal cancer cases and controls (2 year lag period)				
Metformin exposure	**Cases n (%) (n=488)**	**Controls n (%) (n=4655)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	150 (30.7)	1315 (28.3)	1.0	1.00
Ever Metformin	338 (69.3)	3340 (71.8)	0.90 (0.7-1.1)	0.93 (0.7-1.2)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.14: Number of metformin prescriptions among colorectal cancer cases and controls (2 year lag period)						
# of prescriptions	**Cases n (%)**	**Follow-Up (years) mean, (SD)**	**Controls n (%)**	**Follow-Up (years) mean, (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Never	150 (30.7)	4.9 (2.6)	1315 (28.3)	4.7 (2.3)	1.00	1.00
1 to 7	101 (20.7)	4.1 (2.2)	916 (19.7)	4.2 (2.5)	1.03 (0.8-1.4)	1.03 (0.8-1.4)
8 to 17	72 (14.8)	4.6 (2.0)	797 (17.1)	4.6 (2.2)	0.81 (0.6-1.1)	0.85 (0.6-1.2)
18 to 34	77 (15.8)	6.3 (2.3)	802 (17.2)	6.1 (2.2)	0.85 (0.6-1.2)	0.87 (0.6-1.2)
35 or more	88 (18.0)	8.9 (2.8)	825 (17.7)	8.2 (2.5)	0.90 (0.7-1.3)	0.93 (0.6-1.2)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications						

Table 2.15: Other anti-diabetic medication use among colorectal cancer cases and controls (2 year lag period)				
Sulfonylureas use among colorectal cancer cases and controls				
Sulfonylureas exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Sulfonylureas	153 (31.4)	1613 (34.7)	1.00	1.00
Ever Sulfonylureas	335 (68.7)	3042 (65.4)	1.13 (0.9-1.4)	1.08 (0.8-1.4)
TZD use among colorectal cancer cases and controls				
TDZ exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never TZD	427 (87.5)	4112 (88.3)	1.00	1.00
Ever TZD	61 (12.5)	543 (11.7)	1.12 (0.8-1.5)	1.12 (0.8-1.5)
Insulin use among colorectal cancer cases and controls				
Insulin exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Insulin	449 (92.0)	4307 (92.5)	1.00	1.00
Ever Insulin	39 (8.0)	142 (3.1)	0.97 (0.7-1.4)	1.08 (0.7-1.6)
Alpha-Glucosidase inhibitors use among colorectal cancer cases and controls				
Alpha-Glucosidase Inhibitors exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Alpha-Glucosidase	467 (95.7)	4513 (97.0)	1.00	1.00
Ever Alpha-Glucosidase	21 (4.3)	142 (3.0)	1.4 (0.9-2.3)	1.41 (0.9-2.3)
All other OHA medications: Meglitinides Combination, DPP-4 Inhibitor, GLP-1 Analogs, and Guar Gum use among lung cancer cases and controls				
Other exposure	**Cases n (%)**	**Controls n (%)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Other	481 (98.6)	4596 (98.7)	1.00	1.00
Ever Other	7 (1.4)	59 (1.3)	1.16 (0.5-2.6)	1.16 (0.5-2.6)
****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.16: Ever other drug use with metformin among colorectal cancer cases and controls (2 year lag period)						
Ever Drug	**Cases (n=338)**	**Follow-Up (years) mean (SD)**	**Controls (n=3340)**	**Follow-Up (years) mean (SD)**	**Crude RR**	**Adjusted RR (95% CI)****
Metformin	128 (37.9)	4.0 (1.7)	1402 (42.0)	4.1 (1.69)	1.00	1.00
Metformin+Sulfonylureas	130 (38.5)	6.7 (2.7)	1223 (36.6)	6.5 (2.6)	1.16 (0.9-1.5)	1.13 (0.9-1.5)
Metformin + TZD	16 (4.7)	5.9 (3.4)	164 (4.9)	5.8 (2.3)	1.08 (0.6-1.9)	1.06 (0.9-1.9)
Metformin + Insulin	2 (0.6)	4.1 (1.3)	15 (0.5)	5.6 (2.0)	1.48 (0.3-6.5)	1.63 (0.4-7.2)
Met + TZD + Sulf	31 (9.1)	7.0 (2.6)	263 (7.9)	7.6 (2.6)	1.09 (0.6-1.8)	1.28 (0.8-2.0)
Met + TZD + Insulin	1 (0.3)	6.8	4 (0.1)	6.2 (1.0)	2.79 (0.3-25)	2.9 (0.3-27)
Met + Sulf + Insulin	24 (7.1)	10.1 (3.2)	218 (6.5)	9.0 (3.1)	1.29 (0.8-2.0)	1.03 (0.6-1.8)
Met+Sulf+Insulin+TZD	6 (1.8)	10.3 (3.6)	51 (1.5)	8.9 (2.8)	1.28 (0.5-3.1)	1.26 (0.5-3.1)

