Pioglitazone HCl (ACTOS)

Clinical Study No. 01-03-TL-OPI-524

Cohort Study of Pioglitazone and Bladder Cancer in Patients with Diabetes

Final Report with Data from January 1, 1997 to December 31, 2012

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Executive Summary

Our team of investigators from the University of Pennsylvania's Center for Clinical Epidemiology and Biostatistics, Kaiser Permanente Northern California (KPNC) Division of Research, and Rutgers University conducted this study to determine whether treatment with pioglitazone increases the incidence of bladder cancer among patients with diabetes mellitus. Since the launch of this study more than 10 years ago, five interim reports have been provided to the sponsor and the Food and Drug Administration. In our 5-year interim report (published in Diabetes Care 34:916–922, 2011), we did not observe a statistically significant association between pioglitazone treatment overall and bladder cancer risk. However, we observed a statistically significantly increased risk of bladder cancer among patients with the longest exposure to pioglitazone (i.e., 2+ years), a finding that has been confirmed in several subsequent observational studies. In the 8-year interim report, the magnitudes of our previously observed associations were weaker and no longer statistically significant. This report details the results of the final analysis.

We conducted a retrospective cohort study from 1997 to 2002 and a prospective follow-up from 2003 to 2012 with a nested case-control study among patients included in the KPNC Diabetes Registry. The study cohort included both patients with an established diagnosis of diabetes mellitus prior to January 1, 1997 and those who were newly diagnosed prior to December 31, 2002. Patients were required to be age 40 or older by December 31, 2002. We excluded those with a diagnosis of bladder cancer prior to entry in the cohort or within 6 months of joining KPNC.

We included a total of 16 years of data extending from January 1, 1997 through December 31, 2012. Follow-up started on the first date that the inclusion criteria were met. Follow-up for patients in the cohort ended when the first of the following occurred: 1) a gap of greater than 4 months in either membership or prescription benefits, 2) a new diagnosis of bladder cancer, 3) death from any cause, or 4) end of follow-up (December 31, 2012).

The outcome for this study was an incident diagnosis of bladder cancer identified from the KPNC cancer registry. The primary exposure of interest in this study was treatment with pioglitazone, defined as having filled at least two prescriptions for the drug within a 6-month period according to the KPNC pharmacy database. Exposure to pioglitazone and all other diabetes medications were treated as unidirectional time dependent variables, i.e., once a patient met the exposure definition the patient was considered exposed from that point forward, even if they discontinued the medication. We also examined exposure duration and cumulative dose. Cox regression models were constructed to estimate the hazard ratio and 95% confidence intervals (CI) after adjusting for potential confounders.

A nested case-control study was used to test for confounding by variables that are missing or less completely recorded within the KPNC electronic health record. Case subjects were cohort members diagnosed with incident bladder cancer. Controls who had not been diagnosed with bladder cancer as of the index date of the case subject were randomly selected from the same source cohort and matched to the case subject on sex, age (± 2.5 years), and time from entry into the diabetes registry to index date (± 6 months). Detailed smoking and occupational histories were collected by telephone interview.

After applying the exclusion criteria, the final cohort included 193,099 patients with diabetes. Among patients who ever used pioglitazone as of the end of follow-up, the median time from the first prescription to the end of follow-up was 6.1 years (range 0.2-13.3 years) and the median duration of therapy among pioglitazone treated patients was 2.8 years (range 0.2-13.2 years). By the end of follow-up, approximately one-third of the pioglitazone exposed patients had started pioglitazone more than 8 years earlier, had more than 4 years of use of pioglitazone, and had received more than 40,000 mg in total dose.

Among the 1261 cases of newly diagnosed bladder cancer, 186 were patients who ever used pioglitazone before the bladder cancer diagnosis and 1075 were patients who never used pioglitazone. In the fully adjusted model, ever exposure to pioglitazone was not associated with subsequent diagnosis with bladder cancer (HR 1.06; 95% CI 0.89-1.26). None of the categories of dose or duration were statistically significantly associated with an elevated risk of bladder cancer. For patients with more than 4 years of exposure to pioglitazone, the hazard ratio was 1.16 (95% CI 0.87 – 1.54) in the fully adjusted model. Similar results were observed for more than 8 years since initiation of pioglitazone therapy (HR 1.20, 95% CI 0.83 – 1.75) and more than 40,000 mg of total exposure (HR 1.07, 95% CI 0.79 – 1.44). Tests for trend were not statistically significant.

The nested case-control study included 464 pairs of case and control subjects. Odds ratios from the case-control study were generally similar to the hazard ratios measured in the cohort study and there was little evidence for additional confounding by race, smoking history, high-risk occupations, or prior urinary tract infections. Thus, we believe that the cohort study provides the strongest evidence to address the study aims.

An extensive series of planned and post hoc sensitivity and secondary analyses were done to understand why the results of the final analysis differed from those of the interim analyses done after the initial 5 years of the study, and reproduced by other groups. Notable findings from these analyses were a small decrease in the rates of bladder cancer among patients with more than 2 years of pioglitazone exposure and a small increase in bladder cancer incidence among the pioglitazone unexposed during the follow-up period after April 30, 2008 (the end of follow-up in the published interim analysis). Further subdividing the pioglitazone exposure category of more than 4 years into 4.1 to 6 years and more than 6 years of exposure produced unanticipated results, with the suggestion of an increased risk with 4.1 to 6 years of exposure (HR 1.29, 95% CI 0.91-1.82), but no association with more than 6 years of exposure (HR 0.99, 95% CI 0.63-1.55). In addition, there was no evidence of an increased risk of bladder cancer with short- or long-term pioglitazone use among patients newly diagnosed with diabetes. From these and additional analyses, there is evidence arguing for and against each of the potential explanations for the difference in results between the 5-year interim analysis and the final analysis, which is discussed in detail in the body of the report.

In summary, unlike the 5-year interim result of this study, which was consistent with the results of other short-term cohort studies, in the final analysis of this study we again observed no overall statistically significant increased risk of bladder cancer among patients ever treated with pioglitazone (the primary analysis), but also observed no increased risk with long-term use (the secondary analyses). In prespecified sensitivity analyses, again no statistically significant associations were observed between ever use of

pioglitazone or with longer duration of use. Likewise, none of the other diabetes medications were statistically significantly associated with bladder cancer. Lastly, the case-control study documented little evidence of residual confounding from several bladder cancer risk factors not measurable in the cohort study. In a post hoc analysis, there was an increased risk with 4.1 to 6 years of pioglitazone use, although it was not statistically significant and beyond 6 years there was no evidence of increased risk. As will be described below, there is no single explanation that accounts for all of the observed results. Differences between the final results and those of the midpoint interim analysis of this study and some other published studies could be explained by tumor promoter biology, detection bias, temporal changes in prescribing patterns or bladder cancer screening, or less likely chance. Additional studies with follow-up comparable to this study are needed to confirm these results.

1.0 BACKGROUND

Bladder cancer is an uncommon cancer. Approximately 90% of all bladder cancers are transitional cell tumors. The incidence of bladder cancer is extremely low prior to age 40 and subsequently increases until at least age 80. The demographics of patients developing bladder cancer are similar to those of patients developing type 2 diabetes mellitus.

There are several well-established risk factors for bladder cancer (1). The disease is more common among men than women and more common among Caucasians than African-Americans. There are also several environmental exposures that are associated with an increased incidence of bladder cancer. Some of these were generally related to occupational exposures. Examples of this include aromatic amines to which workers in the dye, rubber, textile, and chemical industries may be exposed. Of note, exposure to these compounds was thought to have approximately a 20-year latency period until the development of bladder cancer.

Non-occupational exposures demonstrated or suggested to be associated with bladder cancer include cigarette smoking, chlornaphazine, phenacetin-containing analgesics, cyclophosphamide, thiotepa, melphalan, and radiation therapy. Most notable of these is cigarette smoking, which is believed to be responsible for 25% to 60% of all bladder cancers among populations in Western industrialized nations (1).

Chronic or repeated infection of the bladder is also believed to contribute to the development of bladder cancer. This is probably best demonstrated with schistosomiasis. However, frequent bacterial infections and chronic indwelling Foley catheters are believed to be associated with an increased risk of bladder cancer as well (1).

Finally, several studies have observed an association between diabetes and bladder cancer 2-4).

Peroxisome proliferators-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of transcription factors whose activities are regulated by high-affinity binding of small, lipophilic ligands such as steroid hormones, vitamin D, retinoids, and thyroid hormone. PPAR alpha, delta and a third subtype called gamma are related sufficiently to be considered members of a subfamily, and have similar properties including DNA binding specificity and heterodimerization with retinoid X receptor (RXR), whose ligands also activate the PPAR/RXR heterodimers. Ligands selective for PPAR gamma include Prostaglandin J_2 (PGJ_2) derivatives, such as 15-deoxy-Δ12,14-PGJ_2 (15d-PGJ_2), and anti-diabetic thiazolidinediones (TZD) compounds, including troglitazone, rosiglitazone, and pioglitazone.

Following guidance from the United States Food and Drug Administration (FDA), Takeda Global Research & Development Center, Inc. (Takeda) requested that our research team design and conduct this study to assess the potential association between pioglitazone and bladder cancer among patients with type 2 diabetes mellitus. The study has been conducted over the course of 10 years, with a series of interim analyses provided to the sponsor (Takeda) and the appropriate regulatory agencies.

To date, we have provided 5 interim reports and 2 supplemental analyses (Table). The data from the planned 5-year interim analysis (2009) was published in Diabetes Care (5).

Date of interim report	Population included	End of study period
August 10, 2005	Cohort study	December 31, 2003
July 25, 2006	Case-control study	January 31, 2006
August 9, 2007	Cohort study	December 31, 2005
November 19, 2009	Cohort and case-control studies	April 30, 2008
May 30, 2012	Cohort study	December 31, 2010
November 30, 2012	Supplement 1 to May 30, 2012 report – additional sensitivity analyses	December 31, 2010
May 31, 2013	Supplement 2 to May 30, 2012 report – proteinuria analysis	December 31, 2010

Here we present the results of the final analysis from 1 January 1997 to 31 December 2012.

2.0 METHODS

2.1 Data Source

The study was conducted within Kaiser Permanente Northern California (KPNC), which provides comprehensive healthcare services to approximately 3.2 million members, representing approximately 30% of the population in its catchment area (6). The KPNC pharmacy database records information on each outpatient prescription dispensed at a KPNC pharmacy and includes medication name, dose, regimen, number of pills and days supply. Prior research demonstrated that 80% to 85% of KPNC members fill all of their prescriptions at Kaiser pharmacies; it is approximately 95% for those with a pharmacy benefit (6).

The source population was identified from the KPNC diabetes registry, which was first constructed in 1993 and has been updated annually since then. The registry identifies patients primarily from four data sources: primary hospital discharge diagnoses of diabetes mellitus (since 1971); two or more outpatient visit diagnoses of diabetes (since 1995); any prescription for a diabetes-related medication (since 1994); or any record of an abnormal hemoglobin A1c (HbA1c) test (>6.7%) (since 1991).

The diabetes registry gathers data from a variety of KPNC electronic medical records (EMR) to build and follow the registry cohort across time. These data include cancer registries, pharmacy records, laboratory records, and inpatient and outpatient medical diagnoses. These data have been widely employed in prior epidemiological studies (6).

2.1.1 Creation of the Study Cohort

The study cohort included both patients with an established diagnosis of diabetes mellitus prior to January 1, 1997 and those who were newly diagnosed prior to December 31, 2002. Patients were eligible for the study cohort if they met any of the following criteria: 1) as of January 1, 1997 they had been diagnosed with diabetes mellitus, were age 40 or older and were members of KPNC, 2) they had been diagnosed with diabetes mellitus, reached age 40 between January 1, 1997 and December 31, 2002 and were KPNC members on their 40th birthday, or 3) had diabetes mellitus and were age 40 or older when they joined KPNC between January 1, 1997 and December 31, 2002. From this cohort of 207,389 we then excluded 823 patients with a diagnosis of bladder cancer prior to entry in the cohort or within 6 months of joining KPNC in order to avoid misclassification of prevalent bladder cancers as incident diagnoses. Likewise, patients without prescription benefits at the time of entry into the cohort (n=6,674) or those with a gap of more than four months in prescription or membership benefits where the gap started within the first four months of entering the cohort (n=6,782) were excluded. These patients would have an extremely limited opportunity to meet our exposure definition (described below). This resulted in 193,099 eligible men and women with diabetes mellitus (full cohort), of whom 59,070 were newly diagnosed with diabetes mellitus between January 1, 1997 and December 31, 2002 (incident subcohort).

