Pioglitazone HCl (ACTOS)

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

Cohort Study of Pioglitazone and Bladder Cancer in Patients with Diabetes

Fourth Interim Analysis (8-Year) Report with Data from January 1, 1997 to December 31, 2010

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1.0 BACKGROUND

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 is being conducted over the course of 10 years, with a series of interim analyses provided to the sponsor (Takeda) and the appropriate regulatory agencies.

In 2011, we published the planned 5-year interim analysis of this study in Diabetes Care [1]. That report included data from 1 January 1997 to 30 April 2008. Following reporting of these data, there was a request from the FDA for an additional fourth interim analysis at 8 years including data from 1 January 1997 to 31 December 2010. In addition, the FDA requested additional sensitivity analysis, which will be completed by 31 December 2012.

2.0 METHODS

2.1 Data Source

The study is being 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 [2]. The KPNC pharmacy database includes information on each outpatient prescription dispensed at a KPNC pharmacy. Prior research has 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 [2].

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 [2].

2.1.1 Creation of the Study Cohort

For this fourth interim analysis, 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.

2.1.2 Follow-up Period for Fourth Interim Analysis

For this interim analysis, we included data from January 1, 1997 through December 31, 2010. Follow-up started on the first date that the inclusion criteria were met. Follow-up for patients in the cohort ended when any 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, 2010).

2.1.3 Primary Outcome

The primary 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, 2010. This was supplemented by case identification through surveillance of electronic pathology reports within KPNC for the period from January 1, 2005 to December 31, 2010, the time period of interviewing for the linked nested case-control study [1]. 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 [3,4].

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.

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.

While the FDA had requested that we include exposure to pioglitazone prior to age 40 in our calculation of cumulative dose and duration of exposure, we have subsequently elected not to implement this change. We reason that inclusion of this follow-up time in the calculation of cumulative dose and duration would introduce immortal time bias [5]. 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). In our prior analyses, all confounders other than smoking were measured using data recorded on or before the start of follow-up. Where appropriate, categorical variables included an additional category for "missing data."

In the current analysis, we have now included several new potential confounder variables as time updating covariates (i.e., once the patient had documented exposure the patient was considered exposed from that point forward and prior to that the patient was considered unexposed). The new variables include use of statins, angiotensin converting enzyme inhibitors or angiotensin receptor blockers, or medications used to treat benign prostatic hypertrophy. Also newly included were urinary incontinence, urinary tract infection or pyelonephritis, urolithiasis, other bladder conditions, prostatic specific antigen (PSA) testing, hemoglobin A1c 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 as a composite variable and as its individual components. 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. As discussed below, these new variables were not included in a fully adjusted model that used the same variables included in the earlier interim reports.

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) [6].

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).

2.1.6 Statistical Analyses

Continuous and categorical variables were compared with the Wilcoxon rank sum test and chi-square test or Fisher's exact test, respectively. 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. 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. For this interim analysis, calendar year of cohort entry was added to 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. To test for confounding, we separately added the other potential confounding variables to the baseline model to assess for a change in the hazard ratio for pioglitazone or for change in the highest category of pioglitazone exposure in models of dose and duration. We decided a priori that any variable resulting in a 10% change would be included in the final model. However, because none of the variables met this definition, the baseline model was the final model. Because the large number of cases of bladder cancer made over fitting the statistical model unlikely, we repeated the analyses using the same variables from our prior reports where we included all potential confounders in the fully adjusted Cox regression models. This did not include the new time updating variables, as it would have been infeasible to include so many time updating variables in a single model.

Several different definitions of HbA1c were tested in the models. These included HbA1c concentration as time updating with each measurement, as time updating with a three year lag period, at baseline with and without an interaction term between HbA1c levels, and whether the measurement coincided with a new diagnosis of diabetes based on the patient newly entering the diabetes registry (this could include true incident diagnosis of diabetes or a patient with diabetes who newly registered with KPNC). Akaike's Information Criterion (AIC) was used to assess relative model fit with the different definitions [7]. The model that treated HbA1c concentration as time updating using each new measurement consistently had the smallest AIC value, although the results were generally similar (data not shown).