****Adjusted for:** BMI, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications

Table 2.17: BMI and HbA1c characteristics of colorectal cancer cases and controls at baseline			
	Cases (n=607)	**Control (n=5837)**	**Crude RR (95% CI)**
HbA1c (%), median (last result prior to cohort entry)	(8.1 n= 289)	(8.3 n= 2837)	
< 6.5%, n (%)	20 (3.3)	181 (3.1)	1.00
6.5-7.4%, n (%)	45 (7.4)	518 (8.9)	0.80 (0.5-1.4)
7.5-8.9%, n (%)	130 (21.4)	1140 (19.5)	1.06 (0.6-1.7)
≥9%, n (%)	94 (15.5)	1012 (17.3)	0.85 (0.5-1.4)
Unknown, n (%)	3118 (52.4)	2986 (51.16)	
Body mass index at base line			
< 30, n (%)	342 (56.3)	3468 (58.4)	1.00
≥ 30, n (%)	248 (40.9)	2261 (38.7)	1.14 (1.0-1.4)
Unknown, n (%)	17 (2.8)	108 (1.9)	
Changes in Body Mass Index (%), (measured at baseline and index date)			
< 30 for baseline and index date, n (%)	318 (52.4)	3116 (53.4)	1.00
< 30 at baseline to ≥ 30 at index date, n (%)	40 (6.6)	335 (5.7)	1.17 (0.8-1.7)
≥ 30 at baseline to < 30 at index date, n (%)	24 (4.0)	352 (6.0)	0.67 (0.4-1.0)
≥ 30 for baseline and index date, n (%)	208 (34.3)	1926 (33.0)	1.09 (0.9-1.3)
Unknown, n (%)	17 (2.8)	108 (1.9)	

Table 2.18: Metformin use among colorectal cancer cases and controls (BMI at baseline)				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.91 (0.7-1.2)
****Adjusted for: BMI at baseline**, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, HbA1c, diabetes duration, and all other diabetes medications				

Table 2.19: Metformin use among colorectal cancer cases and controls (change in BMI measured at baseline and index date)				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.92 (0.7-1.2)
****Adjusted for: change in BMI from baseline to index date**, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, hba1c, diabetes duration, and all other diabetes medications				

Table 2.20: Metformin use among colorectal cancer cases and controls (HbA1c at baseline)				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.97 (0.8-1.2)
****Adjusted for:** BMI at baseline, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, **HbA1c at baseline**, diabetes duration, and all other diabetes medications				

Table 2.21: Metformin use among colorectal cancer cases and controls (BMI at baseline and hba1c at baseline)				
Metformin exposure	**Cases n (%) (n=607)**	**Controls n (%) (n=5837)**	**Crude RR**	**Adjusted RR (95% CI)****
Never Metformin	163 (26.9)	1431 (24.5)	1.0	1.00
Ever Metformin	444 (73.1)	4406 (75.5)	0.90 (0.7-1.1)	0.94 (0.7-1.2)
****Adjusted for: BMI at baseline**, smoking, polyps, cholecystectomy, colonoscopy, statins, NSAIDS, aspirin, alcohol, **HbA1c at baseline**, diabetes duration, and all other diabetes medications				