2.1.2 Follow-up Period

We included a total of 16 years of data extending from January 1, 1997 through December 31, 2012. This report includes an addition of 4 years and 8 months from our

published interim analysis (5). Follow-up started on the first date that the inclusion criteria were met. Follow-up for patients in the cohort ended when the first of the following occurred: 1) a gap of greater than 4 months in either membership or prescription benefits, 2) a new diagnosis of bladder cancer, 3) death from any cause, or 4) end of follow-up (December 31, 2012).

2.1.3 Outcome

The outcome for this study was an incident diagnosis of bladder cancer. Incident bladder cancers were identified from the KPNC cancer registry (one of several sites that submit data to the Surveillance, Epidemiology, and End Results [SEER] program) from January 1, 1997 to December 31, 2012. This was supplemented by case identification through surveillance of electronic pathology reports within KPNC for the period from January 1, 2005 to March 23, 2012, the time period of interviewing for the linked nested case-control study (5). We did not make any distinction regarding the histology of the bladder cancer and included patients diagnosed with in situ bladder cancer as well as papillary urethral neoplasm of low malignant potential (PUNLMP) from 2005 onward (7, 8).

2.1.4 Exposure Definition

The primary exposure of interest in this study was treatment with pioglitazone, defined as having filled at least two prescriptions for the drug within a 6-month period according to the KPNC pharmacy database. Identical definitions were used to determine exposure to other categories of diabetes medications.

Exposure to pioglitazone and all other diabetes medications were treated as unidirectional time dependent variables, i.e., once a patient met the exposure definition the patient was considered exposed from that point forward, even if they discontinued the medication. Diabetes medications were categorized as pioglitazone, other TZDs, metformin, sulfonylureas, insulin, and other (e.g., miglitol and acarbose). In addition, indicator variables were created separately for patients who had not received any diabetes medication prescriptions and for those who received at least one prescription but had not met the definition of exposure (i.e., did not fill two prescriptions for the same medication within a 6-month period). Each of these was considered as a separate variable. Due to the numerous combinations of diabetes medications that are used by patients within the cohort and the absence of an *a priori* hypothesis that certain combinations would be more or less harmful, we did not attempt to create variables to describe the different combinations (e.g., sulfonylurea plus pioglitazone).

Cumulative duration of exposure was measured by counting the number of days between prescriptions. If the next prescription was filled within 30 days of the expected end date of the previous prescription, we assumed that therapy was uninterrupted. However, if there were no refills within the 30 days after the expected end date of the previous prescription, we assumed a gap in therapy starting 30 days after the date that the previous prescription should have ended (based on days supply variable in prescription database).

Cumulative dose of pioglitazone was calculated in a similar fashion. For any prescription that was completed prior to an event date, the total prescribed dose (i.e., number of pills in the prescription multiplied by the dose of the pills) was assumed to have been consumed. For prescriptions that were still active on the date of an event, the total

consumed dose was reduced to reflect the proportion of pills expected to have been consumed by that date.

After review of our 5-year interim report, the FDA requested that we include exposure to pioglitazone prior to age 40 in our calculation of cumulative dose and duration of exposure. As noted in our 8-year interim report, we have not implemented this change since inclusion of this follow-up time in the calculation of cumulative dose and duration would introduce immortal time bias (9). Specifically, had any patient developed bladder cancer prior to age 40 while taking pioglitazone, they would have been excluded from the study. Therefore, the follow-up time prior to age 40 would include only follow-up time where there are no events, thus decreasing the apparent incidence of bladder cancer among the pioglitazone cohort, and introducing bias. Of note, we documented that there were only 183 subjects with exposure to pioglitazone prior to age 40 in the cohort.

2.1.5 Potential Confounding Variables

For the cohort study, data on the potential confounders listed in Table 1 were extracted from the EMR. We selected as potential confounders variables believed to be associated with one or more of the following: the risk of bladder cancer (e.g., age, race, sex, smoking, socioeconomic status), the possibility of detection of bladder cancer (e.g., urinary diseases or symptoms including urinary tract infections, urinary incontinence, urolithiasis, and prior history of other cancers), or the likelihood of being prescribed pioglitazone (e.g., diabetes duration, HbA1c levels, congestive heart failure, and renal insufficiency). Most confounders other than smoking were measured using data recorded on or before the start of follow-up. The following variables were treated as time updating covariates: use of statins, angiotensin converting enzyme inhibitors or angiotensin receptor blockers, or medications used to treat benign prostatic hypertrophy, urinary incontinence, urinary tract infection or pyelonephritis, urolithiasis, other bladder conditions, prostatic specific antigen (PSA) testing, HbA1c concentration, and complications of diabetes. Complications of diabetes included diabetic retinopathy, peripheral neuropathy, diabetic nephropathy, microalbuminuria or proteinuria, and coronary artery disease. This variable was analyzed both as a composite variable and as its individual components. Where appropriate, categorical variables included an additional category for “missing data.”

Although body mass index was proposed as a potential confounder in the protocol, this was not analyzed since the variable was missing in approximately 50% of the patients.

Smoking status was categorized as current, not current, or missing. Data on smoking have been recorded in the electronic databases since the middle of 1998. For patients who entered the cohort prior to this time, electronic smoking data is incomplete. To account for this, we also used data on smoking from a patient survey that was completed during the years 1994 to 1996 by members of the diabetes registry. Thus, patients were categorized as smokers if they were identified as current smokers in the outpatient EMR or by the survey. Patients who were censored prior to July 1, 1998, had not completed the survey, and lacked smoking data in the electronic record were considered to have missing data on smoking (n=6,905, 3.6%). In the multivariable analyses described below, patients with missing data on smoking were grouped with non-smokers.

Similarly, diabetes duration was assessed from a patient survey that was completed during the years 1994 to 1996 by members of the diabetes registry. Using this source we obtained diabetes duration at the baseline (or date of entry in the cohort) for 47,051 people. For the remaining people we attempted to calculate diabetes duration at the baseline using the date of entry in the diabetes registry. We were able to do so for the 106,281 diabetes registry members who had been in the health plan for at least two years prior to the date of entry in the diabetes registry. However, for the remaining 39,778 people who had been in the health plan for less than two years before the date of entry in the diabetes registry, we were unable to assess diabetes duration.

Renal insufficiency was determined from measured creatinine concentrations. We used the sex-specific threshold levels suggested as a contraindication to metformin therapy to define renal insufficiency (≥1.5 mg/dL in males and ≥1.4 mg/dL in females) (10). Diabetic nephropathy was defined as a creatinine concentration >2.0 mg/dL for both men and women.

Median annual household income in the census block was used as a measure of socioeconomic status. We dichotomized this measure as high or low, based on whether the census block median annual household income was above or below the average census block median income for the cohort ($59,000) at the time of the first interim analysis.

2.1.6 Statistical Analyses for the Cohort Study

Continuous and categorical variables were compared with the Wilcoxon rank sum test and chi-square test or Fisher's exact test, respectively.

2.1.6.1 Primary analysis

For the cohort study, Cox proportional hazards models were used for all calculations of the relative hazard (HR) of bladder cancer with pioglitazone, adjusted for the covariates. The reference group for calculation of the relative hazard associated with ever use of pioglitazone was never use of pioglitazone. Never use of pioglitazone includes patients on no diabetes medications and those treated with medications other than pioglitazone. Identical methods were used to determine relative hazards associated with exposure to other categories of diabetes medications.

We decided *a priori* to include age (categorized as 40-49, 50-59, 60-69, and 70 years or older) and sex in all baseline models, given the known association of these variables with increased risk of bladder cancer. Calendar year of cohort entry was included in the baseline model to account for trends in treatment patterns. Other categories of diabetes medications were included in the baseline model to assess both for confounding and the association of the other medications with bladder cancer. As in prior reports, to test for confounding, we performed analyses that included smoking and other variables measured at baseline in a fully adjusted Cox regression model. This fully adjusted model included the following as covariates: age, sex, race/ethnicity, other diabetes medications, smoking, other bladder conditions, median household income, congestive heart failure, cancer other than bladder cancer, renal insufficiency, HbA1c and the interaction with new diagnosis of diabetes, duration of diabetes, and year of cohort entry. The fully adjusted model did not include the time updating variables, as it would have been infeasible to

include so many time updating variables in a single model. Rather, we tested each time updating variable separately by adding the variable to the base model to determine whether inclusion of the variable changed the HR for the association with pioglitazone by 10% or more. None of the variables resulted in a 10% or greater change, and as such none meet our definition of confounding. However, the largest changes in the HR were seen when the variables related to proteinuria or diabetic nephropathy were included. Thus, we created an additional model that included the variables measured at baseline, smoking, and the results of testing for microalbuminuria. As described previously (11), the latter variable excluded urine tests that included testing for hematuria and was categorized as positive, negative, or no testing during the past year.

2.1.6.2 Secondary Analysis - Assessment of Relation between Pioglitazone Dose and Duration and Bladder Cancer Incidence

Additional analyses were performed to explore for evidence of an increasing risk of bladder cancer with increasing exposure to pioglitazone as compared to never use of pioglitazone. We measured exposure in terms of the following: time since initiation of therapy, cumulative dose of pioglitazone, and cumulative duration of therapy. Calculation of the total dose or duration of treatment was computed starting with the first prescription that defined ever use of pioglitazone. Each of these variables was categorized into three levels (tertiles) such that we had three groups of approximately similar size. The reference group for these analyses was never use of pioglitazone.

2.1.6.3 Test for effect modification

We also examined whether the association between bladder cancer and pioglitazone exposure (ever vs. never and by duration, dose, and time since initiation of pioglitazone) differed according to sex or by smoking status. Sex was selected to examine for effect modification based on the apparent difference in bladder cancer risk among male and female rats treated with pioglitazone. Smoking was selected because of the strong association between smoking and bladder cancer risk.

2.1.6.4 Additional prespecified analyses of patients with complete history of drug exposure and association of other diabetes medications with bladder cancer risk

An analysis limited to patients who were newly diagnosed with diabetes during the run in period from 1997 to 2002. The definition of newly diagnosed diabetes required that the patient was a member of KPNC for a minimum of 2 years before their first diabetes diagnosis. This analysis eliminated the potential for left censoring. This analysis also allowed us to examine the duration effect of other medications.

2.1.6.5 Analysis considering temporal effects on the association of pioglitazone and bladder cancer

We initially had planned a test of interaction using the date September 17, 2010. However, there is very limited follow-up in the cohort after that date. Therefore, we conducted an analysis that examined the impact of study period on the hazard of bladder cancer with long-term pioglitazone exposure using the date April 30, 2008 (i.e., the end

of follow-up of our 5-year interim report analysis). The analysis was limited to those with 2+ years of pioglitazone exposure. The variable of interest was the year when the patient first reached 2 years of pioglitazone exposure, with this variable dichotomized as before or after April 30, 2008. This analysis was adjusted for age at which the patient had 2 years of exposure, sex, race, smoking, and other medications. The analysis was then repeated among patients without exposure to pioglitazone. Patients who later started pioglitazone were included in this analysis with follow-up censored at the time of the first exposure to pioglitazone. This analysis allowed us to examine the effect of time period on bladder cancer incidence rates.

2.1.6.6 Excluding the first two years of follow-up

A sensitivity analysis had been planned that would exclude the first 2 years of follow-up if there was evidence of an early increased risk of bladder cancer to look for evidence of detection bias. However, because in the interim analyses the only evidence of a possible association between pioglitazone and bladder cancer was with longer duration of exposure, this analysis was not included in the final analyses.