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 and 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.

2.1.7 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 exposed. 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.

3.0 RESULTS

3.1 Descriptive Analyses

The registry contained 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.

Patients who ever used pioglitazone during the study period (n=33,416) 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 ever used pioglitazone as of the end of follow-up, the median time from the first prescription to the end of follow-up was 4.8 years (range 0.2-11.3 years) and the median duration of therapy among pioglitazone treated patients was 2.6 years (range 0.2-11.2 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 6.5 years earlier, had more than 4 years of use of pioglitazone, and had received more than 35,000 mg in total dose.

3.2 Overall Analyses of Anti-diabetic Drugs and Bladder Cancer

During the follow-up period, there were 1089 cases of newly diagnosed bladder cancer, 137 among patients who ever used pioglitazone and 952 among patients who never used pioglitazone. In a completely unadjusted model, there was no association between pioglitazone use and the incidence of bladder cancer (HR=0.98, 95% CI 0.81-1.18). After adjusting only for age, sex, calendar year of cohort entry, and use of other categories of diabetes medications the results were similar (HR=1.06, 95% CI 0.87-1.30). None of the other potential covariates met our definition of a confounder and as such, this can be viewed as the final model. However, as with our prior reports, we repeated the analysis using all confounders included in the 5-year interim report and the results were similar (HR=1.07, 95% CI 0.87-1.30) (Table 3).

Like pioglitazone, in the fully adjusted model none of the other diabetes medications (i.e., other TZDs, metformin, sulfonylureas, insulin, or other diabetes medications) were significantly associated with bladder cancer, with hazard ratios ranging from 1.03 to 1.12.

In the fully adjusted model, there was a strong association between bladder cancer incidence and older age (40-49 years reference group: 50-59 years HR=4.51, 95% CI 3.00 – 6.80; 60-69 years HR=13.0, 95% CI 8.81 - 19.3; 70 years or older HR=20.2, 95% CI 13.6 - 30.0)), and male sex (HR=4.69, 95% CI 3.99 – 5.51), and a modest association with current smoking (HR=1.51, 95% CI 1.31 - 1.74).

3.3 Dose and Duration Analyses

When we examined the association between bladder cancer incidence and increasing levels of pioglitazone exposure (Table 3), the risk of bladder cancer increased with increasing dose and duration of pioglitazone use. After adjusting only for age, sex, and calendar year, the risk of bladder cancer was 20% higher among those whose duration of pioglitazone therapy was 1.5 to 4 years (HR=1.19, 95% CI 0.91-1.57) and 40% higher among those with more than 4 years of exposure (HR = 1.38, 95% CI 0.97-1.96), as compared to never users of pioglitazone (test for trend p=0.11). The fully adjusted models provided slightly attenuated results (test for trend p=0.24). There was a similar pattern between increasing time since initiation of pioglitazone and bladder cancer risk and for cumulative dose (fully adjusted test for trend: time since initiation p=0.53; cumulative dose p=0.45). Unlike in our previous analyses, none of the individual HR estimates achieved conventional levels of statistical significance in this fourth interim analysis.

3.4 Interaction by Sex and Smoking

The association between ever use of pioglitazone and bladder cancer risk was similar among men (HR= 1.02, 95% CI 0.82 - 1.27) and women (HR=1.33, 95% CI 0.83 - 2.12) (test for interaction p=0.35). Similarly, the pioglitazone hazard ratios were similar among nonsmokers (HR=1.12, 95% CI 0.89-1.41) and smokers (HR=0.91, 95% CI 0.61-1.36) (test for interaction p=0.79). Dose and duration analyses stratified by sex and smoking status are summarized in Table 4. Although differences across most subgroups were not statistically significant, there was qualitative evidence of a stronger association for duration of therapy among men and nonsmokers.