2.1.6.7 Additional post hoc analyses

We designed a number of post hoc analyses, largely geared at understanding differences in the 5-year interim analysis and the 10-year final analysis (analyses 1-3 below) and checking for potential bias in our design (analyses 3-4 below). These included the following:

1) An analysis that truncated follow-up after April 30, 2008 (the end of follow-up in the published interim analysis) but used the current cut points for exposure duration. This analysis allowed us to assess whether small changes in the computational methods or updates to the clinical and cancer registry data could have impacted the results.
2) An analysis that further divided the longest exposure duration category into 4.1 to 6 years and more than 6 years, fully adjusted as in the primary analyses. This analysis allowed us to assess for a duration response using finer gradation of the duration categories, whether those with the longest exposure to pioglitazone had the highest risk of bladder cancer, and whether the smaller hazard ratio for long term exposure to pioglitazone in the final analysis was uniform across all durations of exposure or unique to only those with very long duration of use.
3) An analysis that censored follow-up after patients discontinue pioglitazone for 1 year. This examined whether our design that considered patients exposed forever even if they discontinued therapy could have biased the results.
4) An analysis that further divided the upper most age category into finer groups to account for potential bias related to less use of pioglitazone among elderly patients, fully adjusted as in the primary analysis.

2.2 Nested case-control study

To account for incomplete or missing EMR data on race/ethnicity, smoking history, duration of diabetes, and occupational exposures, we supplemented the cohort study with

a case-control study nested within the study cohort. From the source cohort, we identified all incident diagnoses of bladder cancer for the period from October 1, 2002 to March 23, 2012. The index date was defined as the date of bladder cancer diagnosis.

For each individual with bladder cancer, one control was randomly selected after matching on sex, age (± 2.5 years), and time from entry into the diabetes registry to index date (± 6 months). In addition, each control subject could not have been diagnosed with bladder cancer or have been censored from the cohort for other reasons as of the date of first diagnosis with bladder cancer of the matched case subject. When a control subject could not be reached for interview (see below) or refused to participate, additional control subjects were selected until a matched control could be enrolled. A minimum of 15 attempts that included a variety of days and times were made to reach all cases and control subjects before determining that the subject was unreachable.

The date that the case subject was first diagnosed with bladder cancer served as the reference date for both the case subject and for the matched control. The additional data for the case-control study (e.g., duration of diabetes, smoking, use of indwelling catheters, frequency of urinary tract infections, and occupational exposures) were collected up to the reference date through telephone interviews using a standardized questionnaire administered by trained interviewers. The questionnaire was administered using computer-assisted telephone interviews (CATI) with direct data entry by interviewer. For a small number of case subjects who were unable to complete the full interview (n=46), a shorter interview was completed by a proxy. For 23 of these case subjects, the matched control subject also completed the shorter proxy version of the survey. In 7 cases with full interviews, the control had a proxy interview.

In the case-control study, smoking was categorized according to total pack-years consumed prior to the reference date. Cigar and/or pipe smoking among non-cigarette smokers were combined as a dichotomous variable for having ever smoked 1 or more cigars or pipes per week for six months or longer. Duration of diabetes was categorized as less than 5 years, 6 to 10 years, more than 10 years, and unknown. Previous and/or current employment in professions associated with increased bladder cancer risk were treated as dichotomous variables. High-risk occupational exposures were defined as any prior history of work as a painter, driver or hairdresser (12-14). Previous urinary tract infection was categorized as none, one to two prior infections, or more than two prior infections.

2.2.1 Statistical analyses for the Nested Case-Control Study

Analysis of the case-control study was conducted in a similar fashion as the cohort study except that conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Within the case-control study, potential confounders that were derived exclusively from EMR data were defined as described for the cohort study (e.g., congestive heart failure was assessed at the start of follow-up in the cohort).

3.0 RESULTS

3.1 Cohort study

The registry included data on 84,336 patients diagnosed with diabetes, who were age 40 or older and were still members of Kaiser Permanente on January 1, 1997. An additional 3,866 patients with diabetes reached age 40 between January 1, 1997 and December 31, 2002. Another 122,342 patients were newly diagnosed with diabetes or had diabetes and joined KPNC between January 1, 1997 and December 31, 2002 and were age 40 or older. After applying the exclusion criteria, the final cohort included 193,099 patients with diabetes. During follow-up, 51,927 (26.9%) cohort members died of causes other than bladder cancer, 74,285 (38.5%) had a lapse in membership or drug benefits, 1,261 (0.65%) were diagnosed with bladder cancer, and 65,626 (34.0%) were bladder cancer free members of KPNC at the end of follow-up. The latter group included 55% of the pioglitazone exposed patients and 29% of the patients never exposed to pioglitazone.

Prevalence of all of the covariates other than female sex differed by treatment group (Table 1; $p<0.01$ for all variables other than sex). Patients who ever used pioglitazone during the study period (n=34,181) were less likely to be age 70 or older and were more likely to have a baseline HbA1c of at least 10% than patients who never used pioglitazone (Table 1). They were also more likely to have been treated with metformin, sulfonylureas, and insulin during the period of observation.

Among patients who were never treated with pioglitazone, the median duration of follow-up was 7.2 years (range 0.1-16.0 years). Among those who ever used pioglitazone as of the end of follow-up, the median follow-up time from cohort entry was 12.4 years (range 0.2 – 16.0 years) and the median time from the first prescription to the end of follow-up was 6.1 years (range 0.2-13.3 years). The median duration of therapy among pioglitazone treated patients was 2.8 years (range 0.2-13.2 years). The pattern of use is described further in Table 2. By the end of follow-up, approximately one-third of the pioglitazone exposed patients had started pioglitazone more than 8 years earlier, had more than 4 years of use of pioglitazone, and had received more than 40,000 mg in total dose. Of all pioglitazone-exposed patients, 5.2% were taking pioglitazone monotherapy when they initiated pioglitazone.

3.1.1 Primary Analyses of Anti-diabetic Drugs and Bladder Cancer

Among the 1261 cases of newly diagnosed bladder cancer, 186 were patients who ever used pioglitazone before the bladder cancer diagnosis and 1075 were patients who never used pioglitazone. In an unadjusted model, there was no association between pioglitazone use and the incidence of bladder cancer (HR 0.99, 95% CI 0.84-1.16). After adjusting only for age, sex, calendar year of cohort entry, and use of other categories of diabetes medications the results were similar (HR 1.09, 95% CI 0.92-1.29). In a model including age, sex, race/ethnicity, other diabetes medications, smoking, other bladder conditions, median household income, congestive heart failure, cancer other than bladder cancer, renal insufficiency, HbA1c, and the interaction with new diagnosis of diabetes, duration of diabetes, and year of cohort entry as covariates, similar results were obtained (HR 1.10, 95% CI 0.92-1.31). Finally, when testing for proteinuria was added to the model, the HR was slightly reduced to 1.06 (95% CI 0.89-1.26) (Table 3).

Table 3A includes a summary of the hazard ratios for each of the variables included in the fully adjusted model. Like pioglitazone, none of the other diabetes medications (i.e., other TZDs, metformin, sulfonylureas, insulin, or other diabetes medications) were significantly associated with bladder cancer risk in the fully adjusted model, with hazard ratios ranging from 0.91 to 1.09. There was a strong association between bladder cancer risk and older age (40-49 years reference group: 50-59 years HR 4.04, 95% CI 2.86 – 5.70; 60-69 years HR 9.98, 95% CI 7.18 – 13.9; 70 years or older HR 14.8, 95% CI 10.6 – 20.7) and male sex (HR 4.69, 95% CI 3.85 – 5.20). There was a modest association with current smoking (HR 1.46, 95% CI 1.28 - 1.66). Asian (HR 0.38, 95% CI 0.30 – 0.48), Hispanic (HR 0.42, 95% CI 0.33 – 0.53), and black patients (HR 0.54, 95% CI 0.44 – 0.68) had a lower risk of bladder cancer than white patients. A positive test for proteinuria was associated with an increased risk of bladder cancer diagnosis (HR 2.50, 95% CI 2.19 – 2.85) while a negative test result was associated with a decreased risk (HR 0.61, 95% CI 0.52 – 0.72).

3.1.1.1 Stage at diagnosis

The vast majority of bladder cancers were diagnosed at an early stage (Table 4). Three percent of cancers in both groups were undetermined stage. Six percent of bladder cancers in the pioglitazone-exposed patients had regional or distant metastasis at the time of diagnosis. In contrast, 9% of bladder cancers in the pioglitazone-unexposed patients had regional or distant metastasis at the time of diagnosis (2-sided Fisher's exact p=0.16 excluding cases with undetermined stage).

3.1.2 Secondary Analysis of Dose and Duration

When we examined the association between bladder cancer incidence and increasing levels of pioglitazone exposure (Table 3), the unadjusted incidence rates of bladder cancer increased with increasing dose and duration of pioglitazone use. However, the 95% CI overlapped across categories. None of the categories of dose or duration were significantly associated with an elevated risk of bladder cancer after adjusting only for age, sex, and calendar year, or in the fully adjusted models. For patients with more than 4 years of exposure to pioglitazone, the hazard ratio was 1.16 (95% CI 0.87 – 1.54) in the fully adjusted model that included testing for proteinuria. Similar results were observed for more than 8 years since initiation of pioglitazone therapy (HR 1.20, 95% CI 0.83 – 1.75) and more than 40,000 mg of total exposure (HR 1.07, 95% CI 0.79 – 1.44). Tests for trend were not significant (p=0.32 for time since initiation, p=0.51 for duration of therapy, p=0.64 for total dose).

3.1.3 Effect modification by Sex and Smoking

The association between ever use of pioglitazone and bladder cancer risk was qualitatively lower among men (HR 1.03, 95% CI 0.85 - 1.24) than women (HR 1.43, 95% CI 0.96 - 2.15), although the test for interaction did not reach traditional levels of statistical significance (p=0.12). The pioglitazone hazard ratios were similar among nonsmokers (HR 1.12, 95% CI 0.92-1.37) and smokers (HR 1.01, 95% CI 0.72-1.41) (test for interaction p=0.93). Dose and duration analyses stratified by sex and smoking status are summarized in Table 5.

3.1.4 Analyses among patients with an incident diagnosis of diabetes during follow-up

We compared results from our basic model with adjustment for age, sex, and calendar year of cohort entry among the full cohort to results for those patients with an incident diagnosis of diabetes during the run in period from 1997 to 2002 (Table 6). As with the full cohort, ever use of pioglitazone was not associated with bladder cancer risk (HR 0.84, 95% CI 0.58-1.21). Among those with more than 4 years of pioglitazone exposure, the hazard ratio was 0.92 (95% CI 0.46 – 1.81) for newly diagnosed patients as compared to 1.22 (95% CI 0.92 – 1.61) in the full cohort. As previously requested by the FDA, we have also included an analysis of duration of use for the other commonly used diabetes medications among the patients newly diagnosed with diabetes during this period (Table 6). Although not statistically significant, the model suggested an increased risk of bladder cancer among patients with long-term insulin exposure.

3.1.5 Analysis considering temporal effects on the association of pioglitazone and bladder cancer

We compared risk of bladder cancer in the period prior to April 30, 2008 to that after April 30, 2008 – separately for those exposed to pioglitazone for more than 2 years and for those not exposed to pioglitazone. After adjusting for age, sex, race, smoking, and use of other diabetes medications, the risk of bladder cancer among patients with more than 2 years of pioglitazone exposure was higher prior to April 30, 2008 than after this time (HR 1.43, 95% CI 0.76-2.68). In contrast, among patients who were not treated with pioglitazone there was a slightly lower risk of bladder cancer in the earlier time period (HR 0.88, 95% CI 0.70-1.10).

3.1.6 Additional post hoc analyses of the cohort study

Supplemental Table A compares the results of the 5-year and 8-year interim analyses to the results of this final report. For the dose and duration analyses, the categories have changed as the population accumulated additional follow-up time. Nonetheless, the estimated associations between pioglitazone exposure and bladder cancer risk consistently moved closer to the null.