3.5 Additional Notes on Testing for Confounders

Although none of the potential confounders other than those in our base model resulted in a 10% or greater change in the hazard ratio, the impact of adjusting for proteinuria and diabetic nephropathy shifted the hazard ratio slightly towards the null. For example, in the base model that included age, sex, year of cohort entry and other diabetes medications, the hazard ratio for ever exposure to pioglitazone shifts from 1.06 (0.87-1.30) to 1.01 (0.83-1.23) with additional adjustment for microalbuminuria. Similarly, in the model assessing duration of therapy, the hazard ratio for more than 4 years of therapy decreased from 1.38 (0.97-1.96) to 1.27 (0.90-1.81) after adjusting for microalbuminuria or proteinuria (micro or macro) and to 1.33 (0.93-1.88) when adjusting for diabetic nephropathy. After adjusting for age, sex, and year of cohort entry, microalbuminuria, proteinuria, and diabetic nephropathy were each strongly associated with bladder cancer, with hazard ratios of 4.80 (4.11-5.61), 5.49 (95% CI 4.65-6.47), and 1.55 (95% CI 1.33-1.81), respectively. Likewise, they were more common among patients treated with pioglitazone (Table 1).

The vast majority of bladder cancers were diagnosed at an early stage (Table 5). Five 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=0.14 excluding cases with undetermined stage).

4.0 DISCUSSION

This report describes the fourth (8-year) interim results of a 10-year cohort study being 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 [8]. 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 [9]. However, a more recent study in a different animal model proposed that rosiglitazone, another TZD, may be a tumor promoter even in late stages of bladder cancer development [10]. Regardless, in the absence of controlled studies in humans, it is not possible to know with certainty whether pioglitazone therapy increases or decreases the risk of bladder cancer in humans.

In our third (5-year) interim report we noted that we did not observe a significant association between any pioglitazone exposure 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 and in those with the highest cumulative dose and a significant dose response in the trend analysis [1]. In the current analysis, the magnitude of the previously observed associations were weaker and no longer statistically significant, although there remained a fairly consistent albeit statistically nonsignificant pattern of increasing hazard ratios with longer duration of exposure and greater cumulative dose (see Table 6 for a comparison of the results from the two sequential analyses). Of note, our methods were nearly identical, with the exception of a slight change in how we measured duration of exposure. Prior to performing this analysis, we also 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.

We urge caution in over interpreting serial interim analyses. We have now conducted four interim analyses of the 10-year cohort study. The associations observed in the 5-year interim analysis were statistically significant, but only marginally so at a p value of 0.05. In the 8-year analysis, the tentative signal from the 5-year analysis has not become stronger, in contrast to what one may have predicted as more time has accumulated and patients have even longer duration of exposure to pioglitazone. Of course, we cannot predict what the results will be after the full follow-up is complete. Thus, we believe that any formal conclusions should be reserved until the study is fully completed.

In the current analysis, we have also demonstrated that there may be differential effects among men and women across the dose strata, but effect modification by smoking was not demonstrated. In the coming months we will test for interaction between sex and pioglitazone exposure and smoking and pioglitazone exposure in the trend analyses described above.

Although none of the potential confounders other than those in our base model resulted in a 10% or greater change in the hazard ratio, we noted that the impact of adjusting for proteinuria and diabetic nephropathy shifted the hazard ratio slightly towards the null. A possible explanation for this finding is that the presence of proteinuria or diabetic nephropathy may prompt a treating physician to order a complete urinalysis, which in turn may detect hematuria and prompt an

evaluation that leads to the diagnosis of bladder cancer. Since pioglitazone is prescribed to patients with more advanced diabetes who are more likely to have proteinuria or renal insufficiency (since the first line diabetes medication metformin is contraindicated in these patients), this could lead to increased detection of bladder cancer. This is consistent with a small and non-significant excess of early stage bladder cancers among the pioglitazone treated patients. We noted this possibility in our prior reports, but had not adjusted for proteinuria in those reports. Some have also hypothesized that renal insufficiency and albuminuria may directly contribute to the incidence of bladder cancer [11-14]. An alternative explanation is that symptoms of bladder cancer prompt detection of proteinuria, which occurs before the diagnosis of bladder cancer is confirmed [14]. In that case, adjusting for proteinuria in our model would potentially bias us toward the null, thereby potentially missing a true association between pioglitazone and bladder cancer.