We repeated the analysis of duration of exposure to pioglitazone using the same dose categories as in the final analysis but truncating follow-up to April 30, 2008 as was done in the interim analysis (Supplemental Table B). In this analysis, more than 4 years of exposure to pioglitazone was associated with a 50% increase in bladder cancer incidence in the fully adjusted model without inclusion of proteinuria testing, although this did not reach traditional levels of statistical significance (HR 1.50, 95% 0.88 – 2.51).

Further subdividing the pioglitazone exposure category of more than 4 years into 4.1 to 6 years and more than 6 years of exposure produced unanticipated results, with the suggestion of an increased risk with 4.1 to 6 years of exposure (HR 1.29, 95% CI 0.91-1.82), but no association with more than 6 years of exposure (HR 0.99, 95% CI 0.63-1.55) (Supplemental Table C). A test for linear trend was not statistically significant (p=0.52). These data should be interpreted with caution due to the relatively small number of cancers in each duration category, and this being a post hoc analysis. There

were only 21 patients with more than 6 years of pioglitazone exposure who were diagnosed with bladder cancer.

To account for the possibility that any increase in the risk of bladder cancer with pioglitazone exposure is present only while the patient is currently taking pioglitazone, we repeated the analysis censoring follow-up of pioglitazone exposed patients when one year elapsed without refilling a pioglitazone prescription. The results were comparable to the primary analysis. Neither ever exposure to pioglitazone (HR 1.07, 95% CI 0.88 – 1.32) nor more than 4 years of exposure (HR 1.05, 95% CI 0.74 – 1.78) was associated with an increased risk of bladder cancer (Supplemental Table D).

In an analysis using finer adjustment for age in the oldest category, the results were essentially unchanged from the primary analysis (Supplemental Table E).

3.2 Nested Case-Control Study Results

Between October 1, 2002 and March 23, 2012 there were 702 eligible patients from the source cohort identified with bladder cancer. A total of 464 case subjects (66%) completed the interview, of which 108 (37%) were diagnosed prior to January 1, 2005 (the start of interviewing). The reasons for exclusion or non-participation are summarized in Figure 1. When the case and control subjects could be contacted and were deemed able to provide consent, participation rates were 464/583 (80%) and 464/673 (69%), respectively.

The case subjects were more likely than controls to have a history of heavy smoking (23% vs. 13%), to have participated in occupations associated with bladder cancer (44% vs. 34%) and to be non-Hispanic white (73% vs. 58%) (Table 7). The proportion of case and control subjects with greater than 10 years duration of diabetes was similar (37% vs. 37%). Case subjects who participated in the interview were slightly more likely to have ever used pioglitazone than control subjects who participated in the interview (19.6% versus 17.5%).

The case-control analysis resulted in similar estimates of association between ever use of pioglitazone and bladder cancer to that observed in the cohort analysis (unadjusted OR 1.14, 95% CI 0.79-1.65) (Table 8). This association was similar after adjusting for race, smoking history, high-risk occupations, urinary tract infections, and HbA1c concentration (OR 1.18, 95% CI 0.78-1.80). None of the other categories of diabetes medications were significantly associated with an increased bladder cancer risk (adjusted ORs: metformin 1.15, 95% CI 0.82 – 1.61; sulfonylureas 1.27, 95% CI 0.84 – 1.93; insulin 0.65, 95% CI 0.44 – 0.97). In analysis of varying levels of pioglitazone exposure, there was no clear evidence of an increased risk with increasing duration or dose. The unadjusted and adjusted odds ratios were generally similar, although for use of pioglitazone for 1.5 to 4 years duration, adjustment for the confounders met our definition of a 10% change in the odds ratio (unadjusted OR 1.55 – adjusted OR 1.78). None of the individual strata of dose or duration of pioglitazone exposure was significantly associated with increased bladder cancer risk (Table 8).

We examined the characteristics of the participants and non-participants in the nested case-control study (Table 9). Unlike in the 5-year interim analysis, the proportion of non-participants with pioglitazone exposure prior to the index date was 16% in both cases and

controls, although the distribution was skewed toward pioglitazone exposure among refusers in the controls and toward non-participation for other reasons in the cases.

4.0 DISCUSSION

In the final analysis of this cohort study, with the longest duration of follow-up of any study to date, we did not observe a statistically significant association between treatment with pioglitazone and the risk of bladder cancer. This study was conducted at the request of the FDA in response to animal studies suggesting a possible increased risk of bladder cancer among patients treated with pioglitazone. This association was initially observed in male rats, but not in female rats or in mice of either sex (15). Subsequent research suggested that this effect in male rats can be prevented with dietary modification, suggesting a mechanism related to the bladder anatomy and acid milieu of urine in male rats (16). Dual PPARα and PPARγ agonists have also caused bladder neoplasia in animal models, possibly through a similar mechanism (17). However, in a different animal model, rosiglitazone, another TZD, acted as a tumor promoter even in late stages of bladder cancer development (18). Thus, if there is any association of pioglitazone, or TZDs as a class, with an increased incidence of bladder cancer, the mechanism is not well defined.

In our 5-year interim report we did not observe a statistically significant association between ever exposure to pioglitazone and bladder cancer risk in our cohort study, overall. However, we observed an increased risk of bladder cancer among patients with the longest exposure to pioglitazone (i.e., 2+ years) and a significant dose response in the linear trend analysis (5).

In a subsequent (8-year) interim analysis, the magnitude of the previously observed association was weaker and no longer statistically significant, although there remained a fairly consistent pattern of increasing hazard ratios with longer duration of use and greater cumulative dose.

Now, in the final analysis of this cohort study, there again is no statistically significant association between ever use of pioglitazone and subsequent bladder cancer diagnosis (the primary analysis). Furthermore, even in those with 4 or more years of use (the secondary analyses), the association was weak and not statistically significant; there also was no statistically significant linear trend of increasing risk with increasing duration or cumulative dose of therapy. In sensitivity analyses, no statistically significant associations were observed between ever exposure to pioglitazone or with longer duration of use. Likewise, none of the other diabetes medications were statistically significantly associated with bladder cancer (Tables 3A and 6). Lastly, the case-control study documented little evidence of residual confounding from several bladder cancer risk factors not measurable in the cohort study.

Because the initial animal studies observed an increased risk of bladder cancer in male rats, but not in females, we assessed for effect modification by sex. Among women, the hazard ratio for having ever been exposed to pioglitazone was 1.43 (95% CI 0.96-2.15), while among men the hazard ratio was 1.03 (95% CI 0.85-1.24). However, the test for interaction was not statistically significant and there was no consistent association with duration among women, as the largest hazard ratio was observed among women with less than 1.5 years of exposure. Several other studies have considered an interaction by sex. Mamtani et al. did not observe effect modification by sex in a study comparing TZDs to sulfonylureas (19). Neumann et al. (20) observed a significantly increased risk of bladder

cancer among men treated with pioglitazone (HR 1.28, 95% CI 1.09-1.51), but not among women (HR 0.78, 95% CI 0.44-1.37). Finally, a meta-analysis by He et al. observed similar pooled estimates of the association among men and women (21). Thus, there is no consistent pattern of effect modification by sex across prior studies.

Of note, our methods remained nearly identical throughout the conduct of 5 separate cohort analyses reported to the sponsor and the FDA. Prior to performing this analysis, we revised the categories for dose and duration analyses to avoid having most of the pioglitazone exposed patients in the longest duration and highest dose categories.

A key question is why our results have changed over the course of the second 5 years of the study. To address this, we conducted a number of exploratory analyses. As will be described below, there is no single explanation that accounts for all of the observed results. Hypotheses that were considered included detection bias, tumor promoter biology, temporal changes in prescribing patterns or bladder cancer screening, and chance.

We first applied the dose and duration categories employed in the final analyses using only the data available for the 5-year interim analysis. The results were again consistent with the prior interim report, showing a stronger trend of increasing bladder cancer incidence with longer duration of therapy. Thus, the differing results in the 5-year interim report and this final report were not due to the small changes in the programming or updates to the clinical and cancer registry data.

We next examined finer gradation of the longest duration of exposure. This produced unexpected results, with a pattern of possible increased risk of bladder cancer with 4.1 to 6 years of use (albeit not statistically significant), but not for more than 6 years of exposure.

In an analysis designed to ensure complete capture of diabetes treatment, there was no evidence of an increased risk of bladder cancer with short- or long-term pioglitazone use among patients newly diagnosed with diabetes.

In an analysis looking at changes in rates of bladder cancer incidence over time during the study period, the risk of bladder cancer among those with more than 2 years of pioglitazone treatment was higher prior to April 30, 2008 than in the later study period. The opposite was observed for patients without pioglitazone use.

These analyses suggest: 1) that the elevated risk associated with pioglitazone exposure may plateau or even decline beyond 6 years of exposure to pioglitazone and/or 2) the risk of bladder cancer associated with long duration of pioglitazone use differs by duration of diabetes, with risk being elevated among those with a long history of diabetes but not among those recently diagnosed. However, diabetes duration was not itself associated with the risk of bladder cancer and the authors are not aware of a biologically plausible explanation for such an interaction.

A possible explanation for the former is that pioglitazone acts as a tumor promoter but does not influence the formation of de novo bladder cancer. In this model, pioglitazone would shorten the time between tumor formation and the tumor becoming clinically apparent. This would lead to earlier diagnosis of bladder cancer in the first few years after starting pioglitazone and could produce an initial duration-response relationship as seen

in this study and others. Because all of the tumors would ultimately have become symptomatic, with very long-term follow-up, the incidence rates and cumulative incidence would become more similar over time between the pioglitazone exposed and unexposed. Thus, if the promoter effect took up to 6 years for the very earliest stage of cancer to become symptomatic, one could see a pattern such as that observed in this study (i.e. increasing risk with up to 6 years of exposure but not beyond that). However, we would have expected to have seen similar effects among the subgroup of patients who were newly diagnosed with diabetes and in the analysis that truncates follow-up when people discontinue therapy with pioglitazone, although of course these analyses had less follow-up time. However, the absence of this argues somewhat against the tumor promoter hypothesis.

Detection bias could lead to an apparent increased risk of bladder cancer in the early years of the study that would gradually disappear in a manner similar as you might see with a tumor promoter. However, detection bias would not be expected to produce a duration response. Also arguing against detection bias was the lack of stage shift and the apparent duration response in the earlier analyses.

A related explanation would be a change in the way physicians caring for patients with diabetes look for bladder cancer. We hypothesized that perhaps the publicity around the 5-year report led clinicians to screen for bladder cancer among all patients with diabetes, thereby increasing the detection of asymptomatic bladder cancers to a greater extent in the unexposed group than in the pioglitazone exposed patients after 2008. In our exploratory analysis, the risk of bladder cancer among patients without pioglitazone use was slightly higher after April 30, 2008 than in the earlier period. However, it is not clear why this would preferentially affect the unexposed group unless there had been earlier detection of bladder cancer among the pioglitazone-exposed patients prior to 2008. Our proteinuria analysis suggested that this was possible, but the magnitude of effect was small (11).

One of the most informative of our exploratory analyses compared the incidence of bladder cancer among patients with 2 or more years of pioglitazone treatment before and after April 30, 2008. In this analysis, the risk of bladder cancer among those with more than 2 years of pioglitazone treatment was higher prior to April 30, 2008 than in the later study period. It is difficult to identify a biological reason or study design feature that explains the observed decline in bladder cancer incidence. As noted above, our methods were essentially the same during both time periods. It seems unlikely that physicians preferentially prescribed pioglitazone to "lower risk" patients after April 30, 2008. Publicity regarding bladder cancer did not start until September 2010. Patients starting pioglitazone after that time would have limited opportunity to experience 2 or more years of pioglitazone use before the end of our follow-up period. Furthermore, we adjusted the analysis for age, sex, race and smoking – the key risk factors for bladder cancer. Had the publicity around pioglitazone and bladder cancer led to a detection bias among the pioglitazone exposed, the effect would have been the opposite of what we observed.

Those who first had 2 years of exposure after April 30, 2008 would also be more likely to have been newly diagnosed with diabetes during the study period, consistent with the absence of a duration response in that subset of the patients. One could hypothesize that some of the patients in the earlier time period had use of pioglitazone prior to the start of

follow-up, such that their duration of use was systematically misclassified to be shorter than it truly was (i.e., left censoring). If more than 2 years of pioglitazone exposure was required to increase the risk of bladder cancer, one might expect a pattern of bladder cancer incidence similar to that which we observed. In our analysis using finer gradation of duration of exposure, we observed increasing incidence rates up with up to 6 years of exposure, however not with longer exposure. Thus, left censoring leading to misclassification of duration of exposure does not completely explain all of the results.

Ultimately, we cannot rule out chance as a cause either for the observed increased risk of bladder cancer with pioglitazone exposure in our earlier analyses or the lack of such an effect in the later analyses, although the latter is less likely given our ample statistical power. Further, that other studies have also seen positive associations between pioglitazone exposure and bladder cancer, all with shorter duration of therapy than we now have, makes the positive associations seen in the first half of the study less likely to be due to chance.

Thus, there is evidence arguing for and against each of the potential explanations for the difference in results between the 5-year interim analysis and the final analysis.

Another difference between the final analysis and the 5-year interim analysis was the degree to which the nested case-control study results matched that of the full cohort. In the 5-year interim analysis, there was evidence of a selection bias related to participation rates of case and control subjects that yielded inflated estimates of the association between pioglitazone and bladder cancer in the nested case-control study. Using multiple imputation and weighted analyses we were able to account for the selection bias. However, there was no obvious explanation for why the participation rates among case and control subjects should have been differentially associated with pioglitazone exposure rates. Thus, we hypothesized that this was a chance occurrence. Consistent with this hypothesis, by the end of the study, the participation rates among case and control subjects were no longer differentially associated with pioglitazone exposure. As such, multiple imputation and weighted analyses were not required to accurately interpret the case-control study results in the final analysis.

There are several major strengths of this study. The study followed the principles of Good Pharmacoepidemiology Practice (22, 23) and contains the longest follow-up of any study addressing this question to date. There are a number of other strengths that are unique to KPNC. First, the KPNC diabetes registry includes a large population of persons with diabetes available for analysis of medication exposure and a relatively rare outcome. The diabetes registry employs active surveillance based on diagnoses, laboratory tests, and pharmacy data, and as such is able to also identify persons with diabetes who are not treated with medications. We used the KPNC cancer registry to identify patients with bladder cancer. This well established cancer registry, which contributes data to SEER, is held to SEER's very high quality standards. This study is also strengthened by the availability of the KPNC pharmacy data. Pharmacoepidemiology studies require accurate data on medication consumption. By requiring patients to fill two prescriptions within a six-month period, we have minimized misclassification of unexposed patients as exposed. Patients who filled only a single pioglitazone prescription (n=5,474) or who filled two or more prescriptions that were never within six months of each other (n=1,255) were not categorized as exposed according to our definition. Some of these patients may have

actually been exposed to pioglitazone. However, this misclassification is unlikely to be important given that such a small duration of therapy would be unlikely to change the risk of cancer. Furthermore, because these patients represented a small proportion of patients who filled at least one pioglitazone prescription and an even smaller proportion of the population categorized as unexposed, their potential impact on the estimated hazard ratio is limited.

We have also conducted a number of sensitivity analyses to test the impact of our study design. We tested whether the effect may diminish after pioglitazone was discontinued, the impact of left censoring, and the impact of our choice of age categories. None of these analyses pointed to a bias that would mask a true association of pioglitazone with bladder cancer incidence. Many studies of the effect of diabetes medications on risk of cancer have used designs that predispose to immortal time bias (24). We were careful to use time updating exposures to avoid such bias. We conducted a nested case-control study to test for unmeasured confounding in the cohort study, particularly by race, smoking history and occupational exposures. There was little evidence of confounding by these, thereby allowing us to focus on the cohort study with its increased statistical power. Likewise, the large number of patients who have been prescribed pioglitazone and that approximately one-third of these have taken the medication for more than 4 years is a major strength of the study.

Since we initiated this study approximately 10 years ago, there have been several other observational studies addressing the question of whether pioglitazone or TZDs in general increase the risk of incident bladder cancer. These studies and several clinical trials have been summarized in at least 7 meta-analyses focusing on pioglitazone (Supplemental Table G). Despite using slightly different statistical methods and inclusion criteria, the meta-analyses of observational studies have consistently estimated approximately 20% and 45% increased risk of bladder with ever use and more than 24 months of pioglitazone use, respectively. The estimated relative risk from meta-analysis of RCTs was higher, but was driven almost entirely from the results of the PROactive Study with an average follow-up of 34.5 months (25). In the PROactive study, more than 50% of the bladder cancers were diagnosed in the first year of follow-up. Our study is unique in the ability to assess much longer duration of exposure to pioglitazone. In our post hoc analysis we observed an approximately 30% increased hazard of bladder cancer with use for more than 4 year but less than 6 years (HR 1.32, 95% CI 0.94-1.87). Interestingly, we observed no increased hazard beyond 6 years of exposure, although we could not exclude up to 56% increased incidence.

We did not adjusted for multiple comparisons either within the final analysis or accounting for the multiple interim analyses as might be done in a clinical trial. At the outset of this study, it was agreed that interim reports would be provided to the sponsor and the FDA, but that the study would continue for 10 years regardless of the results of the interim reports. As such, we elected not to reduce the threshold for declaring statistical significance. Since none of the results from the final analyses had a p value less than 0.05, this would not have affected our conclusions.

We had less statistical power for some of our sensitivity and subgroup analyses. In particular, the analyses of patients who were newly diagnosed with diabetes mellitus were conducted in a cohort approximately 70% smaller than the full cohort. Nonetheless,

this subgroup allowed us to assess the association of other diabetes medications with bladder cancer risk using the same methods as employed for pioglitazone and without the potential for bias from left censoring. In this subgroup of the cohort, there was no association of bladder cancer risk with pioglitazone use or the other diabetes medications.

In summary, unlike the 5-year interim result of this study, which was consistent with the results of other short-term cohort studies, in the final analysis of this study we again observed no overall statistically significant increased risk of bladder cancer among patients ever treated with pioglitazone (the primary analysis), but also observed no increased risk with long-term use (the secondary analyses). In prespecified sensitivity analyses, again no statistically significant associations were observed between ever use of pioglitazone or with longer duration of use. Likewise, none of the other diabetes medications were statistically significantly associated with bladder cancer. Lastly, the case-control study documented little evidence of residual confounding from bladder cancer risk factors not measurable in the cohort study. In a post hoc analysis, there was an increased risk with 4.1 to 6 years of pioglitazone use, although it was not statistically significant and beyond 6 years there was no evidence of increased risk. Differences between the final results and those of the midpoint interim analysis of this study and other published studies could be explained by tumor promoter biology, detection bias, temporal changes in prescribing patterns or bladder cancer screening, or less likely chance. No single explanation is consistent with all of the observed results. Additional studies with follow-up comparable to this study are needed to confirm these results.

Table 1. Demographics of the study cohort according to pioglitazone treatment at any time during follow-up[§]

	Ever treated with pioglitazone (n=34,181)	Never treated with pioglitazone (n=158,918)
Age at baseline		
40-49 years	9,992 (29.2%)	35,072 (22.1%)
50-59 years	11,284 (33.0%)	40,623 (25.6%)
60-69 years	8,791 (25.7%)	41,698 (26.2%)
70 years and older	4,114 (12.0%)	41,525 (26.1%)
Female sex	15,902 (46.5%)	73,941 (46.5%)
Race/Ethnicity		
White	17,543 (51.3%)	83,085 (52.3%)
Black	3,497 (10.2%)	17,199 (10.8%)
Asian	5,069 (14.8%)	20,268 (12.8%)
Hispanic	4,589 (13.4%)	16,890 (10.6%)
Other	2,001 (5.9%)	9,033 (5.7%)
Missing	1,482 (4.3%)	12,443 (7.8%)
Current smoker	6,982 (20.4%)	27,615 (17.4%)
Renal function at baseline		
Normal creatinine	26,455 (77.4%)	122,598 (77.1%)
Elevated creatinine*	1,360 (4.0%)	13,881 (8.7%)
Missing	6,366 (18.6%)	22,439 (14.1%)
Congestive heart failure at baseline	1,010 (3.0%)	10,997 (6.9%)
Income		
Low[‡]	16,336 (47.8%)	80,347 (50.6%)
High	14,627 (42.8%)	64,331 (40.5%)
Missing	3,218 (9.4%)	14,240 (9.0%)
Baseline HbA1c		
< 7%	5,859 (17.1%)	45,421 (28.6%)
7-7.9%	6,271 (18.3%)	30,701 (19.3%)
8-8.9%	4,383 (12.8%)	16,598 (10.4%)
9-9.9%	3,303 (9.7%)	11,200 (7.0%)
≥10%	8,182 (23.9%)	27,165 (17.1%)
Missing	6,183 (18.1%)	27,833 (17.5%)

Newly diagnosed with DM at the start of follow-up[#]	17,376 (50.8%)	92,050 (57.9%)
Diabetes duration at baseline		
0-4 years	20,530 (60.1%)	99,749 (62.8%)
5-9 years	3,155 (9.2%)	9,499 (6.0%)
10 or more years	3,116 (9.1%)	17,272 (10.9%)
Missing	7,380 (21.6%)	32,398 (20.4%)
Other cancer prior to baseline	1,056 (3.1%)	8,460 (5.3%)
Treated with pioglitazone but no other diabetes medications	1,764 (5.2%)	0 (0%)
Other diabetes medications[¥]		
Other TZDs	2,816 (8.2%)	2,427 (1.5%)
Metformin	28,985 (84.8%)	72,980 (45.9%)
Sulfonylureas	30,682 (89.8%)	97,325 (61.2%)
Other oral hypoglycemic drugs	2,174 (6.4%)	2,214 (1.4%)
Insulin	17,997 (52.7%)	46,659 (29.4%)
Never treated with any diabetes medication	0 (0%)	22,673 (14.3%)
Statin Use	30,372 (88.9%)	93,357 (58.7%)
ACE inhibitors or ARB	31,561 (92.3%)	110,589 (69.6%)
BPH medications[†]	5,090 (27.8%)[μ]	16,919 (19.9%)[μ]
Urinary Incontinence	2,545 (7.4%)	8,580 (5.4%)
UTI/Pyelonephritis	12,055 (35.3%)	47,162 (29.7%)
Urolithiasis	2,758 (8.1%)	7,949 (5.0%)
Other Bladder conditions[Σ]	11,898 (34.8%)	41,465 (26.1%)
PSA Testing	16,721 (91.5%)[μ]	60,449 (71.1%)[μ]
Diabetes complications[β]	28,794 (94.7%)	110,996 (81.6%)
Diabetic retinopathy	18,079 (52.9%)	51,693 (32.5%)
Peripheral neuropathy[∞]	25,636 (75.0%)	87,266 (54.9%)
Proteinuria[Ω]	26,370 (77.1%)	95,041 (59.8%)
Diabetic nephropathy[π]	9,077 (26.6%)	33,709 (21.2%)
Coronary artery disease	17,095 (50.0%)	73,275 (46.1%)

[§] All variables are at any time during follow-up except for some baseline variables noted.