In a previous report of data through December 31, 2005 (the second interim analysis of the cohort study), we did not observe evidence of a strong dose or duration response with sulfonylureas or metformin. Although these medications may typically be used in patients with earlier stage diabetes than those treated with pioglitazone, were detection contributing to the higher incidence of bladder cancer with longer duration of therapy, we may have seen an increase in bladder cancer risk with sulfonylureas and metformin. Specifically, we anticipate that proteinuria, particularly microalbuminuria, should become more common with longer follow-up on any of the medications. Thus, the absence of this finding, while not definitive, argues against increased diagnosis due to more frequent microalbuminuria in long-term users of pioglitazone.

In the coming months, we will work to try to understand which of these scenarios is the most plausible. We have now planned several additional analyses to explore this issue.

1. We will assess the prevalence of proteinuria at cohort entry and at various points of time during follow-up. This will allow us to understand better the timing of proteinuria diagnosis.
2. We will assess the incidence of testing for proteinuria over time in the study cohort. This will allow us to understand whether there has been increased surveillance for proteinuria during the course of the study.
3. We will assess whether patients are more likely to have a full urinalysis completed following a positive test for microalbuminuria vs. following a negative test for microalbuminuria. If we see such an association, it will suggest that the higher prevalence of microalbuminuria among patients treated with pioglitazone could have led to increased detection of bladder cancer in this population.
4. If microalbuminuria is associated with performance of a full urinalysis, we will assess whether patients treated with pioglitazone are more likely to have testing for microalbuminuria than patients treated with other diabetes therapies.

There are several major strengths of this study. 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=4,669) or who filled two or more prescriptions that were never within six months of each other (n=733) 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. Finally, 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.

We have not adjusted for multiple comparisons in these analyses and as such some statistical associations might be expected by chance alone. However, two of these subgroups, duration of therapy and cumulative dose, are expected to be strongly correlated. Time since initiation of pioglitazone is likely also correlated with duration of therapy and cumulative dose, albeit less strongly.

The stage data are reassuring that the mortality impact of any increase in bladder cancer incidence that occurs within the first eleven years of pioglitazone therapy, if real, should be small. We will continue to monitor the distribution of early and late stage cancers throughout the remainder of this ongoing cohort study.

In summary, in our cohort analysis, there is no overall statistically significant increased risk of bladder cancer among patients ever treated with pioglitazone. The analyses addressing increasing exposure to pioglitazone suggest a possible small increased risk with longer-term therapy. However, the tentative signal from the 5 year study has not gotten any stronger, which one would have expected as more time has accumulated. In absolute terms, the incidence of bladder cancer among patients who received 4 or more years of pioglitazone was 115 per 100,000 person-years. Furthermore, it remains reassuring that only seven of 137 bladder cancers diagnosed in patients treated with pioglitazone were advanced stage. With longer-term follow-up of this cohort, more precise estimates will be obtained.

Table 1 Demographics of the Study Cohort According to Pioglitazone Treatment at any Time During Follow-up [§]