All comparisons have p-values <0.01 except female sex (p=0.99)

***** Creatinine ≥1.4 mg/dL for women and ≥1.5 mg/dL for men

‡ Low income defined as median household income in census block below the cohort average ($59,000)

Includes newly diagnosed patients and patients who newly enrolled in Kaiser Permanente with an existing diagnosis of diabetes mellitus

¥ Includes use of any other diabetes medications during follow-up

 Never received 2 or more prescriptions for a diabetes medication within a 6-month period

† BPH medications to treat benign prostatic hypertrophy

μ Number and percentage among males

Σ Other bladder conditions include hematuria, retention, urgency, neurogenic bladder, catheter and other bladder/urethral symptoms

β Diabetes complications include diabetic retinopathy, peripheral neuropathy, proteinuria, diabetic nephropathy or coronary artery disease

∞ Includes diabetic neuropathy, foot ulcer, or amputation

Ω Includes microalbuminuria or macroalbuminuria

π Creatinine ≥2.0 mg/dL for both men and women

Table 2. Pioglitazone exposures as of the end of follow-up

Category	
Ever exposed, n	34,181
Time since starting pioglitazone (median, range)	6.1 yr (0.2-13.3)
Less than 4.5 years (n, %)	11,795 (34.5%)
4.5-8.0 years (n, %)	11,504 (33.7%)
More than 8 years (n, %)	10,882 (31.8%)
Duration of therapy (median, range)	2.8 yr (0.2-13.2)
Less than 1.5 years (n, %)	10,419 (30.5%)
1.5-4.0 years (n, %)	11,504 (33.6%)
More than 4.0 years (n, %)	12,258 (35.8%)
Cumulative dose, mg (median, range)	24,000 mg (450-156,000)
1 – 14000 mg (n, %)	11,683 (34.2%)
14001 – 40000 mg (n, %)	11,319 (33.1%)
>40000 mg (n, %)	11,179 (32.7%)

Table 3. Incidence rate and hazard ratios assessing the association between pioglitazone treatment and risk of bladder cancer

	Cases of bladder cancer	Person-years of follow-up time	Bladder cancer incidence rate (per 100,000 person-years)	Unadjusted (HR, 95% CI)	Adjusted for age, sex and year of cohort entry (HR, 95% CI)	Adjusted for age, sex, year of cohort entry and smoking (HR, 95% CI)	Fully adjusted[†] (HR, 95% CI)	Fully adjusted adding the proteinuria testing variable[††] (HR, 95% CI)
Unexposed to pioglitazone	1,075	1,417,196	75.9 (71.3-80.4)	Reference	Reference	Reference	Reference	Reference
Ever exposed to pioglitazone	186	207,112	89.8 (76.9-102.7)	0.99 (0.84-1.16)	1.09 (0.92-1.29)*	1.09 (0.92-1.30)*	1.10 (0.92-1.31)	1.06 (0.89-1.26)
Time since starting pioglitazone								
Less than 4.5 years	88	129,017	68.2 (54.0-82.5)	0.81 (0.65-1.01)	0.93 (0.75-1.17)	0.94 (0.75-1.17)	0.93 (0.74-1.17)	0.89 (0.71-1.12)
4.5-8.0 years	65	58,247	111.6 (84.5-138.7)	1.15 (0.89-1.49)	1.30 (1.00-1.68)	1.30 (1.00-1.68)	1.26 (0.97-1.65)	1.21 (0.93-1.59)
More than 8 years	33	26,234	125.8 (82.9-168.7)	1.20 (0.84-1.73)	1.29 (0.90-1.86)	1.29 (0.90-1.87)	1.22 (0.84-1.78)	1.20 (0.83-1.75)
Test for trend					P=0.07		P=0.17	P=0.32
Duration of therapy								
Less than 1.5 years	60	88,839	67.5 (50.4-84.6)	0.80 (0.61-1.04)	0.95 (0.73-1.24)	0.95 (0.73-1.23)	0.93 (0.71-1.22)	0.88 (0.68-1.16)

1.5-4.0 years	69	78,059	88.4	0.97	1.09	1.09	1.08	1.03
			(67.5-109.3)	(0.76-1.24)	(0.85-1.40)	(0.85-1.40)	(0.83-1.39)	(0.80-1.33)
More than 4 years	57	50,145	113.7	1.13	1.22	1.23	1.19	1.16
			(84.2-143.2)	(0.86-1.49)	(0.92-1.61)	(0.93-1.62)	(0.89-1.58)	(0.87-1.54)
Test for trend					P=0.19		P=0.29	P=0.51
Cumulative dose								
1 – 14000 mg	66	95,534	69.1	0.80	0.95	0.95	0.94	0.90
			(52.4-85.8)	(0.63-1.03)	(0.74-1.22)	(0.74-1.22)	(0.73-1.22)	(0.69-1.16)
14001 – 40000 mg	69	71,198	96.9	1.05	1.17	1.17	1.15	1.10
			(74.0-119.8)	(0.82-1.35)	(0.91-1.50)	(0.91-1.50)	(0.89-1.49)	(0.85-1.42)
>40000 mg	51	50,310	101.4	1.03	1.14	1.14	1.09	1.07
			(73.5-129.2)	(0.77-1.37)	(0.85-1.52)	(0.85-1.53)	(0.81-1.47)	(0.79-1.44)
Test for trend					P=0.23		P=0.41	P=0.64

[†]Fully adjusted refers to inclusion of all potential confounders in the statistical model from the 5-year interim report plus year of cohort entry: age, sex, race/ethnicity, other diabetes medications, smoking, other bladder conditions, median household income, congestive heart failure, cancer other than bladder cancer, renal insufficiency, HbA1c and the interaction with new diagnosis of diabetes, duration of diabetes, and year of cohort entry

[††] Fully adjusted model adding the 3-level time updated proteinuria testing variable (no testing, negative and positive testing for proteinuria), excluding same day test for hematuria

*Also adjusted for use of other diabetes medication

Table 3A. Hazard ratios and confidence intervals for all variables included in the analysis of ever exposure to pioglitazone

All covariates	Fully Adjusted HR (95% CI)
Diabetes Medications	
Pioglitazone	1.06 (0.89-1.26)
Other TZDs	1.09 (0.78-1.53)
Metformin	1.02 (0.89-1.18)
Insulin	1.03 (0.88-1.20)
Sulfonylureas	1.03 (0.87-1.23)
Other oral hypoglycemic agents	0.91 (0.59-1.40)
Never treated with any diabetes medications	1.09 (0.87-1.37)
Received at least one prescription for a diabetes medication but never met the definition of exposure	0.98 (0.70-1.39)
Male sex	4.47 (3.85-5.20)
Age	
Age 40-49	Reference
Age 50-59	4.04 (2.86-5.70)
Age 60-69	9.98 (7.18-13.9)
Age >=70	14.8 (10.6-20.7)
Cohort Entry	
Entered cohort in 1997	Reference
Entered cohort in 1998	0.86 (0.64-1.14)
Entered cohort in 1999	1.02 (0.78-1.34)
Entered cohort in 2000	0.92 (0.69-1.22)
Entered cohort in 2001	0.76 (0.58-1.01)
Entered cohort in 2002	0.75 (0.56-1.01)
Race	
Caucasian	Reference
Black	0.54 (0.43-0.68)
Asian	0.38 (0.30-0.48)
Hispanic	0.42 (0.33-0.53)
Other Race	0.72 (0.56-0.92)
Smoking Before Censor Date	1.46 (1.28-1.66)
Any Bladder Conditions on or Prior to Baseline	0.92 (0.78-1.09)
Income	
Median Household Income Above Average ($59K)	0.99 (0.88-1.11)
Income Census Data Missing	0.80 (0.61-1.04)
Congestive Heart Failure Prior to or on Baseline	1.09 (0.87-1.37)
Had Cancer Other than Bladder Cancer Prior to Baseline	1.18 (0.95-1.46)

Table 3A. Hazard ratios and confidence intervals for all variables included in the analysis of ever exposure to pioglitazone (continued)

All covariates	Fully Adjusted HR (95% CI)
Serum Creatinine	
Abnormal at Baseline	1.03 (0.84-1.26)
Missing at Baseline	1.03 (0.86-1.23)
Baseline HbA1c	
<7	Reference
7-7.9	0.79 (0.63-0.99)
8-8.9	0.97 (0.75-1.25)
9-9.9	0.74 (0.54-1.02)
>=10	0.91 (0.70-1.18)
Missing	0.72 (0.54-0.94)
Newly Diagnosed Diabetic	1.26 (0.95-1.67)
Interaction Term	
HbA1c 7-7.9 and Newly Diagnosed Diabetic	1.04 (0.76-1.43)
HbA1c 8-8.9 and Newly Diagnosed Diabetic	0.87 (0.59-1.28)
HbA1c 9-9.9 and Newly Diagnosed Diabetic	1.01 (0.62-1.65)
HbA1c >=10 and Newly Diagnosed Diabetic	0.75 (0.52-1.08)
HbA1C Missing and Newly Diagnosed Diabetic	0.88 (0.61-1.28)
Diabetes Duration	
<5 Years	Reference
5-9 Years	1.11 (0.89-1.38)
10+ Years	1.05 (0.86-1.28)
Missing	0.91 (0.76-1.08)
Proteinuria Testing	
No testing	Reference
Negative test result	0.61 (0.52-0.72)
Positive test result	2.50 (2.19-2.85)

Table 4. Cancer stage by pioglitazone treatment

Cancer stage	Ever treated with pioglitazone (n=186 cases)	Never treated with pioglitazone (n=1,075 cases)
PUNLMP*	2 (1%)	11 (1%)
In situ	93 (50%)	521 (49%)
Local	74 (40%)	409 (38%)
Regional	8 (4%)	65 (6%)
Distant	3 (2%)	34 (3%)
Undetermined	6 (3%)	35 (3%)

* Papillary urethral neoplasm of low malignant potential

Table 5. Hazard ratios[‡] assessing the association between pioglitazone treatment and bladder cancer risk by sex and smoking status

	Men	**Women**	**Smokers**	**Non-smokers**
Person-years of follow-up time unexposed to pioglitazone	740,801	676,395	270,848	1,146,348
Person-years of follow-up time ever exposed to pioglitazone	108,632	98,480	42,277	164,835
Cases of bladder cancer among pioglitazone unexposed	899	176	257	818
Cases of bladder cancer among pioglitazone exposed	151	35	48	138
	HR, 95% CI	**HR, 95% CI**	**HR, 95% CI**	**HR, 95% CI**
Ever exposed to pioglitazone	1.03 (0.85-1.24)	1.43 (0.96-2.15)	1.01 (0.72-1.41)	1.12 (0.92-1.37)
Interaction p value	0.12		0.93	
Time since starting pioglitazone*				
Less than 4.5 years	0.94 (0.74-1.20)	0.88 (0.50-1.56)	0.89 (0.58-1.37)	0.95 (0.73-1.23)
4.5-8.0 years	1.20 (0.89-1.61)	1.77 (1.02-3.07)	1.14 (0.68-1.93)	1.35 (1.00-1.82)
More than 8 years	1.23 (0.81-1.85)	1.60 (0.72-3.56)	1.21 (0.60-2.43)	1.33 (0.86-2.03)
Test for trend p values	0.25	0.08	0.65	0.07
Interaction p values[†]	0.13		0.82	
Duration of therapy*				
Less than 1.5 years	0.81 (0.59-1.11)	1.58 (0.97-2.59)	0.87 (0.52-1.48)	0.97 (0.72-1.32)
1.5-4.0 years	1.15 (0.88-1.50)	0.78 (0.38-1.61)	0.99 (0.61-1.62)	1.13 (0.84-1.50)
More than 4 years	1.21 (0.89-1.64)	1.29 (0.65-2.58)	1.17 (0.68-2.02)	1.24 (0.90-1.72)
Test for trend p values	0.23	0.55	0.76	0.17
Interaction p values[†]	0.58		0.78	
Cumulative dose*				
1 – 14000 mg	0.79 (0.59-1.07)	1.66 (1.05-2.63)	0.84 (0.51-1.40)	0.99 (0.74-1.32)
14001 – 40000 mg	1.27 (0.98-1.66)	0.62 (0.27-1.41)	1.29 (0.82-2.03)	1.12 (0.83-1.51)
>40000 mg	1.12 (0.81-1.54)	1.28 (0.62-2.65)	0.85 (0.46-1.58)	1.25 (0.90-1.74)
Test for trend p values	0.26	0.67	0.96	0.17
Interaction p values[†]	0.17		0.97	

[‡] Adjusted for age, sex, and calendar year of cohort entry

* Reference group is unexposed to pioglitazone

[†] Interaction p values are for the interaction between the exposure and sex or smoking in the test for trend analysis

Table 6. Analysis of duration of other diabetes therapies among the 59,070 patients who were newly diagnosed [§] with diabetes during 1997-2002

	Pioglitazone HR (95% CI)	**Metformin HR (95% CI)**	**Sulfonylureas HR (95% CI)**	**Insulin HR (95% CI)**
N exposed	9,046	33,926	39,836	11,724
N exposed cases/ N exposed by end of follow-up				
Duration of therapy				
Less than 1.5 years	13/2,589	37/5,760	57/7,448	14/4,857
1.5-4.0 years	12/3,198	58/8,229	68/9,564	13/3,730
More than 4 years	9/3,259	72/19,937	101/22,824	11/3,137
Model 1				
Ever/Never Exposed	0.84 (0.58-1.21)	0.93 (0.75-1.16)	0.90 (0.73-1.11)	0.95 (0.67-1.34)
Duration of therapy				
Never exposed	Reference	Reference	Reference	Reference
Less than 1.5 years	0.82 (0.47-1.44)	0.81 (0.57-1.15)	1.01 (0.75-1.36)	0.67 (0.39-1.14)
1.5-4.0 years	0.75 (0.42-1.34)	0.94 (0.70-1.27)	0.90 (0.68-1.20)	0.99 (0.56-1.73)
More than 4 years	0.89 (0.45-1.76)	0.90 (0.67-1.22)	0.73 (0.56-0.97)	1.49 (0.81-2.77)
Model 2				
Duration of therapy				
Never exposed	Reference	Reference	Reference	Reference
Less than 1.5 years	0.85 (0.49-1.50)	0.82 (0.58-1.17)	1.01 (0.75-1.37)	0.70 (0.41-1.20)
1.5-4.0 years	0.77 (0.43-1.39)	0.96 (0.71-1.30)	0.91 (0.68-1.21)	1.03 (0.59-1.82)
More than 4 years	0.92 (0.46-1.81)	0.93 (0.68-1.26)	0.75 (0.56-0.99)	1.53 (0.83-2.84)

[§] The definition of newly diagnosed with diabetes required that the patient was a member of KPNC for a minimum of 2 years before the first diabetes diagnosis.
Model 1 - Results of age, sex, race, smoking and calendar year of cohort entry adjusted models.
Model 2- Adjusted for model 1 variables plus each of the other three diabetes therapies where the other therapy is treated as a time updating variable for never versus ever exposed.

Table 7. Characteristics of bladder cancer case and control subjects

	Cases (n=464)	**Controls (n=464)**
Age at reference date		
40-59 years	18 (3.9%)	19 (4.1%)
60-69 years	118 (25.4%)	126 (27.2%)
70-79 years	210 (45.3%)	210 (45.3%)
80 years and older	118 (25.4%)	109 (23.5%)
Female sex	70 (15.1%)	70 (15.1%)
Time in registry		
0-5 years	127 (27.4%)	122 (26.3%)
6-10 years	165 (35.6%)	172 (37.1%)
More than 10 years	172 (37.1%)	170 (36.6%)
Race/ethnicity		
Non-Hispanic White	340 (73.3%)	270 (58.2%)
Black or African American	31 (6.7%)	51 (11%)
Hispanic	32 (6.9%)	57 (12.3%)
Asian or Pacific Islander	19 (4.1%)	52 (11.2%)
Other	40 (8.6%)	30 (6.5%)
Missing	2 (0.4%)	4 (0.9%)
Cigarette smoking history		
Never smoked	155 (33.4%)	200 (43.1%)
20 or fewer pack-years	87 (18.8%)	111 (23.9%)
21-40 pack-years	93 (20%)	66 (14.2%)
>40 pack-years	106 (22.8%)	61 (13.1%)
Missing	23 (5%)	26 (5.6%)
Pipe or Cigar Smoker		
No	329 (70.9%)	341 (73.5%)
Yes	84 (18.1%)	91 (19.6%)
Missing	51 (11%)	32 (6.9%)
Renal function		
Normal creatinine	384 (82.8%)	368 (79.3%)
Elevated creatinine*	23 (5%)	28 (6%)
Missing	57 (12.3%)	68 (14.7%)

Table 7 - Continued

	Cases (n=464)	Controls (n=464)
Urinary tract infections		
None	284 (61.2%)	312 (67.2%)
1-2	64 (13.8%)	60 (12.9%)
3+	43 (9.3%)	41 (8.8%)
Missing	73 (15.7%)	51 (11%)
Urinary incontinence		
No	357 (76.9%)	353 (76.1%)
Yes	57 (12.3%)	75 (16.2%)
Missing	50 (10.8%)	36 (7.8%)
Catheter use		
No	394 (84.9%)	415 (89.4%)
Yes	22 (4.7%)	17 (3.7%)
Missing	48 (10.3%)	32 (6.9%)
Manufacturing industry	123 (26.5%)	110 (23.7%)
High risk occupation†	204 (44%)	157 (33.8%)
Congestive heart failure	21 (4.5%)	13 (2.8%)
Annual household income		
<$40,000	177 (38.1%)	154 (33.2%)
$40,000-$74,000	172 (37.1%)	159 (34.3%)
≥ $75,000	91 (19.6%)	114 (24.6%)
Missing	24 (5.2%)	37 (8%)
Baseline HbA1c		
< 7%	176 (37.9%)	167 (36%)
7-7.9%	80 (17.2%)	104 (22.4%)
8-8.9%	53 (11.4%)	42 (9.1%)
≥ 9%	90 (19.4%)	79 (17%)
Missing	65 (14%)	72 (15.5%)
Newly diagnosed with DM at entry into the cohort.	288 (62.1%)	287 (61.9%)
Diabetes duration		
0-5 years	95 (20.5%)	92 (19.8%)
6-10 years	103 (22.2%)	118 (25.4%)
>10 years	204 (44%)	209 (45%)
Missing	62 (13.4%)	45 (9.7%)

Table 7 - Continued

	Cases (n=464)	Controls (n=464)
Diabetes medication exposure		
Pioglitazone	91 (19.6%)	81 (17.5%)
Other TZD	14 (3%)	10 (2.2%)
Any TZD	96 (20.7%)	88 (19%)
Metformin	258 (55.6%)	252 (54.3%)
Sulfonylureas	313 (67.5%)	296 (63.8%)
I Insulin	107 (23.1%)	123 (26.5%)
Other OHA	11 (2.4%)	9 (1.9%)
Never took any DM drugs	71 (15.3%)	66 (14.2%)
None of the above	17 (3.7%)	15 (3.2%)
Recentness of starting pioglitazone		
Non-user	373 (80.4%)	383 (82.5%)
Used < 4.5 years ago	46 (9.9%)	36 (7.8%)
Used 4.5-8.0 years ago	32 (6.9%)	26 (5.6%)
Used more than 8 years ago	13 (2.8%)	19 (4.1%)
Total duration of pioglitazone use		
None	373 (80.4%)	383 (82.5%)
< 1.5 years	25 (5.4%)	24 (5.2%)
1.5-4.0 years	39 (8.4%)	27 (5.8%)
More than 4 years	27 (5.8%)	30 (6.5%)
Total dose of pioglitazone		
None	373 (80.4%)	383 (82.5%)
<14,000 mg.	31 (6.7%)	27 (5.8%)
14,001 mg-40,000mg	33 (7.1%)	27 (5.8%)
>40,000 mg.	27 (5.8%)	27 (5.8%)

*Creatinine ≥1.4 for women and ≥1.5 for men.

† High risk occupation includes painter, driver or barber

Table 8. Association of pioglitazone treatment and bladder cancer in the nested case-control study

	Cases (n)	Controls (n)	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Never use pioglitazone	373	383	reference	reference
Ever exposed	91	81	1.14 (0.79-1.65)	1.18 (0.78-1.80)
Time since starting pioglitazone				
Less than 4.5 years	46	36	1.36 (0.84-2.21)	1.42 (0.80-2.52)
4.5-8.0 years	32	26	1.33 (0.75-2.36)	1.20 (0.62-2.32)
More than 8 years	13	19	0.65 (0.29-1.43)	0.70 (0.27-1.78)
Duration of therapy				
Less than 1.5 years	25	24	1.10 (0.62-1.96)	1.16 (0.59-2.25)
1.5-4.0 years	39	27	1.55 (0.90-2.67)	1.78 (0.93-3.40)
More than 4 years	27	30	0.94 (0.54-1.64)	0.81 (0.42-1.55)
Cumulative dose				
1 – 14000 mg	31	27	1.19 (0.70-2.03)	1.26 (0.69-2.33)
14001 – 40000 mg	33	27	1.27 (0.75-2.15)	1.27 (0.68-2.36)
>40000 mg	27	27	1.06 (0.59-1.88)	0.98 (0.50-1.93)

* Adjusted for other diabetes meds, race, smoking history, high risk occupations, urinary tract infections, and HbA1c concentration

Table 9. Comparison of survey respondents and non-respondents by case-control status

	Case participant (n=464)	Case refuser (n=119)	Case non-participant for other reason (n=117)	Control participant (n=464)	Control refuser (n=209)	Control non-participant for other reason (n=360)
Age at reference date (%)						
40-59	18 (3.9%)	6 (5%)	7 (6%)	19 (4.1%)	4 (1.9%)	9 (2.5%)
60-69	118 (25.4%)	18 (15.1%)	28 (23.9%)	126 (27.2%)	58 (27.8%)	71 (19.7%)
70-79	210 (45.3%)	54 (45.4%)	38 (32.5%)	210 (45.3%)	78 (37.3%)	139 (38.6%)
80+	118 (25.4%)	41 (34.5%)	44 (37.6%)	109 (23.5%)	69 (33%)	141 (39.2%)
Sex (Female)	70 (15.1%)	20 (16.8%)	31 (26.5%)	70 (15.1%)	35 (16.7%)	63 (17.5%)
Congestive heart failure (%)	21 (4.5%)	12 (10.1%)	7 (6%)	13 (2.8%)	8 (3.8%)	16 (4.4%)
Elevated creatinine* (%)	23 (5%)	9 (7.6%)	17 (14.5%)	28 (6%)	13 (6.2%)	30 (8.3%)
Prevalent case (%)	109 (23.5%)	35 (29.4%)	15 (12.8%)	110 (23.7%)	64 (30.6%)	91 (25.3%)
Pioglitazone (%)	91 (19.6%)	12 (10.1%)	26 (22.2%)	81 (17.5%)	42 (20.1%)	49 (13.6%)
Other TZDs (%)	14 (3%)	3 (2.5%)	4 (3.4%)	10 (2.2%)	7 (3.3%)	9 (2.5%)
Metformin (%)	258 (55.6%)	58 (48.7%)	56 (47.9%)	252 (54.3%)	105 (50.2%)	162 (45%)
Sulfonylurea (%)	313 (67.5%)	69 (58%)	77 (65.8%)	296 (63.8%)	122 (58.4%)	229 (63.6%)
Insulin (%)	107 (23.1%)	31 (26.1%)	36 (30.8%)	123 (26.5%)	50 (23.9%)	68 (18.9%)
No diabetes medications (%)	71 (15.3%)	22 (18.5%)	18 (15.4%)	66 (14.2%)	43 (20.6%)	67 (18.6%)
Current Smoker**	116 (25%)	34 (28.6%)	30 (25.6%)	77 (16.6%)	44 (21.1%)	60 (16.7%)

*Creatinine $\geq$1.4 for women and $\geq$1.5 for men ** As defined in the cohort study data

Supplemental Table A. Comparison of the results of the final analysis and the 5-year and 8-year interim reports