	Pioglitazone treated (n=33,416)	No pioglitazone treatment (n=159,683)
Age at baseline		
40-49 years	9,729 (29.1%)	35,335 (22.1%)
50-59 years	11,066 (33.1%)	40,841 (25.6%)
60-69 years	8,573 (25.7%)	41,916 (26.2%)
70 years and older	4,048 (12.1%)	41,591 (26.0%)
Female sex	15,586 (46.6%)	74,257 (46.5%)
Race/Ethnicity		
White	17,203 (51.5%)	83,425 (52.2%)
Black	3,426 (10.3%)	17,270 (10.8%)
Asian	4,914 (14.7%)	20,423 (12.8%)
Hispanic	4,466 (13.4%)	17,012 (10.7%)
Other	1,951 (5.8%)	9,083 (5.7%)
Missing	1,456 (4.4%)	12,470 (7.8%)
Current smoker	6,851 (20.5%)	27,746 (17.4%)
Renal function at baseline		
Normal creatinine	25,844 (77.3%)	123,209 (77.2%)
Elevated creatinine*	1,334 (4.0%)	13,907 (8.7%)
Missing	6,238 (18.7%)	22,567 (14.1%)
Congestive heart failure at baseline	999 (3.0%)	11,008 (6.9%)
Income		
Low[‡]	15,985 (47.8%)	80,698 (50.5%)
High	14,268 (42.7%)	64,690 (40.5%)
Missing	3,163 (9.5%)	14,295 (9.0%)
Baseline HbA1c		
< 7%	5,646 (16.9%)	45,634 (28.6%)
7-7.9%	6,116 (18.3%)	30,856 (19.3%)
8-8.9%	4,295 (12.9%)	16,686 (10.4%)
9-9.9%	3,259 (9.8%)	11,244 (7.0%)
≥10%	8,023 (24.0%)	27,324 (17.1%)
Missing	6,077 (18.2%)	27,939 (17.5%)
Newly diagnosed with DM at the start of follow-up[#]	16,905 (50.6%)	92,521 (57.9%)
Diabetes duration at baseline		
0-4 years	19,961 (59.7%)	100,318 (62.8%)
5-9 years	3,108 (9.3%)	9,546 (6.0%)
10 or more years	3,068 (9.2%)	17,320 (10.8%)
Missing	7,279 (21.8%)	32,499 (20.4%)
Other cancer prior to baseline	1,032 (3.1%)	8,484 (5.3%)

Footnotes are on last table page.

Table 1 Demographics of the Study Cohort According to Pioglitazone Treatment at any Time During Follow-up [§] (continued)

	Pioglitazone treated (n=33,416)	No pioglitazone treatment (n=159,683)
Other diabetes medications[¥]		
Other TZDs	2,792 (8.4%)	2,446 (1.5%)
Metformin	28,084 (84.0%)	71,861 (45.0%)
Sulfonylureas	29,767 (89.1%)	96,518 (60.4%)
Other oral hypoglycemic drugs	1,945 (5.8%)	2,119 (1.3%)
Insulin	16,049 (48.0%)	44,673 (28.0%)
Statin Use	29,457 (88.2%)	92,863 (58.2%)
ACE inhibitors or ARB	30,678 (91.8%)	110,114 (69.0%)
BPH medications[†]	4,531 (25.4%)[μ]	15,881 (18.6%)[μ]
Urinary Incontinence	2,103 (6.3%)	7,626 (4.8%)
UTI/Pyelonephritis	10,878 (32.6%)	44,385 (27.8%)
Urolithiasis	2,449 (7.3%)	7,353 (4.6%)
Other Bladder conditions[Σ]	10,611 (31.8%)	38,628 (24.2%)
PSA Testing	16,180 (90.8%)[μ]	60,384 (70.7%)[μ]
Diabetes complications [β]	31,437 (94.1%)	131,101 (82.1%)
Diabetic retinopathy	16,647 (49.8%)	49,277 (30.9%)
Peripheral neuropathy[∞]	23,888 (71.5%)	84,379 (52.8%)
Proteinuria[Ω]	24,822 (74.3%)	92,096 (57.7%)
Microalbuminuria	24,312 (72.8%)	87,325 (54.7%)
Macroalbuminuria	10,821 (32.4%)	36,179 (22.7%)
Diabetic nephropathy[π]	7,898 (23.6%)	31,398 (19.7%)
Coronary artery disease	15,216 (45.5%)	69,035 (43.2%)