	5-Year Cohort Analysis Categories	5-Year Cohort Analysis Fully adjusted HR† (95% CI)	8-Year Cohort Analysis Categories	8-Year Cohort Analysis Fully adjusted HR† (95% CI)	10-Year Cohort Analysis Categories	10-Year Cohort Analysis Fully adjusted HR† (95% CI)
Ever exposed to pioglitazone		1.17 (0.92-1.49)		1.07 (0.87-1.30)		1.10 (0.92-1.31)
Time since starting pioglitazone						
	Less than 1.5 years	1.17 (0.79 - 1.74)	Less than 3.5 years	0.96 (0.74-1.24)	Less than 4.5 years	0.93 (0.74-1.17)
	1.5 to 3 years	1.37 (0.91 - 2.06)	3.5-6.5 years	1.07 (0.77-1.48)	4.5 – 8.0 years	1.26 (0.97-1.65)
	More than 3 years	1.27 (0.89 - 1.82)	More than 6.5 years	1.19 (0.78-1.80)	More than 8 years	1.22 (0.84-1.78)
Duration of therapy						
	Less than 1 year	0.83 (0.55 - 1.26)	Less than 1.5 years	0.78 (0.57-1.05)	Less than 1.5 years	0.93 (0.71-1.22)
	1 to 2 years	1.40 (0.92 - 2.13)	1.5-4.0 years	1.15 (0.87-1.53)	1.5-4.0 years	1.08 (0.83-1.39)
	More than 2 years	1.44 (1.03 - 2.02)				
	More than 4 years*	1.62 (0.96 - 2.74)	More than 4 years	1.30 (0.91-1.86)	More than 4 years	1.19 (0.89-1.58)
Cumulative dose						
	1 – 10500 mg	1.02 (0.71 - 1.47)	1 – 13000 mg	0.89 (0.67-1.20)	1 – 13000 mg	0.94 (0.73-1.22)
	10501 – 28000 mg	1.18 (0.80 - 1.75)	13001 – 35000 mg	0.98 (0.71-1.35)	14001 – 40000 mg	1.15 (0.89-1.49)

>28000 mg	1.43 (0.96 - 2.12)	>35000 mg	1.25 (0.91-1.74)	>40000 mg	1.09 (0.81-1.47)

†Fully adjusted refers to inclusion of all potential confounders in the statistical model from the 5 year interim report: age, sex, race/ethnicity, other diabetes medications, smoking, other bladder conditions, median household income, congestive heart failure, cancer other than bladder cancer, renal insufficiency, HbA1c and the interaction with new diagnosis of diabetes, and duration of diabetes. In the 8 and 10 year analysis, the fully adjusted model also includes year of cohort entry.
* More than 4 years was a post hoc subset of the more than 2 year category.

Supplemental Table B. Truncating follow-up at April 30, 2008

	Fully adjusted model (HR, 95% CI)	Fully adjusted model adding the proteinuria testing variables (HR, 95% CI)
Unexposed to pioglitazone	Reference	Reference
Ever exposed to pioglitazone	1.15 (0.90-1.46)	1.09 (0.85-1.38)
Duration of therapy		
Less than 1.5 years	1.03 (0.73-1.46)	0.95 (0.68-1.35)
1.5-4.0 years	1.27 (0.89-1.82)	1.18 (0.83-1.69)
More than 4 years	1.50 (0.88-2.51)	1.42 (0.84-2.40)

Supplemental Table C. Analysis of duration of exposure to pioglitazone with additional categories of long term exposure

	Cases of bladder cancer	Fully adjusted model (HR, 95% CI)	Fully adjusted model adding the proteinuria testing variables (HR, 95% CI)
Unexposed to pioglitazone	1,075	Reference	Reference
Duration of therapy			
Less than 1.5 years	60	0.93 (0.71-1.22)	0.88 (0.68-1.16)
1.5-4.0 years	69	1.08 (0.83-1.39)	1.03 (0.80-1.33)
4.1-6.0 years	36	1.32 (0.94-1.87)	1.29 (0.91-1.82)
More than 6 years	21	1.00 (0.64-1.56)	0.99 (0.63-1.55)

Supplemental Table D. Censoring follow-up if pioglitazone was discontinued for at least 1 year

	Cases of bladder cancer	Fully adjusted adding the proteinuria testing variable[††] (HR, 95% CI)
Unexposed to pioglitazone	1,075	Reference
Ever exposed to pioglitazone	117	1.07 (0.88-1.32)
Duration of therapy among current users		
Less than 1.5 years	36	0.90 (0.64-1.27)
1.5-4.0 years	44	1.07 (0.78-1.45)
More than 4 years	37	1.05 (0.74-1.48)

Supplemental Table E. Analysis using finer gradation of the oldest age category[#]

	Adjusted for age, sex and year of cohort entry (HR, 95% CI)	Fully adjusted[†] (HR, 95% CI)	Fully adjusted adding the proteinuria testing variable[††] (HR, 95% CI)
Unexposed to pioglitazone	Reference	Reference	Reference
Ever exposed to pioglitazone	1.09 (0.92-1.29)*	1.10 (0.93-1.31)	1.06 (0.89-1.26)
Time since starting pioglitazone			
Less than 4.5 years	0.94 (0.75-1.17)	0.94 (0.75-1.18)	0.89 (0.71-1.12)
4.5-8.0 years	1.30 (1.00-1.68)	1.27 (0.97-1.66)	1.22 (0.93-1.59)
More than 8 years	1.29 (0.90-1.86)	1.24 (0.85-1.80)	1.20 (0.83-1.75)
Test for trend	P=0.06	P=0.13	P=0.28
Duration of therapy			
Less than 1.5 years	0.95 (0.73-1.24)	0.94 (0.72-1.23)	0.89 (0.68-1.16)
1.5-4.0 years	1.09 (0.85-1.40)	1.09 (0.84-1.40)	1.03 (0.80-1.34)
More than 4 years	1.22 (0.92-1.61)	1.19 (0.90-1.59)	1.16 (0.87-1.54)
Test for trend	P=0.16	P=0.26	P=0.47
Cumulative dose			
1 – 14000 mg	0.95 (0.74-1.23)	0.95 (0.73-1.22)	0.90 (0.69-1.16)
14001 – 40000 mg	1.17 (0.91-1.50)	1.16 (0.90-1.50)	1.10 (0.86-1.42)
>40000 mg	1.14 (0.85-1.52)	1.09 (0.81-1.48)	1.06 (0.79-1.44)
Test for trend	P=0.20	P=0.35	P=0.59

[#] Age categories were 40-49, 50-59, 60-69, 70-79, 80-89, 90 and older.

[†]Fully adjusted refers to inclusion of all potential confounders in the statistical model from the last report plus year of cohort entry: age, sex, race/ethnicity, other diabetes medications, smoking, other bladder conditions, median household income, congestive heart failure, cancer other than bladder cancer, renal insufficiency, HbA1c and the interaction with new diagnosis of diabetes, duration of diabetes, and year of cohort entry

[††] Fully adjusted model adding the 3-level time updated proteinuria testing variable (no testing, negative and positive testing for proteinuria), excluding same day test for hematuria

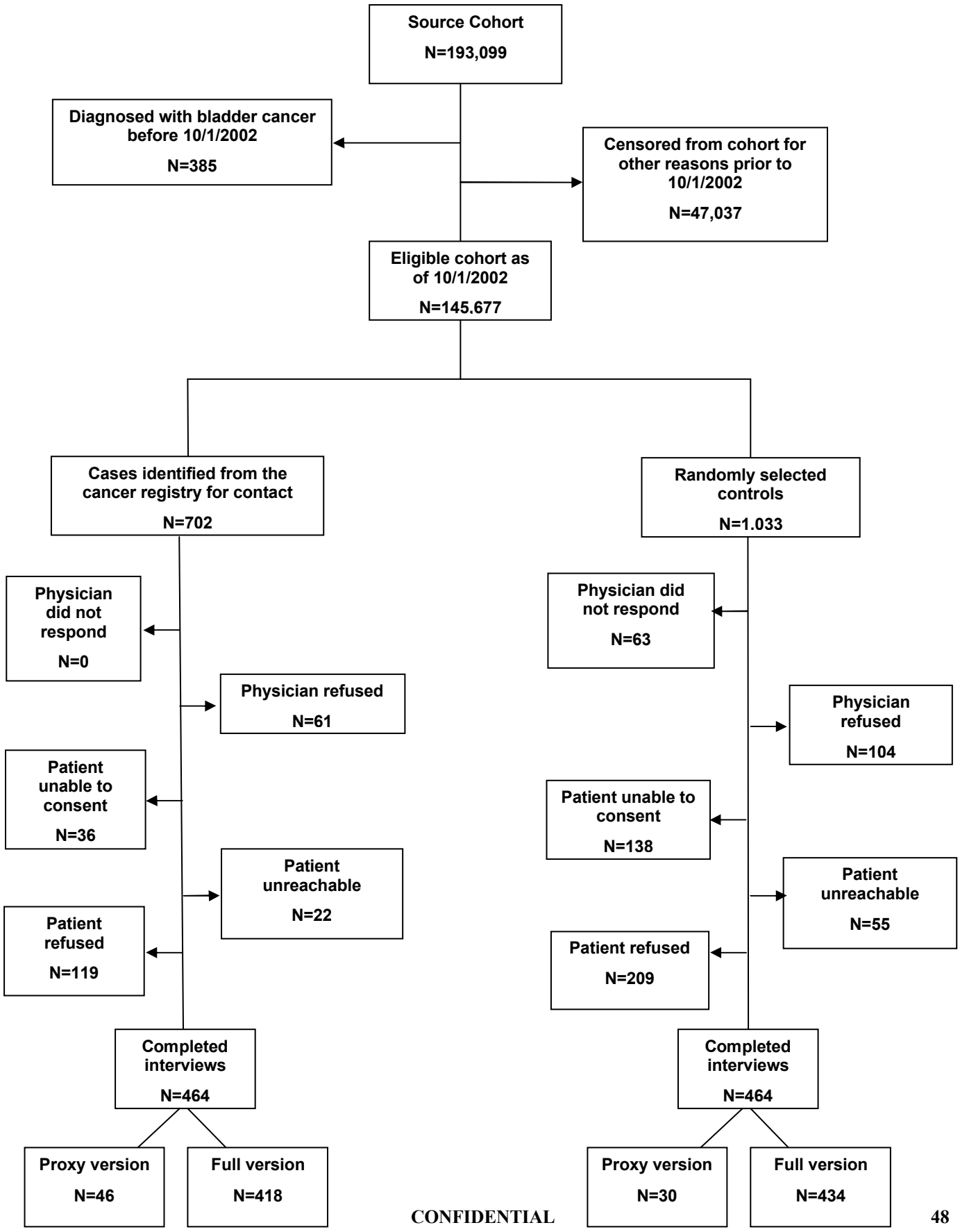
*Also adjusted for use of other diabetes medication

Supplemental Table F. Prior meta-analyses of the association of pioglitazone with the risk of bladder cancer

Study	Summary estimate for ever having been treated with pioglitazone	Summary estimate for more than 24 months of exposure	Studies
Colmers (2012)(26)	1.22 (1.07 – 1.39)	N/R	3 CS
Zhu (2012)(27)	1.17 (1.03 – 1.32)	1.38 (1.12 – 1.70)	1 RCT, 1 CS, 1 CC
Bosetti (2013)(28)	1.20 (1.07 – 1.34)	1.42 (1.17 – 1.72)	4 CS, 2 CC
Ferwana (2013)(29)	1.23 (1.09 – 1.39)	1.44 (1.19 – 1.74)	1 RCT, 4 CS, 1 CC
Monami (2013)(30)	2.05 (0.84 – 5.02)	N/R	4 RCT
He (2014)(21)	1.48 (1.09 – 2.00)	1.48 (1.23 – 1.79)	1 RCT, AERS, 5 CS, 3 CC
Turner (2014)(17)	RCTs 2.51 (1.09 – 5.80) CS 1.21 (1.09 – 1.35)	N/R 1.51 (1.26 – 1.81)	3 RCT 6 CS, 2CC

N/R – not reported; RCT – randomized clinical trial; CS – cohort study; CC – case-control study

Figure 1. Creation of the study population for the nested case-control study



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