[§] 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=0.64).
* 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.
[†] 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	33,416
Time since starting pioglitazone (median, range)	4.8 yr (0.2-11.3)
Less than 3.5 years (n, %)	10,916 (32.7%)
3.5-6.5 years (n, %)	11,372 (34.0%)
More than 6.5 years (n, %)	11,128 (33.3%)
Duration of therapy (median, range)	2.6 yr (0.2-11.2)
Less than 1.5 years (n, %)	10,755 (32.2%)
1.5-4.0 years (n, %)	12,277 (36.7%)
More than 4 years (n, %)	10,384 (31.1%)
Cumulative dose, mg (median, range)	21,900 mg (450-140,000)
1 – 13000 mg (n, %)	11,176 (33.4%)
13001 – 35000 mg (n, %)	10,723 (32.1%)
>35000 mg (n, %)	11,517 (34.5%)

Table 3 Incidence Rate and Relative Hazard of Bladder Cancer with Pioglitazone Exposure

	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)
Unexposed to pioglitazone	952	1,311,069	72.6 (68.0 – 77.2)	Reference	Reference	Reference	Reference
Ever exposed to pioglitazone	137	166,556	82.3 (68.5 – 96.0)	0.98 (0.81-1.18)	1.06 (0.87 - 1.30)*	1.07 (0.88 – 1.30)*	1.07 (0.87-1.30)
Time since starting pioglitazone							
Less than 3.5 years	67	99,058	67.6 (51.4 – 83.8)	0.84 (0.65-1.08)	0.98 (0.76-1.26)	0.98 (0.76-1.26)	0.96 (0.74-1.24)
3.5-6.5 years	43	48,955	87.8 (61.6 – 114.1)	1.00 (0.73-1.37)	1.12 (0.82-1.54)	1.13 (0.82-1.54)	1.07 (0.77-1.48)
More than 6.5 years	27	24,718	109.2 (68.0 – 150.4)	1.21 (0.81-1.80)	1.29 (0.87-1.93)	1.30 (0.87-1.94)	1.19 (0.78-1.80)
Test for trend					P=0.23		P=0.53
Duration of therapy							
Less than 1.5 years	46	84,199	54.7 (38.9 – 70.4)	0.67 (0.50-0.91)	0.80 (0.60-1.08)	0.80 (0.60-1.08)	0.78 (0.57-1.05)
1.5-4.0 years	56	61,223	91.5 (67.5 – 115.4)	1.07 (0.81-1.41)	1.19 (0.91-1.57)	1.20 (0.91-1.58)	1.15 (0.87-1.53)
More than 4 years	35	30,537	114.6 (76.6 – 152.6)	1.28 (0.90-1.81)	1.38 (0.97-1.96)	1.39 (0.98-1.97)	1.30 (0.91-1.86)
Test for trend					P=0.11		P=0.24

Footnotes are on last table page.

Table 3 Incidence Rate and Relative Hazard of Bladder Cancer with Pioglitazone Exposure (continued)

	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)
Cumulative dose							
1 – 13000 mg	50	79,833	62.6 (45.3 - 80.0)	0.77 (0.58-1.03)	0.91 (0.69-1.22)	0.91 (0.69-1.22)	0.89 (0.67-1.20)
13001 – 35000 mg	43	55,326	77.7 (54.5 – 101.0)	0.91 (0.67-1.24)	1.01 (0.74-1.38)	1.01 (0.74-1.38)	0.98 (0.71-1.35)
>35000 mg	44	41,115	107.0 (75.4 - 138.6)	1.21 (0.88-1.65)	1.34 (0.98-1.83)	1.34 (0.98-1.84)	1.25 (0.91-1.74)
Test for trend					P=0.21		P=0.45

†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.
*Also adjusted for use of other diabetes medication.

Table 4 Results of Age, Sex, and Calendar Year of Cohort Entry Adjusted Models Stratified by Sex and Smoking Status.

	Men	**Women**	**Smokers**	**Non-smokers**
Person-years of follow-up time unexposed to pioglitazone	687,083	623,986	251,567	1,059,503
Person-years of follow-up time ever exposed to pioglitazone	87,161	79,395	34,209	132,347
Cases of bladder cancer among pioglitazone unexposed	797	155	225	727
Cases of bladder cancer among pioglitazone exposed	112	25	33	104
	HR, 95% CI	**HR, 95% CI**	**HR, 95% CI**	**HR, 95% CI**
Ever exposed to pioglitazone Interaction p value	1.02 (0.82-1.27) 0.35	1.33 (0.83-2.12)	0.91 (0.61-1.36) 0.79	1.12 (0.89-1.41)
Time since starting pioglitazone*				
Less than 3.5 years	0.96 (0.73-1.27)	1.05 (0.57-1.96)	0.85 (0.51-1.43)	1.02 (0.77-1.36)
3.5-6.5 years	1.16 (0.82-1.62)	0.96 (0.42-2.20)	1.00 (0.54-1.87)	1.17 (0.81-1.68)
More than 6.5 years	1.12 (0.70-1.80)	2.09 (0.98-4.46)	0.96 (0.41-2.22)	1.43 (0.91-2.26)
Interaction p values†	0.72, 0.81, 0.07		0.78, 0.93, 0.83	
Duration of therapy*				
Less than 1.5 years	0.70 (0.49-0.99)	1.31 (0.74-2.32)	0.64 (0.34-1.21)	0.86 (0.61-1.21)
1.5-4.0 years	1.24 (0.92-1.67)	0.96 (0.44-2.06)	1.10 (0.64-1.87)	1.23 (0.89-1.70)
More than 4 years	1.41 (0.96-2.06)	1.29 (0.52-3.22)	1.12 (0.54-2.33)	1.49 (1.00-2.22)
Interaction p values†	0.04, 0.70, 0.91		0.59, 0.83, 0.91	
Cumulative dose*				
1 – 13000 mg	0.78 (0.55-1.09)	1.55 (0.90-2.66)	0.73 (0.40-1.35)	0.98 (0.71-1.36)
13001 – 35000 mg	1.04 (0.74-1.45)	0.87 (0.38-1.98)	1.19 (0.68-2.06)	0.94 (0.65-1.38)
>35000 mg	1.43 (1.03-1.99)	0.86 (0.31-2.36)	0.82 (0.40-1.68)	1.56 (1.10-2.21)
Interaction p values†	0.02, 0.86, 0.47		0.59, 0.23, 0.29	

* Reference group is unexposed to pioglitazone.
† Interaction p values are for the interaction between the exposure and sex or smoking within strata defined by the dose level or duration level.

Table 5 Cancer Stage by Exposure Status.

Cancer stage	Pioglitazone treated (n=137 cases)	No pioglitazone treatment (n=952 cases)
PUNLMP*	1 (1%)	7 (1%)
In situ	65 (47%)	467 (49%)
Local	61 (45%)	363 (38%)
Regional	6 (4%)	58 (6%)
Distant	1 (1%)	28 (3%)
Undetermined	3 (2%)	29 (3%)

* Papillary urethral neoplasm of low malignant potential.

Table 6 Comparison of the Results of the Current Analysis and the 5-Year Interim Report

	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)
Ever exposed to pioglitazone		1.17 (0.92-1.49)		1.07 (0.87-1.30)
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)
	1.5 to 3 years	1.37 (0.91 - 2.06)	3.5-6.5 years	1.07 (0.77-1.48)
	More than 3 years	1.27 (0.89 - 1.82)	More than 6.5 years	1.19 (0.78-1.80)
Duration of therapy				
	Less than 1 year	0.83 (0.55 - 1.26)	Less than 1.5 years	0.78 (0.57-1.05)
	1 to 2 years	1.40 (0.92 - 2.13)	1.5-4.0 years	1.15 (0.87-1.53)
	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)
Cumulative dose				
	1 – 10500 mg	1.02 (0.71 - 1.47)	1 – 13000 mg	0.89 (0.67-1.20)
	10501 – 28000 mg	1.18 (0.80 - 1.75)	13001 – 35000 mg	0.98 (0.71-1.35)
	>28000 mg	1.43 (0.96 - 2.12)	>35000 mg	1.25 (0.91-1.74)

†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 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.

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